Deviations from Michaelis–Menten Kinetics

THE POSSIBILITY OF COMPLICATED CURVES FOR SIMPLE KINETIC SCHEMES AND THE COMPUTER FITTING OF EXPERIMENTAL DATA FOR ACETYLCHOLIN-ESTERASE, ACID PHOSPHATASE, ADENOSINE DEAMINASE, ARYLSULPHATASE, BENZYLAMINE OXIDASE, CHYMOTRYPSIN, FUMARASE, GALACTOSE DEHYDROGENASE, β -GALACTOSIDASE, LACTATE DEHYDROGENASE, PEROXIDASE AND XANTHINE OXIDASE

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1. The possible graph shapes for one-site/two-state and substrate-modifier models are discussed. 2. The two-state model is a version of the Monod–Wyman–Changeux model and gives a rate equation with 240 denominator terms. Discussion in terms of K and V effects is not possible. A simplified version of the mechanism can be shown to give v -versus- $[S]$ curves that are either sigmoid or non-sigmoid. They may show substrate inhibition or no final maximum, and the double-reciprocal plots can be concave up or down. The corresponding binding model is determined by only two constants and gives a linear double-reciprocal plot. 3. The substrate-modifier mechanism is a simple example of a mechanism where inclusion of catalytic steps leads to a genuine increase in degree of the rate equation. The v -versus- $[S]$ curve can show such complexities as two maxima and a minimum, and the double-reciprocal plot can cross its asymptote twice, proving the rate equation to be 4:4. 4. A simplified version is 3:3, and analysis shows that at least 18 of the 27 double-reciprocal plots that can arise with 3:3 functions are possible with this particular mechanism. 5. Representative double-reciprocal and Scatchard plots are presented for several sets of rate-constant values. 6. It is concluded that relatively simple mechanisms give pseudo-steady-state rate equations of high degree and considerable complexity. With extended ranges of substrate concentrations there is every reason to believe that experimental data would show the sort of deviations from Michaelis–Menten kinetics seen with calculated curves for such simple mechanisms. Narrow ranges of substrate concentration, on the other hand, would lead to inflexions and curvature being overlooked. 7. It is not possible to discuss such deviations from Michaelis–Menten kinetics in terms of kinetic constants such as K_m and V , and, in general, it is also difficult to see any simple way to explain intuitively such features as sigmoidicity, substrate inhibition, double-reciprocal convexity and decrease in degree by cancellation of common factors between numerator and denominator of rate equations. 8. These conclusions apply with even more force when catalytic steps are included, for then the rate equations are, for multi-site mechanisms, of higher degree, allowing increasingly complex curve shapes. 9. A number of enzymes were studied and initial-rate data were fitted by computer. 10. Most of the enzymes studied showed pronounced deviations from Michaelis–Menten kinetics, but in some cases the deviations were slight. Non-linear regression of the experimental data gave adequate fits for rational functions of degree 2:2, 3:3 or 4:4. 11. In some cases the last numerator term was not significant, indicating dead-end substrate inhibition, i.e. 1:2 or 2:3 functions. 12. It is suggested that, in general, computer fitting of enzyme kinetic data over extended ranges of substrate concentration will require rational functions of degrees 2:2, 3:3 or 4:4, and interpretation in terms of K_m and V will not be possible.

In a recent review of enzyme-kinetic publications, it was pointed out that over 800 enzymes are known to give deviations from Michaelis–Menten kinetics, and that the number of cases known to obey hyperbolic kinetics over a wide variety of experimental conditions is actually small (Hill *et al.*, 1977). It was also observed that over 100 enzymes are currently claimed to have multiple turning points in v -versus- $[S]$ plots or inflexions in double-reciprocal space, features that necessitate third-degree terms in rate equations (Bardsley & Childs, 1975; Bardsley, 1976). Since even relatively simple model mechanisms lead to rate equations of high degree and great complexity, especially if catalytic steps are included (Waight & Bardsley, 1977), it was concluded that probably the steady-state equations required to fit experimental data will be of higher degree than commonly supposed, and that Michaelis–Menten types of kinetics, even in multi-substrate form (Cleland, 1970), will be, in general, inapplicable.

The usual approaches to steady-state enzyme kinetics assume that buried under the experimental noise are kinetic constants K_m and V , which have to be extracted by statistical processing of data and then interpreted. Clearly the previous conclusions are at variance with this accepted approach. Under such circumstances, a number of important questions must be answered, and we commence by attempting to formulate these.

Question 1. What are the possible graph shapes that can be given by simple realistic models for enzyme-catalysed reactions?

Question 2. One of the accepted tenets of enzyme kinetics is that inclusion of catalytic steps does not change the form of rate equations, but this is not true for multi-site models. Does the increase in degree that occurs when catalytic steps are included in such mechanisms lead to new possible curve shapes?

Question 3. How many of the reported examples of non-Michaelian kinetics are due to artifacts such as micelle formation, impurities, ionic-strength effects, non-linear instrument responses or experimental conditions allowing enzyme aggregation or hysteretic effects, and how many are genuine cases of high-degree rate equations?

Question 4. If reasonable safeguards are taken to avoid artifacts, and non-linear regression techniques are used in the attempt to obtain objective estimates of the minimum-degree rate equation involved, then what sort of degrees will usually be required?

Question 5. What new insight into molecular mechanism can be gained by eschewing the use of simplified models if statistical processing of experimental data indicates that high-degree rate equations are required, and in what ways might the use of oversimplified models lead unwittingly to false conclusions?

These questions fall clearly into three categories. Questions 1 and 2 are purely theoretical, questions 3 and 4 concern details of practical technique and question 5 is of a philosophical nature. Accordingly in the present paper we attempt to answer questions 1 and 2 in a theoretical section and then we turn to questions 3 and 4 with the use of experimental data. Subsequently an attempt will be made to answer question 5.

Part I: Theoretical Studies

We commence with some general comments on complex mechanisms and then proceed to analyse two special simple mechanisms, a one-site/two-state model analogous to the Monod–Wyman–Changeux allosteric model (Monod *et al.*, 1965), and the homotropic substrate-modifier mechanism, which has been studied by numerous workers (see references cited in Plowman, 1972).

We assume a pseudo-steady state with product concentration zero and absence of significant enzyme association–dissociation, whereupon the steady-state equation is of the form:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \dots + \alpha_n[S]^n}{\beta_0 + \beta_1[S] + \beta_2[S]^2 + \dots + \beta_{n+r}[S]^{n+r}}$$

and mathematical facts concerning such functions are used below in accordance with theoretical studies published elsewhere (Bardsley & Childs, 1975; Bardsley, 1976, 1977a,b,c,d,e; Bardsley & Waight, 1978).

Now the 1:1 function:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S]}{\beta_0 + \beta_1[S]}$$

is appropriate for those mechanisms usually considered in enzyme kinetics that do not require branching pathways or several dependent sites (Cleland, 1970), and we do not discuss this possibility further except to note that, under restrictive conditions, most complex mechanisms may actually decrease in degree down to an equation like this, namely:

$$\frac{v}{[E]_0} = \frac{\alpha_1\alpha_n[S]}{\alpha_n\beta_0 + \alpha_1\beta_n[S]}$$

which is one formula for the theoretical parent hyperbola (Bardsley, 1977c,e; Bardsley & Waight, 1977). So we might begin our enquiry with the 2:2 function:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2}{\beta_0 + \beta_1[S] + \beta_2[S]^2}$$

which can be used to describe a variety of molecular

mechanisms of the modifier or random type (Botts & Morales, 1953; King, 1956; Botts, 1958; Ferdinand, 1966; Pettersson, 1969; Plowman, 1972; Whitehead, 1976). It is well known that this function has four possible distinct shapes in the v -versus- $[S]$ plots and five possible $(1/v)$ -versus- $(1/[S])$ plots, and usually theoretical mechanisms can give all of these types. A good example of a study of such a mechanism was that discussed by Ferdinand (1966), but, as pointed out elsewhere, the 2:2 function is a conic section in the Lineweaver-Burk, Scatchard and Hanes plots and hence there can be no inflexions or multiple turning points (Bardsley & Childs, 1975; Childs & Bardsley, 1976). Since our particular interest is in such features, we have to turn to mechanisms giving rise to the 3:3 function:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \alpha_3[S]^3}{\beta_0 + \beta_1[S] + \beta_2[S]^2 + \beta_3[S]^3}$$

and theoretical study of such functions is now possible after the classification of all 27 double-reciprocal plots (Bardsley, 1977a). So far in the theoretical study of complex kinetics, a number of important attempts have been made to analyse complex equations (Whitehead, 1976; Ricard *et al.*, 1974), but there has never been a systematic attempt to see what curve shapes are possible for particular model mechanisms giving 3:3 rate equations. Such an analysis is presented below in this paper.

A common technique used to simplify rate equations is to assume that all or some part of the mechanisms is at equilibrium (Cha, 1968), and this device is employed most frequently to analyse data in terms of contemporary allosteric binding models. We emphasize that allosteric mechanisms are of enormous complexity and that the equations obtained by multiplying saturation functions by maximal velocities to obtain supposed velocity equations involve far more drastic assumptions than commonly realized. Let us take up now the subject of a steady-state one-site/two-stage mechanism analogous to the Monod-Wyman-Changeux model.

*One-site/two-state version of the Monod-Wyman-Changeux model (Monod *et al.*, 1965)*

The essential feature of this mechanism is that there is an R and a T conformational state, and so the pseudo-steady-state scheme is as in Fig. 1(a) involving substrate addition, catalytic, conformational and product-release steps. The rate equation is given in the Appendix (eqn. 1a) and is a 2:2 function of the type:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2}{\beta_0 + \beta_1[S] + \beta_2[S]^2}$$

Fig. 1. Version of the Monod-Wyman-Changeux pseudo-steady-state model (Monod *et al.*, 1965) for the special case of one substrate and one site

(a) The explicit scheme [eqn. 1(a) of the Appendix].

This is the simplest realistic scheme involving substrate- and product-addition and -release steps, steps for the interconversion of conformational forms R and T and also catalytic steps. The catalytic steps involve interconversion of substrate and product. (b) The implicit scheme [eqn. 1(b) of the Appendix]. Simplification of the previous scheme results when the catalytic steps are left out altogether. The rate equation is still of degree 2:2 but is far simpler. Monod-Wyman-Changeux models with more than one site actually have a higher degree when catalytic steps are included, i.e. the explicit scheme is of a different form to the implicit one. (c) The Dalziel (1968) version [eqn. 1(c) of the Appendix]. This approximate treatment still has four nodes, as has the previous implicit one, but interconversion of R and T forms is only possible for the free enzyme. The rate equation is like the binding equation, extremely simple and of degree 1:1.

At low substrate concentration, the best linear approximation is not simply obtained by neglecting quadratic terms, but is given by the osculating hyperbola at zero as defined elsewhere (Bardsley & Waight, 1978), i.e.:

$$\text{OH}(0) = (-\alpha_1^2/\psi_{11}^{20})[S]/\{(-\alpha_1\beta_0/\psi_{11}^{20}) + [S]\}$$

where $\psi_{11}^{20} = \alpha_2\beta_0 - \alpha_1\beta_1$. Similarly at high substrate concentration the best approximation does not simply involve dropping linear terms to give a Hill equation, but is the osculating hyperbola at infinity with equation:

$$\text{OH}(\infty) = (\alpha_n/\beta_n)[S]/\{(\phi_{n,n-1}/\alpha_n\beta_n) + [S]\}$$

where $n = 2$ and $\phi_{n,n-1} = \alpha_n\beta_{n-1} - \alpha_{n-1}\beta_n$.

The important point now is that in v -versus- $[S]$, double-reciprocal, Scatchard, Hanes or any other graphical space, the actual experimental curve will always give $\text{OH}(0)$ and $\text{OH}(\infty)$ as asymptotes (Bardsley & Waight, 1978). Hence, if the double-reciprocal plot be extrapolated to get K_m and V from the supposed pseudo-linear extremities, then the meaning of the presumed kinetic constants in terms of the rate constants can be found from the value of α_i, β_i given for this mechanism in the Appendix. Here we make the first point. The curve shapes possible for this model depend entirely on the sign of the three functions:

$$\psi_{11}^{20} = \alpha_2\beta_0 - \alpha_1\beta_1$$

$$\phi_{21} = \alpha_2\beta_1 - \alpha_1\beta_2$$

$$R_0^{2:2} = -(\alpha_2\psi_{11}^{20} + \alpha_1^2\beta_2) = -(\alpha_2^2\beta_0 - \alpha_1\phi_{21})$$

and the values of extrapolated slopes and intercepts are expressed in terms of α_i, β_i and $\psi_{11}^{20}, \phi_{21}$. These can be calculated as functions of k_i and are expressions of considerable complexity. They bear no resemblance at all to kinetic constants of classical theory, and so it is impossible to discuss the behaviour of this system in terms of K and V effects as proposed by Monod *et al.* (1965). We might ask then what features differentiate this mechanism from the saturation function originally proposed for two-state equilibrium-binding models? The answer is that there are three distinct differences as follows: (i) the saturation function for this system is 1:1, whereas that for the steady-state model is 2:2; (ii) the saturation function is expressible entirely in terms of the constants L and c , whereas the steady-state model requires 14 independent rate constants; (iii) a 2:2 Monod-Wyman-Changeux saturation function only shows positive co-operativity and the double-reciprocal plot is always concave up, whereas the steady-state model can be either concave up ($R_0^{2:2} < 0$) or concave down

($R_0^{2:2} > 0$); it can also show substrate inhibition, a feature not possible with saturation functions.

It is evident that if this model were assumed it would be impossible to obtain estimates of the rate constant values from steady-state studies alone, and so it is reasonable to ask what value is it to study this type of complex kinetics from a theoretical point of view? If an experimentalist at present had evidence for only one binding site on a monomer and yet the double-reciprocal plot were concave down, then he might be tempted to eliminate the Monod-Wyman-Changeux model from consideration, since it is believed that this model only shows positive co-operativity. The present results show that this conclusion would be unjustified.

There is yet one further important point to be faced. It might be stated that, even though theoretical calculations show that such curve-shape complexities are possible, it may be that there are constraints built into the rate laws by microscopic reversibility, so that, for reasonable values of the rate constants, decrease in degree occurs, i.e. the Sylvester resultant $R_0^{2:2}$ vanishes (Bardsley, 1977c,e), and the mechanism can only give 1:1 behaviour. Alternatively it may be that certain curve shapes are not possible. Now we could in theory attempt to answer this question by analysis of the steady-state rate equation given in the Appendix (eqn. 1a), but the computational labour involved is enormous. Instead we briefly analyse a simpler scheme with fewer degrees of freedom, namely the mechanism of Fig. 1(b). This involves replacing a catalytic and product-release step by a composite rate constant leading to an implicit rather than explicit formulation. We showed elsewhere that, for some mechanisms, there is a decrease in degree when this is done, but this particular mechanism is not characterized by this feature (Waight & Bardsley, 1977). Monod-Wyman-Changeux models with more than one site do, however, decrease in degree. For instance the two-site Monod-Wyman-Changeux model is 6:6 in the explicit formulation, but gives a 4:4 implicit scheme that can actually give curves not possible for degree 3:3 (P. Leff & W. G. Bardsley, unpublished work).

The rate equation for mechanism 1b is given as eqn. 1(b) of the Appendix and is amenable to analysis. It is in fact easy to show that the double-reciprocal plots can be either concave up or down, and that v -versus- $[S]$ curves can be sigmoid or non-sigmoid and may or may not have a maximum. We hence presume that the comprehensive scheme described in Fig. 1(a) with more degrees of freedom can give all of the graph shapes possible for the simplified scheme of mechanism 1b.

To give some idea of the complexity involved in the analysis of such mechanisms, which are already highly simplified, we give the expression that deter-

mines whether there will be no substrate inhibition ($\phi_{21} > 0$) or substrate inhibition ($\phi_{21} < 0$):

$\phi_{21} < 0$, and this argument carries even more force for the full expression for ϕ_{21} calculated in accordance

$$\begin{aligned} \phi_{21} = & k_{+1}k_{+3}\{(k_{-1}k_{+3}k_{-4}^2 + k_{-1}k_{+2}k_{+3}k_{-4} + k_{+1}k_{-3}k_{+4}k_{-4} - k_{+1}k_{-2}k_{-3}k_{+4})k_p \\ & + (k_{-1}k_{+3}k_{+4}k_{-4} + k_{+1}k_{-2}k_{-3}k_{+4} + k_{+1}k_{-3}k_{+4}^2 - k_{-1}k_{+2}k_{+3}k_{-4})k_q + k_{+3}k_{-4}(k_{+2} + k_{-4})k_p^2 \\ & + [k_{+1}k_{+4}(k_{-4} - k_{-2}) + k_{+3}k_{-4}(k_{+4} - k_{+2})]k_p k_q + k_{+1}k_{+4}(k_{-2} + k_{+4})k_q^2\} \end{aligned}$$

Similar but more complicated expressions can be calculated for ψ_{11}^{20} and $R_0^{2:2}$. Actually, it can be shown that $R_0^{2:2}$ has a linear factor $(k_{-1}k_q - k_{-3}k_p)$ corresponding to the decrease in degree under the special constraints of generalized microscopic reversibility as defined by Whitehead (1976) and Ricard (1978). Fig. 2 shows that this is only one of several possible conditions.

The terms in k_p and k_q can be shown to be positive by using $k_{+1}k_{-2}k_{-3}k_{+4} = k_{-1}k_{+2}k_{+3}k_{-4}$ and hence it is easily seen that $k_{-2} > k_{-4}$ and/or $k_{+2} > k_{+4}$ are necessary but not sufficient requirements for substrate inhibition. Also it is evident that the cases $k_p = 0$, $k_q \neq 0$, or $k_p \neq 0$, $k_q = 0$ cannot give substrate inhibition. It is difficult to see how any intuitive explanation for substrate inhibition could be extracted from the necessary and sufficient condition

with eqn. (1a) of the Appendix. In general we expect to abandon all hope of simple intuitive explanations of curve-shape phenomena and instead concentrate on this question: can such a mechanism give all the mathematically possible curve shapes? This question cannot easily be answered by algebraic analysis, and we now present a simple device that we have found useful in the attempt to answer questions of this sort.

Whenever a question about curve shape is posed for such a complex mechanism we are always led to consider the signs of homogenous polynomials in the rate constants k_i , and frequently it is impossible to say much analytically about the possibility of the signs of several such polynomials together, i.e. for any chosen set of k_i values. One device we can resort to is random search by com-

Fig. 2. Possible curve shapes for mechanism 1b of Fig. 1.

The rate equation [eqn. 1(b) of the Appendix] is 2:2 of the type:

$$\frac{v}{[E_0]} = \frac{\alpha_1[S] + \alpha_2[S]^2}{\beta_0 + \beta_1[S] + \beta_2[S]^2}$$

and there are four v -versus- $[S]$ plots and five double-reciprocal plots as follows: $\phi_{21} > 0 \Leftrightarrow$ no maximum in v -versus- $[S]$ plot; $\phi_{21} < 0 \Leftrightarrow$ maximum in v -versus- $[S]$ plot; $\psi_{11}^{20} > 0 \Leftrightarrow v$ -versus- $[S]$ plot is sigmoid; $\psi_{11}^{20} < 0 \Leftrightarrow v$ -versus- $[S]$ plot is non-sigmoid; $R_0^{2:2} = 0 \Leftrightarrow$ the degree is 1:1 by cancellation of common factors between numerator and denominator; $R_0^{2:2} > 0 \Leftrightarrow$ the double-reciprocal plot is concave down; $R_0^{2:2} < 0 \Leftrightarrow$ the double-reciprocal plot is concave up. The plane curves $\phi_{21}(k_p, k_q) = 0$, $\psi_{11}^{20}(k_p, k_q) = 0$ and $R_0^{2:2}(k_p, k_q) = 0$ are plotted for the following fixed values of k_i : $k_{+1} = 0.1$, $k_{-1} = 0.1$, $k_{+2} = 100$, $k_{-2} = 1$, $k_{+3} = 0.1$, $k_{-3} = 10$, $k_{+4} = 5$, $k_{-4} = 5$. These are subject to microscopic reversibility and are chosen to give a demonstration of a systematic technique used for locating possible curve shapes. Alternative k_i values give other possible curve shapes. (a) is a large-scale representation and (b) a detailed expansion of (a) near the origin. In each case the string of signs on the diagrams refers to the sequence of signs of ϕ_{21} , ψ_{11}^{20} , $-R_0^{2:2}$. One branch of $R_0^{2:2} = 0$ is the linear factor corresponding to generalized microscopic reversibility (Whitehead, 1976; Ricard, 1978), but the other branch shows that decrease in degree is also possible under other constraints.

Fig. 3. Curve shapes for the one-site/two-state versions of the Monod-Wyman-Changeux mechanism of Fig. 1(b)

Four of the five possible double-reciprocal plots can be demonstrated by using the technique of Fig. 2, but the combination of $\phi_{21} < 0$, $\psi_{11}^{20} > 0$ has not been found by the method. Typical plots are shown together with the v -versus- $(v/[S])$ representation. $[E_0] = 1$ in each case. (a) $\phi_{21} > 0$, $\psi_{11}^{20} < 0$, $-R_0^{2:2} > 0$: $k_{+1} = 0.1$, $k_{-1} = 0.1$, $k_{+2} = 100$, $k_{-2} = 1$, $k_{+3} = 0.1$, $k_{-3} = 10$, $k_{+4} = 5$, $k_{-4} = 5$, $k_p = 40$, $k_q = 50$; $\alpha_1 = 24835$, $\alpha_2 = 4.5$; $\beta_0 = 293557$, $\beta_1 = 558.05$, $\beta_2 = 0.1$. (b) $\phi_{21} < 0$, $\psi_{11}^{20} < 0$, $-R_0^{2:2} > 0$: $k_{+1} = 0.1$, $k_{-1} = 0.1$, $k_{+2} = 100$, $k_{-2} = 1$, $k_{+3} = 0.1$, $k_{-3} = 10$, $k_{+4} = 5$, $k_{-4} = 5$, $k_p = 200$, $k_q = 500$; $\alpha_1 = 1.04605 \times 10^6$, $\alpha_2 = 35$, $\beta_0 = 1.06657 \times 10^7$, $\beta_1 = 2508.05$, $\beta_2 = 0.1$. (c) $\phi_{21} > 0$, $\psi_{11}^{20} > 0$, $-R_0^{2:2} > 0$: $k_{+1} = 10$, $k_{-1} = 2$, $k_{+2} = 0.1$, $k_{-2} = 0.1$, $k_{+3} = 1$, $k_{-3} = 0.5$, $k_{+4} = 2$, $k_{-4} = 5$, $k_p = 500$, $k_q = 10$; $\alpha_1 = 8524$, $\alpha_2 = 25200$, $\beta_0 = 1560.4$, $\beta_1 = 2788.05$, $\beta_2 = 70$. (d) $\phi_{21} < 0$, $\psi_{11}^{20} < 0$, $-R_0^{2:2} < 0$: $k_{+1} = 10$, $k_{-1} = 2$, $k_{+2} = 0.1$, $k_{-2} = 0.1$, $k_{+3} = 1$, $k_{-3} = 0.5$, $k_{+4} = 2$, $k_{-4} = 5$, $k_p = 100$, $k_q = 100$; $\alpha_1 = 11840$, $\alpha_2 = 7000$, $\beta_0 = 2192.4$, $\beta_1 = 2638.4$, $\beta_2 = 70$.

puter for the signs of these functions, but this can be a tedious and arbitrary method. Another approach is to fix the values of all but two arbitrary constants and vary these two independently. Let us turn to this task for mechanism 1b fixing k_{+1} , k_{-1} , k_{+2} , k_{-2} , k_{+3} , k_{-3} , k_{+4} and k_{-4} subject to $k_{+1}k_{-2}k_{-3}k_{+4} = k_{-1}k_{+2}k_{+3}k_{-4}$ and varying k_p and k_q . In Fig. 2 are plotted the plane curves $\psi_{11}^{21}(k_p, k_q) = 0$, $\phi_{21}(k_p, k_q) = 0$, $R_0^{2:2}(k_p, k_q) = 0$, which divide up the (k_p, k_q) plane into regions where these functions have opposite signs. From such a graph we immediately see that at least four of the five possible double-reciprocal plots can occur. The only one not clearly possible is $\psi_{11}^{20} > 0$, $\phi_{21} < 0$, i.e. a sigmoid v -versus- $[S]$ curve with a maximum, and analysis shows that this curve shape is difficult to obtain with this mechanism.

Typical curves are shown in Fig. 3 for mechanism 1b, and in concluding this section we refer to the rate equation proposed by Dalziel (1968) as a steady-state Monod-Wyman-Changeux equation for mechanism 1c. Evidently the failure to allow the conformational change to occur between all enzyme species occupied by substrate or product in Dalziel's (1968) treatment leads to an equation (eqn. 1c of the Appendix) that is not really representative of the full curve-shape potential of the Monod-Wyman-Changeux model. The order of complexity implied by explicit $\gg$ implicit $\gg$ Dalziel equation $>$ Monod *et al.* (1965) equilibrium treatment becomes even more acute as we proceed from the one-site case to several sites.

Homotropic substrate-modifier mechanism

The previous mechanism, like all mechanisms that have ever been analysed for possible curve shapes, is 2:2, and hence either concave up or concave down in double-reciprocal space. However, over 100 enzymes have been claimed to require rate or binding equations of third, fourth or even higher degrees, and such equations will usually lead to double-reciprocal plots with inflexions or multiple turning points. We now analyse for the first time a mechanism that gives such features.

Suppose it be asked: what is the simplest mechanism by which an enzyme with one active site, one conformational state and one substrate can give substrate inhibitory or activatory effects? The answer must surely be that, in addition to combining at the active site, there must be another binding site available for substrate, and that when this site is occupied the catalytic characteristics of the enzyme are altered, leading to activation, inhibition or, in a limiting case, abortive dead-end complexes. Such a mechanism is the explicit form of the homotropic substrate-modifier mechanism of Fig. 4(a). The rate equation is eqn. (4a) of the Appendix and

(a) Mechanism 4a

(b) Mechanism 4b

Fig. 4. Substrate-modifier mechanism

(a) The explicit scheme. There are six nodes (enzyme species) and catalytic steps. The rate equation is of degree 4:4 [eqn. 4(a) of the Appendix]. (b) The implicit scheme. Catalytic steps are omitted and the resulting scheme has only four nodes and a rate equation of degree 3:3 [eqn. 4(b) of the Appendix]. The central feature of this mechanism is that, in addition to binding at a catalytically active site, the substrate can also bind to a modifier site, with resulting modification in substrate binding, product release and/or catalysis.

of view, eqn. (4a) of the Appendix either contains common factors in numerator and denominator leading to decrease in degree by cancellation or has in-built restrictions, so that for realistic k_i values subject to the two independent conditions $k_{+1}k_{-2}k_{-3}k_{+4} = k_{-1}k_{+2}k_{+3}k_{-4}$ and $k_{-4}k_{+5}k_{-6}k_{+7} = k_{+4}k_{-5}k_{+6}k_{-7}$ the mechanism cannot give more than two turning points in v -versus- $[S]$ space or multiple intersections with the asymptote in double-reciprocal space, features possible with 4:4 but not 3:3 functions.

To disprove this point of view we have calculated the v -versus- $[S]$ curve for this mechanism for sets of k_i values, and a computer-generated curve is shown for a selected set of k_i values in Fig. 5. The example shown has two maxima and a minimum in the v -versus- $[S]$ curve and crosses its asymptote twice in double-reciprocal space. Hence it is conclusively established that the rate equation is genuinely 4:4 and can definitely give rise to highly complex curves. It would be of some interest to know the full range of curve shapes possible for this mechanism, but this formidable task so far eludes analysis. Accordingly we now turn to the implicit representation of Fig. 4(b). Note first that setting $k_{+2} = k_{-2} = k_{+3} = k_{-3} = k_q = 0$ in Fig. 4(b) gives us the usual model for 1:2 substrate inhibition due to the abortive complex (SES/SEP).

The rate equation for mechanism 4b is eqn. (4b) of the Appendix, and here we come to another striking conclusion. In the classical theory of linear kinetics (Cleland, 1970) it was shown that inclusion of catalytic steps only led to an increase in complexity but not to an alteration in the form of the rate equation. Hence central complexes have frequently been ignored in the belief that information about them could never be obtained by kinetic studies alone. We pointed out elsewhere that, in studying kinetic isotope effects (Bardsley & Waight, 1976) or allosteric mechanisms (Waight & Bardsley, 1977), by steady-state kinetics it was necessary to include catalytic steps explicitly, and here now is a concrete example where unambiguous increase in degree and complexity of kinetic curve shapes results from inclusion of such steps.

We now attempt to answer the question as to what curve shapes are possible for mechanism 4b by using the analysis of 3:3 curve shapes published elsewhere (Bardsley, 1977b,d). From this analysis it was concluded that 26 shapes could result, and to decide on possible curve shapes it was necessary to know the sign of 13 epsilon functions ϵ_i . As a first approach to this problem we carried out a computer search for values of ϵ_i for random values of k_i , subject to the one independent constraint $k_{+1}k_{-2}k_{-3}k_{+4} = k_{-1}k_{+2}k_{+3}k_{-4}$, and found numerous curve shapes, including one shape overlooked in the previous theoretical enquiry. Also, several new sign-change

is of great complexity. Furthermore, the surprising finding is that this equation is 4:4, not 1:2 as usually assumed for substrate inhibition, or 2:2 as generally presumed for substrate activation. It would be reasonable to object that there may be unknown constraints applying to this mechanism that are due to detailed balancing and topological reasons and, in fact, the degree as calculated is inflated. There are actually some mechanisms for which this does occur, and the rate equation as calculated contains redundant factors in numerator and denominator. According to this point

Fig. 5. Complex curve shape for mechanism 4a

(a) The double-reciprocal plot intersects its asymptote twice and has multiple turning points (see Fig. 5b). (b) This curve has two maxima and one minimum in v -versus- $[S]$ space, and this is shown in a semi-logarithmic plot for clarity. Note that, since $dv/d\log[S] = [S]dv/d[S]$, then for $[S] > 0$ $dv/d\log[S] = 0 \Leftrightarrow dv/d[S] = 0$. These features prove that mechanism 4a leads to eqn. 4(a), which is genuinely of degree 4:4, i.e. there are no common factors between numerator and denominator since the curve-shape features in (a) and (b) cannot occur for 3:3 functions. For $|E_0| = 1$, the rate constants selected to obey microscopic reversibility were: $k_{+1} = 100$, $k_{-1} = 1$, $k_{+2} = 100$, $k_{-2} = 1$, $k_{+3} = 0.01$, $k_{-3} = 1$, $k_{+4} = 0.1$, $k_{-4} = 10$, $k_{+5} = 100$, $k_{-5} = 1$, $k_{+6} = 1$, $k_{-6} = 1$, $k_{+7} = 0.1$, $k_{-7} = 1$, $k_p = 0.5$, $k_q = 0.5$.

patterns were discovered, and these, along with the 27th shape, are collected in Table 2.

Proceeding more systematically we also fixed all k_i except k_p and k_q and plotted the plane curves $\varepsilon_i(k_p, k_q) = 0$ as discussed previously for the Monod-Wyman-Changeux implicit mechanism, and the results of this enquiry are now discussed.

Fig. 6 shows a typical plot for $R_0^{3:3}(k_p, k_q) = 0$ and $R_1^{3:3}(k_p, k_q) = 0$ in accordance with the analysis of Sylvester resultants published separately (Bardsley, 1977c,e). Along the locus $R_0^{3:3} = 0$ the rate equation is actually 2:2 by cancellation of a linear factor, and hence this condition is not a unique one. Where $R_0^{3:3} = 0$ and $R_1^{3:3} = 0$ intersect, then a quadratic factor cancels and the rate equation is hyperbolic. Textbook discussions of this sort of factorization (Plowman, 1972) point out how decrease in degree occurs when there are certain symmetries in the rate constants, but this analysis shows these conditions to be special ones. In other words, decrease in degree can occur under a multitude of conditions of which the symmetry restrictions are but one isolated example.

Fig. 7 shows an example of $\varepsilon_i(k_p, k_q) = 0$ curves for the ε_i needed to determine eight of the 3:3 curve

shapes in double-reciprocal space. Generation of a number of maps of this kind indicates that at least 18 of the possible 27 shapes may be given by mechanism 4b. A random search for other curve shapes did not reveal any alternative curves, and at present we do not know whether the missing nine curves are possible for k_i values missed in our computer work or whether there are constraints, unknown to us at present, that prevent the necessary sign-change patterns occurring in the ε_i functions.

Before presenting examples of the actual calculated curves, we mention that any classification of double-reciprocal plots constitutes a classification of Scatchard and Hanes plots by use of the method of ordering functions, curve-shape determinants and intersections with the osculating hyperbolae (Bardsley, 1976, 1977a; Bardsley & Waight, 1978). However, whereas it is possible to choose a range in double-reciprocal space that exaggerates the low- $[S]$ data or one in Hanes space that exaggerates the high- $[S]$ points, making the plot appear linear, that is not so easy in Scatchard space. Accordingly we now give examples of 27 calculated shapes in both double-reciprocal and Scatchard axes for

Fig. 6. Sylvester resultants $R_0^{3:3}$ and $R_1^{3:3}$ for mechanism 4b

The condition $R_0^{3:3} = 0$ leads to a rate equation of degree 2:2, whereas the condition $R_0^{3:3} = R_1^{3:3} = 0$ implies that the rate equation is hyperbolic (Bardsley 1977c). A fixed set of k_i values was chosen and $R_0^{3:3}(k_p, k_q) = 0$ and $R_1^{3:3}(k_p, k_q) = 0$ are plotted. The graph illustrates how, for any fixed choice of k_i , there will in general be an infinity of positive pairs of k_p and k_q leading to decrease in degree everywhere along the locus $R_0^{3:3} = 0$. However, only at the intersection of this curve with the locus $R_1^{3:3} = 0$ will the steady-state rate equation really be of degree 1:1. The k_i values chosen were: $k_{+1} = 10^3$, $k_{-1} = 10$, $k_{+2} = 10^2$, $k_{-2} = 10$, $k_{+3} = 10^2$, $k_{-3} = 10$, $k_{+4} = 10$, $k_{-4} = 1$. Neither $R_0^{3:3} = 0$ nor $R_1^{3:3} = 0$ corresponds to the line representing generalized microscopic reversibility (Whitehead, 1976; Ricard, 1978). Algebraic curves of the form $f(x, y) = 0$ may be compound, i.e. they may factorize into simpler curves of lower degree as, for instance:

$$f(x, y) = (ax + by + c) \cdot g(x, y)$$

Fig. 7. Fundamental epsilon functions $\epsilon_i(k_p, k_q)$ for mechanism 4b

The rate equation is of degree 3:3, and hence a choice as to which of the 27 possible curve shapes can result requires a knowledge of the signs of 13 ϵ_i functions (Bardsley, 1977a). The plane curves $\epsilon_i(k_p, k_q) = 0$ are plotted for ten of the ϵ_i functions required to classify the double-reciprocal plot, and the signs of the functions are listed in each segment marked out by the curves $\epsilon_i(k_p, k_q) = 0$. By using the maps constructed in this way for different choices of k_i values we have been able to find 18 of the 27 possible shapes. It is not known whether the missing nine shapes are possible for this mechanism, but they have not been found by computer search for ϵ_i signs for random values of k_i . The k_i values chosen for purposes of illustration in this case were: $k_{+1} = 10^3$, $k_{-1} = 10$, $k_{+2} = 10^2$, $k_{-2} = 10$, $k_{+3} = 10^2$, $k_{-3} = 10$, $k_{+4} = 10$, $k_{-4} = 1$.

Such a curve $f(x, y) = 0$ would be satisfied by the line $ax + by + c = 0$, and hence the graph would contain a line. In this instance, generalized microscopic reversibility does not seem to lead to decrease in degree by cancellation of common factors, as it did with the previous mechanism.

Fig. 8. 3:3 curve shapes in double-reciprocal and v -versus- $(v/[S])$ axes

In each example the classification is that used elsewhere (Bardsley, 1977a). Where the curve can be generated by mechanism 4b a set of k_i values is given in Table 1. Otherwise arbitrary values of α_i , β_i were used to generate the curves; these too are given in Table 1. (a) Class I shapes; (b) class II shapes; (c) class III shapes; (d) class IV shapes. [N.B.: parts (b)–(d) are on the following pages.]

comparison. Fig. 8 shows typical plots [with interchanged axes in the Scatchard plot as is customary for velocity studies (Childs & Bardsley, 1976)], and Table 1 gives the particular α_i , β_i and k_i values.

Part II: Experimental Investigations

In this section we present results obtained when initial-velocity data were obtained for several enzymes over a wide range of substrate concentra-

Fig. 8—continued

tions, due precautions being taken for maintaining constant pH and ionic strength.

Computer fitting of data

A number of workers have suggested ways for detecting higher-degree terms in rate equations

(Pettersson & Pettersson, 1970; Mannervik, 1975; Katsumata & Goldman, 1976) assuming a rational function to be the appropriate function with no assumptions as to the possible molecular mechanism involved. For this purpose we have employed several non-linear regression programs, finally

Fig. 8—continued

adopting a variation of the one used previously for alkaline phosphatase (Waight *et al.*, 1977), which is now described.

The first part of the program was a Monte Carlo searching subroutine in which the function of squared deviations was evaluated a number of times as specified by us. For each call the values of the variable parameters were chosen randomly according to uniform distributions centred on the best previous values, of widths depending on the best parameter errors and constraints. Although this procedure does not converge, it is likely to approach the sum-of-squares minimum. This was followed by a Nelder & Mead (1967) method by using a simplex routine, and the final step was a minimization routine based on a variable-matrix method by Davidon (1968) that converges on the

minimum by making successive approximations to the co-variance matrix.

We supplied experimental data with error estimates, constraints and errors, the number of Monte Carlo calls to the function and the relevant model subroutine. The computer supplied the best-fit parameter values with error estimates, the co-variance matrix correlation coefficients and the fitted data values. A model was accepted if it converged to a solution and the fitted data values were within experimental limits.

The degree of the rational function was specified as 1:1, 2:2, 3:3 or 4:4 and values of α_i, β_i were constrained to be zero or positive. In this way mechanisms with $\alpha_1 = 0$ or $\alpha_n = 0$ are diagnosed by the magnitude of the first or last numerator coefficient with respect to the others. Final best-fit

Table 1. Values of k_i , α_i and β_i for mechanism 4b producing the graphs illustrated in Fig. 8

The shape numbers 1–27 correspond to the individual graphs in Figs. 8(a)–8(d), and the numbering is that used in the classification of 3:3 graph shapes (Bardsley, 1977a). The definition of the epsilon functions ϵ_i in terms of α_i , β_i is also given in that paper, and the sign sequences determine the curve shape. Where there are no values of k_i given, this is because we did not discover any combinations corresponding to the ϵ_i sign patterns required for these particular curve shapes. k_{+1} , k_{+2} , k_{+3} and k_{+4} are second-order rate constants with units $M^{-1} \cdot s^{-1}$, but other rate constants are first-order with units s^{-1} .

Shape	ϵ_1	ϵ_2	ϵ_3	ϵ_4	ϵ_5	ϵ_9	ϵ_{10}	ϵ_{11}	ϵ_{12}	k_{+1}	k_{-1}	k_{+2}	k_{-2}	k_{+3}	k_{-3}	k_{+4}	k_{-4}	k_p	k_q
1	+	+	+	—	—	—	+	+	+	10^3	10	10^2	10	10^5	10^3	10	1	20	20
2(i)	+	+	+	—	—	—	+	+	—	10^3	10	10^2	10	10^5	10^3	10	1	10	25
3(i)	—	—	+	—	—	—	+	+	—	10^5	10^3	10^2	10	10^3	10	10	1	500	500
4	—	—	—	—	—	—	+	+	+	10^3	10	10^2	10	10^5	10^3	10	1	30	10
5	—	—	—	—	—	—	+	+	+	10^5	10^3	10^2	10	10^3	10	10	1	80	35
6	+	+	+	+	+	+	+	+	+	10^5	10	10^2	10	10^3	10	10	1	0.1	100
7(i)	+	+	+	+	—	—	+	+	—	10^3	10	10^2	10	10^5	10^3	10	1	15	40
8(i)	—	—	+	+	—	—	+	+	—					—					
9	+	—	—	+	—	—	+	+	+	10^3	10	10^2	10	10^5	10^3	10	1	100	25
10	+	—	—	+	+	—	+	+	+					—					
11(i)	+	+	+	—	—	—	—	+	+	10^5	10^3	10^2	10	10^3	10	10	1	0.49	10
12(ii)	+	+	+	—	+	—	—	+	+					—					
13(ii)	+	—	+	—	+	—	—	+	+					—					
14(i)	+	—	—	—	—	—	—	+	+	50	10^2	10^2	10	0.05	10^2	0.1	10^2	50	1
15	+	—	—	—	—	—	—	+	+					—					
16	+	+	+	+	+	+	—	+	+	10^5	10^3	10^2	10	10^3	10	10	1	0.9	100
17	+	+	+	+	+	—	—	+	+					—					
18	+	—	+	+	+	—	—	+	+					—					
19	+	—	—	+	+	—	—	+	+					—					
20	+	—	—	+	+	—	—	+	+					—					
21	+	+	+	—	—	—	—	—	—	10^5	10^3	10^2	10	10^3	10	10	1	0.5	1
22(i)	+	+	+	—	—	—	—	—	—	10^5	10^3	10^2	10	10^3	10	10	1	50	100
23(i)	+	+	—	—	—	—	—	—	+	10	1	10	1	1	10^2	10	10^3	10	2
24(i)	+	+	+	—	—	—	+	—	—	10^5	10^3	10^2	10	10^3	10	10	1	70	100
25	+	+	+	—	—	—	+	—	—	10^5	10^3	10^2	10	10^3	10	10	1	10	9.7
26	—	+	+	—	—	—	+	—	—	10^5	10^3	10^2	10	10^3	10	10	1	10^3	10^3
27	—	+	+	—	—	—	+	—	—	10^5	10^3	10^2	10	10^3	10	10	1	10^3	1500

values of the coefficients are given $\pm$ the square roots of the diagonal entries of the co-variance matrix (i.e. estimated standard deviations). Computed curves according to the best-fit parameters for each degree were drawn through the experimental data and subjective decisions as to best fit were given for each individual enzyme–substrate pair on the basis of visual inspection of trends. The units were as follows: β_0 , dimensionless; α_i , $mm^{-1} \cdot s^{-1}$; β_i , mm^{-1} , the reaction velocity being measured as rate of absorbance change at the appropriate wavelength, i.e. in units of $\Delta A \cdot s^{-1}$ at fixed enzyme concentration.

Materials and methods

For each enzyme studied we give details of source and method of assay and then discuss the experimental findings displayed in the appropriate Figure. Special details are given as appropriate. The Figures are all in v -versus- $(v/[S])$ space and the error bars represent 95% confidence limits at the individual substrate concentrations. The dotted lines (.....) and dashed lines (----) are 1:1 or 2:2 fits, but the best fits are continuous lines (—)

Table 2. New sign-change patterns for the 3:3 function

During these investigations we discovered nine patterns of ϵ_i signs that were overlooked in the original publication (Bardsley, 1977a). The sign changes and shapes to which they refer are collected in this Table. In particular, shape 27 was missing altogether from the original classification.

Shape	ϵ_1	ϵ_2	ϵ_3	ϵ_4	ϵ_5	ϵ_9	ϵ_{10}	ϵ_{11}	ϵ_{12}	ϵ_{13}
11(ii)	+	+	+	—	+	+	—	+	+	+
16	+	+	+	+	—	—	—	+	+	+
16	+	+	+	+	+	+	—	+	+	+
22(i)	+	+	+	—	—	+	—	—	—	+
22(i)	+	+	+	—	+	—	—	—	—	+
22(ii)	+	+	+	—	—	+	—	—	+	+
23(ii)	+	+	+	—	+	+	—	—	+	+
24(ii)	—	+	+	—	—	+	+	—	—	+
27	—	+	+	—	—	—	+	—	—	+

as appropriate. The definition of $n:m$ functions follows Bardsley & Childs (1975) and Bardsley (1976). Also, we attach no special significance to the numerical estimates for α_i, β_i . The aim of

this work is simply to obtain best fits to experimental data with the lowest-degree rational functions with positive coefficients. More detail and knowledge of other experimental approaches would be required to reach conclusions as to mechanisms, and this is beyond the scope of the present paper. All assays were conducted with inert salt (NaCl or KCl) to maintain ionic strength where appropriate, and constant temperature was maintained by a circulator. Substrate solutions were preincubated to the same temperature and absorbance changes were measured with a Cary 118C spectrophotometer. Enzymes were obtained from Boehringer Corporation or prepared as described. Substrates and buffers were of the highest available purity.

Acetylcholinesterase (EC 3.1.1.7). The *Electrophorus electricus* enzyme was used with acetylthiocholine and dithiobisnitrobenzoate reading at 405 nm in 0.05 M-sodium/potassium phosphate buffer, pH 7.35, at 23°C. The KCl concentration was 0.1 M and substrate concentration range was 0.002–80 mM. Best-fit parameters were:

$$1:1 \quad 'V' = 0.02 \pm 0.0002; 'K_m' = 0.02 \pm 0.0003$$

$$2:2 \quad \alpha_1 = 10.6 \pm 0.1; \alpha_2 = 0.02 \pm 0.02; \\ \beta_0 = 3.9 \pm 0.27; \beta_1 = 114 \pm 3.7; \\ \beta_2 = 12.7 \pm 1.1$$

$$3:3 \quad \alpha_1 = 0.03 \pm 0.02; \alpha_2 = 35.9 \pm 2.0; \\ \alpha_3 = 0.00017 \pm 0.04; \beta_0 = 0.1 \pm 0.03; \\ \beta_1 = 36.4 \pm 2.2; \beta_2 = 434 \pm 28.8; \\ \beta_3 = 38.2 \pm 1.8$$

From these values and the fitted curves shown in Fig. 9, it is evident that considerable improvement results on moving up in degree from 2:2 to 3:3. However, α_2 in the 2:2 fit and α_3 in the 3:3 fit are not significant, and we conclude that acetylcholinesterase is an enzyme showing dead-end substrate inhibition and requiring a rate equation of minimum degree 2:3. The ϵ sign-change pattern is +---+---++++, indicating shape 9 (Bardsley, 1977a).

Acid phosphatase (EC 3.1.3.2). This was prepared from human semen (Kavanagh & Bardsley, 1979) and was investigated with *p*-nitrophenyl phosphate (0.08–75 mM) as substrate and an enzyme concentration of 0.1 μ g/ml. Over a large variety of experimental circumstances (pH, ionic strength etc.), the minimum degree was found to be 2:2, 3:3 or 4:4, but the data illustrated in Fig. 10 refer to pH 7, 0.1 M-Tris/HCl, 24.5°C and the presence of phosphate (1.0 mM). NaCl concentration was 0.2 M in the absence of substrate and ionic-strength corrections were made assuming a pK_a of 5.7 (Van Etten & McTigue, 1977), and initial rates were measured at 405 nm. Best-fit parameters were:

Fig. 9. *Acetylcholinesterase* (computer-fitted kinetic data)

This and subsequent Figures give the steady-state results obtained under the conditions described in the text. At least five determinations were obtained at each substrate concentration, and sometimes as many as ten were obtained. Standard error was calculated at each substrate concentration, 95% confidence limits were obtained by using *t* tables and the error bars are plotted along with the mean. The curves are calculated by using the non-linear regression program described in the text. In all cases the units of velocity (*v*) are as described in the text and substrate concentration (*[S]*) is in mM. The best-fit curves were: ·····, 1:1; ----, 2:2; —, 3:3.

$$1:1 \quad 'V' = 72.5 \pm 2.7; 'K_m' = 0.8 \pm 0.03$$

$$2:2 \quad \alpha_1 = 238 \pm 75; \alpha_2 = 3621 \pm 1148; \\ \beta_0 = 2.8 \pm 0.8; \beta_1 = 31 \pm 9; \beta_2 = 63 \pm 21$$

$$3:3 \quad \alpha_1 = 0.0008 \pm 3; \alpha_2 = 8765 \pm 1316; \\ \alpha_3 = 1.7 \pm 3.8; \beta_0 = 0.2 \pm 0.03; \beta_1 = 75 \pm 14; \\ \beta_2 = 135 \pm 146; \beta_3 = 0.9 \pm 3$$

$$4:4 \quad \alpha_1 = 47 \pm 7.5; \alpha_2 = 7752 \pm 940; \\ \alpha_3 = 9029 \pm 1153; \alpha_4 = 2994 \pm 277; \\ \beta_0 = 0.9 \pm 0.1; \beta_1 = 51 \pm 6; \beta_2 = 304 \pm 33.5; \\ \beta_3 = 35 \pm 46; \beta_4 = 78 \pm 16$$

From visual inspection of Fig. 10, the 3:3 and 4:4 fits are better than the 2:2; however, the α_1 and α_3 of the 3:3 fit are not significant. Over a wide range of conditions not reported in the present paper we have fitted curves with the lowest-degree and highest-degree α_i term meaningful, and this strengthens our feeling that the minimum degree is 4:4.

Adenosine deaminase (EC 3.5.4.4). The calf intestine enzyme was used in 0.1 M-Tris/HCl buffer, pH 8.15, at 24.5°C with adenosine (0.002–0.2 mM) as substrate and an enzyme concentration of 200 ng/ml, and reading at 265 nm. No ionic-strength

Fig. 10. *Acid phosphatase* (computer-fitted kinetic data)
Details were as for Fig. 9. The best-fit curves were:
....., 1:1; ----, 2:2; - · - ·, 3:3; —, 4:4.

Fig. 11. *Adenosine deaminase* (computer-fitted kinetic data)
Details were as for Fig. 9. The best-fit curves were:
....., 1:1; ----, 2:2; —, 3:3.

correction was required because adenosine was only used up to 0.2 mM. Best-fit parameters were:

- 1:1 $'V' = 0.05 \pm 0.0006$; $'K_m' = 0.03 \pm 0.0004$
 2:2 $\alpha_1 = 30.7 \pm 0.1$; $\alpha_2 = 120 \pm 45.5$;
 $\beta_0 = 24 \pm 0.5$; $\beta_1 = 313 \pm 41$;
 $\beta_2 = 5394 \pm 1246$
 3:3 $\alpha_1 = 7 \pm 0.08$; $\alpha_2 = 6 \pm 1.5$; $\alpha_3 = 0.13 \pm 12$;
 $\beta_0 = 0.5 \pm 0.08$; $\beta_1 = 100 \pm 7$; $\beta_2 = 0.6 \pm 51$;
 $\beta_3 = 2391 \pm 368$

The 3:3 fit illustrated in Fig. 11 is rather better than the 2:2 one and has sign-change pattern +-----+ + +, requiring shape 14. It is not possible to proceed to higher substrate concentrations since absorbance then exceeds 2.0, but it does seem that α_3 is not significant and this is therefore an example of 2:3 dead-end substrate inhibition.

Arylsulphatase (EC 3.1.6.1). The *Helix pomatia*

enzyme was employed with *p*-nitrophenyl sulphate in 0.1 M-sodium acetate buffer, pH 6.6, containing 0.1 M-NaCl. The substrate was freed from *p*-nitrophenol impurity by dissolution in buffer and extraction with ether before use. The material treated in this way was homogeneous on high-pressure liquid chromatography. Enzyme concentration was 500 ng/ml, substrate range was 0.004–100 mM and initial rates were determined at 405 nm. The results are illustrated in Fig. 12, and best-fit parameters were:

- 1:1 $'V' = 0.02 \pm 0.0001$; $'K_m' = 0.1 \pm 0.002$
 2:2 $\alpha_1 = 2.5 \pm 0.1$; $\alpha_2 = 0.007 \pm 0.002$;
 $\beta_0 = 17 \pm 0.8$; $\beta_1 = 100 \pm 6$; $\beta_2 = 2 \pm 0.2$

As no further improvement in fit resulted by using degree 3:3, we conclude that arylsulphatase is an example of an enzyme with a dead-end substrate-inhibition complex requiring a minimum degree of 1:2.

Benzylamine oxidase (EC 1.4.3.6). The enzyme was prepared from pig plasma by Dr. Kapil Yadav

Fig. 12. *Arylsulphatase* (computer-fitted kinetic data). Details were as for Fig. 9. The best-fit curves were: ·····, 1:1; —, 2:2.

Fig. 13. *Benzylamine oxidase* (computer-fitted kinetic data). Details were as for Fig. 9. The best-fit curves were: ·····, 1:1; —, 2:2.

and Dr. Peter Knowles (Astbury Department of Biophysics, University of Leeds) by using the method of Taylor *et al.* (1972). The best-fit parameters were:

$$\begin{aligned} 1:1 \quad & 'V' = 0.02 \pm 0.0001; 'K_m' = 0.07 \pm 0.002 \\ 2:2 \quad & \alpha_1 = 2.2 \pm 1.9; \alpha_2 = 42 \pm 70; \beta_0 = 6.7 \pm 5; \\ & \beta_1 = 466 \pm 632; \beta_2 = 2286 \pm 3235 \end{aligned}$$

There was no substantial improvement with 3:3 as opposed to 2:2, which gives an adequate fit and must be regarded as the minimum acceptable degree of rate equation for this enzyme. Other evidence suggests that the degree is actually 3:3 for this enzyme.

Chymotrypsin (EC 3.4.4.5). The bovine pancreatic α -chymotrypsin was used in 0.05 M-sodium/potassium phosphate buffer, pH 7.0, at 24°C with *N*-acetyltyrosine ethyl ester (0.03–1.5 mM) as substrate. Enzyme concentration was 20 μ g/ml and initial rates were measured at 237 nm. The data are plotted in Fig. 14 and best-fit parameters were:

$$\begin{aligned} 1:1 \quad & 'V' = 0.08 \pm 0.003; 'K_m' = 0.8 \pm 0.04 \\ 2:2 \quad & \alpha_1 = 0.10 \pm 0.008; \alpha_2 = 0.6 \pm 1.3; \\ & \beta_0 = 1 \pm 0.0; \beta_1 = 5.7 \pm 11.3; \\ & \beta_2 = 8.8 \pm 16.2 \end{aligned}$$

This enzyme seems, under the conditions used, to be 1:1 or 2:2. Comparison of mean sums of

weighed residuals squared shows little difference, 0.8 and 0.7 for 1:1 and 2:2 respectively. Points obtained above 1.5 mM show pronounced substrate inhibition, but, as the absorbance is high, this could well be an artifact and such points were not fitted.

Fumarase (EC 5.2.1.2). The pig heart fumarase was used with malate (0.01–100 mM) as substrate in 0.1 M-sodium/potassium phosphate buffer, pH 7.1, at 23°C, initial rates being measured at 250 nm with an enzyme concentration of 67 ng/ml. Ionic strength was maintained with a starting concentration of 0.4 M-KCl. The data are plotted in Fig. 15 and best-fit parameters were:

$$\begin{aligned} 1:1 \quad & 'V' = 0.01 \pm 0.0002; 'K_m' = 0.8 \pm 0.02 \\ 2:2 \quad & \alpha_1 = 0.2 \pm 0.001; \alpha_2 = 0.01 \pm 0.02; \\ & \beta_0 = 17 \pm 0.1; \beta_1 = 26 \pm 0.2; \beta_2 = 0.3 \pm 0.01 \\ 3:3 \quad & \alpha_1 = 0.4 \pm 0.02; \alpha_2 = 2 \pm 0.3; \\ & \alpha_3 = 0.2 \pm 0.008; \beta_0 = 30.5 \pm 1.1; \\ & \beta_1 = 158 \pm 24; \beta_2 = 284 \pm 33; \\ & \beta_3 = 7.2 \pm 0.7 \end{aligned}$$

Fig. 15 shows a slightly better fit for 3:3 than for 2:2, although the 2:2 is acceptable. Fumarase presented us with a dilemma. In classical work, fumarase was treated as a Michaelis–Menten enzyme, but it was recognized that the rate equation did actually require second-degree terms (Alberty & Bock, 1953). Previously we found complex

Fig. 14. *Chymotrypsin* (computer-fitted kinetic data) Details were as for Fig. 9. The best-fit curves were: ·····, 1:1; ———, 2:2.

kinetics (Crabbe & Bardsley, 1976) requiring fourth-degree terms, but this was contested (C. Frieden, personal communication). Accordingly we determined afresh the best-fit curves for fumarase over a wide variety of conditions of pH, temperature, ionic strength and alternative buffer systems. In all cases we obtain results of which Fig. 15 is typical, i.e. considerable improvement in proceeding from 1:1 to 2:2 but little substantial improvement on incorporating third-degree terms. We re-investigated the conditions described by Crabbe & Bardsley (1976), but could not reproduce their findings. Accordingly we conclude that fumarase is 2:2 or possibly 3:3, and dismiss the earlier claims by Crabbe & Bardsley (1976), which evidently resulted from using an experimental procedure with some unexplained systematic errors.

Galactose dehydrogenase (EC 1.1.1.43). The

Fig. 15. *Fumarase* (computer-fitted kinetic data) Details were as for Fig. 9. The best-fit curves were: ·····, 1:1; ———, 2:2; ———, 3:3.

enzyme from *Pseudomonas fluorescens* was used at 25°C in 0.1 M-sodium/potassium phosphate buffer, pH 7.0, reading at 340 nm, and purification of NAD⁺ samples as described by Dalziel (1962). A thorough study of error was carried out with this enzyme. First it was found that velocity was linear against total enzyme concentration, and then a single assay was repeated 30 times with NAD⁺ (0.1 mM) and galactose (10 mM) over a period of 4 h. This gave a mean velocity of 0.00564 $\Delta A_{340}/\text{min}$ with a standard error of 3.67×10^{-5} $\Delta A_{340}/\text{min}$, corresponding to an error of $< \pm 1\%$, the distribution being approximately Gaussian. Then kinetic profiles were determined ten times with galactose concentration fixed (5 mM), and variance was found to be irregular. The data are displayed in Fig. 16, and the best-fit parameters are listed below.

(a) NAD⁺ concentration fixed (0.1 mM)

$$\begin{aligned} 1:1 \quad 'V' &= 3.4 \times 10^{-3} \pm 3.3 \times 10^{-5} \\ 'K_m' &= 4.6 \times 10^{-2} \pm 1.6 \times 10^{-3} \end{aligned}$$

Fig. 16. Galactose dehydrogenase (computer-fitted kinetic data)

Details were as for Fig. 9. The best-fit curves were as follows. (a) Galactose concentration fixed (5 mM): ·····, 1:1; ----, 2:2; ———, 3:3; ———, 4:4. (b) NAD⁺ concentration fixed: 0.1 mM: ·····, 1:1; ----, 2:2; ———, 3:3.

$$\begin{aligned}
 2:2 \quad & \alpha_1 = 2.5 \times 10^{-1} \pm 2.7 \times 10^{-2} \\
 & \alpha_2 = 3.4 \times 10^{-2} \pm 3.0 \times 10^{-3} \\
 & \beta_0 = 5.0 \pm 5.1 \times 10^{-1} \\
 & \beta_1 = 38.2 \pm 5.4 \\
 & \beta_2 = 12.1 \pm 8.8 \times 10^{-1}
 \end{aligned}$$

$$\begin{aligned}
 3:3 \quad & \alpha_1 = 3.9 \times 10^{-1} \pm 7.7 \times 10^{-3} \\
 & \alpha_2 = 3.6 \times 10^{-1} \pm 1.1 \times 10^{-1} \\
 & \alpha_3 = 6.3 \times 10^{-2} \pm 1.3 \times 10^{-3} \\
 & \beta_0 = 7.6 \pm 3.3 \times 10^{-1} \\
 & \beta_1 = 71 \pm 4.2 \\
 & \beta_2 = 54 \pm 2.4 \\
 & \beta_3 = 22 \pm 2.4 \times 10^{-1}
 \end{aligned}$$

$$\begin{aligned}
 4:4 \quad & \alpha_1 = 2.0 \times 10^{-1} \pm 4.0 \times 10^{-2} \\
 & \alpha_2 = 5.7 \times 10^{-1} \pm 1.3 \times 10^{-1} \\
 & \alpha_3 = 1.6 \pm 4.8 \times 10^{-1} \\
 & \alpha_4 = 1.1 \times 10^{-1} \pm 4.2 \times 10^{-3} \\
 & \beta_0 = 3.5 \pm 8.7 \times 10^{-1} \\
 & \beta_1 = 57.9 \pm 4.9 \\
 & \beta_2 = 33.8 \pm 1.5 \\
 & \beta_3 = 337 \pm 126 \\
 & \beta_4 = 40 \pm 8.4 \times 10^{-1}
 \end{aligned}$$

(b) Galactose concentrations fixed (5 mM)

$$\begin{aligned}
 1:1 \quad & 'V' = 6.7 \times 10^{-3} \pm 1.4 \times 10^{-4} \\
 & 'K_m' = 5.7 \times 10^{-2} \pm 2.9 \times 10^{-3}
 \end{aligned}$$

$$\begin{aligned}
 2:2 \quad & \alpha_1 = 1.9 \times 10^{-1} \pm 3.1 \times 10^{-2} \\
 & \alpha_2 = 1.7 \times 10^{-1} \pm 1.4 \times 10^{-1} \\
 & \beta_0 = 1.7 \pm 2.6 \times 10^{-1} \\
 & \beta_1 = 27.5 \pm 4.4 \\
 & \beta_2 = 27 \pm 22
 \end{aligned}$$

$$\begin{aligned}
 3:3 \quad & \alpha_1 = 1.1 \times 10^{-4} \pm 4.8 \times 10^{-5} \\
 & \alpha_2 = 6.4 \times 10^{-5} \pm 3.0 \times 10^{-4} \\
 & \alpha_3 = 5.9 \times 10^{-2} \pm 1.7 \times 10^{-3} \\
 & \beta_0 = 7.0 \times 10^{-4} \pm 3.5 \times 10^{-4} \\
 & \beta_1 = 4.8 \times 10^{-2} \pm 1.3 \times 10^{-2} \\
 & \beta_2 = 3.2 \times 10^{-5} \pm 1.4 \times 10^{-1} \\
 & \beta_3 = 9.2 \pm 1.8 \times 10^{-1}
 \end{aligned}$$

In both instances there is a clear increase in goodness of fit for 2:2 as opposed to 1:1. With NAD⁺ concentration varied, the 3:3 fit is satisfactory, but with galactose concentration varied a 3:3 fit is only a slight improvement over 2:2, and a further slight improvement occurs for a 4:4 rational function. Hence the minimum degree is 3:3 or possibly 4:4 with galactose concentration varied. The 3:3 sign-change patterns were: (a) (galactose concentration fixed at 5 mM) +-----+++, i.e. shape 14(i); (b) (NAD⁺ concentration fixed at 0.1 mM) +-+-----++, i.e. shape 23(ii).

β-Galactosidase (EC 3.2.1.23). The *Escherichia coli* enzyme was used, with *p*-nitrophenyl *β*-D-

Fig. 17. β -Galactosidase (computer-fitted kinetic data). Details were as for Fig. 9. The best-fit curves were: 1:1; ———, 2:2.

galactopyranoside (0.05–75 mM) as substrate with 0.1 M-imidazole/HCl buffer, pH 7.1, containing 0.145 M-NaCl and 1 mM-MgSO₄ at 23°C. Initial rates were measured at 405 nm with an enzyme concentration of 100 ng/ml. The experimental data are plotted in Fig. 17, and best-fit parameters were as follows:

- 1:1 $'V' = 0.09 \pm 0.0002$; $'K_m' = 0.07 \pm 0.0004$
 2:2 $\alpha_1 = 13 \pm 1$; $\alpha_2 = 0.01 \pm 0.02$;
 $\beta_0 = 13.5 \pm 2.5$; $\beta_1 = 88 \pm 16$; $\beta_2 = 2.2 \pm 2.2$
 3:3 $\alpha_1 = 3.2 \pm 0.2$; $\alpha_2 = 1.8 \pm 0.3$;
 $\alpha_3 = 0.001 \pm 0.002$; $\beta_0 = 3.2 \pm 0.3$;
 $\beta_1 = 23.6 \pm 2.3$; $\beta_2 = 11.8 \pm 2.6$;
 $\beta_3 = 0.3 \pm 0.01$

The 2:2 fit has a negligible α_2 value and the 3:3 fit a negligible α_3 value [sign-change pattern +-----+++, shape 14(i)], and there is only a slight improvement in fit for 3:3 as opposed to 2:2. We conclude that the rate equation required is at least 1:2 and possibly 2:3.

Lactate dehydrogenase (EC 1.1.1.27). The pig heart (H₄) and pig muscle (M₄) isoenzymes were assayed in 0.1 M-potassium phosphate buffer, pH 7.0, at 24°C, ionic strength being maintained with KCl (0.1 M for pyruvate, 0.2 M for lactate), and reading at 340 nm. Further details were as follows.

(a) Heart isoenzyme. With NADH concentration fixed (0.1 mM) the pyruvate concentration range was 0.005–50 mM, enzyme concentration being 200 ng/ml. With NAD⁺ concentration fixed (1 mM) the lactate concentration range was 0.1–100 mM and the enzyme concentration was 500 ng/ml. Best-fit values were as follows.

As in Fig. 18(a), with lactate concentration varied:

- 1:1 $'V' = 0.03 \pm 0.0006$; $'K_m' = 4 \pm 0.09$
 2:2 $\alpha_1 = 5 \pm 0.08$; $\alpha_2 = 0.0002 \pm 0.001$;
 $\beta_0 = 761 \pm 21$; $\beta_1 = 110 \pm 7$; $\beta_2 = 1 \pm 0.1$
 3:3 $\alpha_1 = 3.4 \pm 0.76$; $\alpha_2 = 50 \pm 21$;
 $\alpha_3 = 0.0002 \pm 0.2$; $\beta_0 = 318 \pm 63$;
 $\beta_1 = 7740 \pm 2810$; $\beta_2 = 955 \pm 420$;
 $\beta_3 = 9.7 \pm 3.7$

This is clearly dead-end substrate inhibition and there is a slight improvement with 3:3 as opposed to 2:2. The 3:3 sign-change pattern is +-----+++ (shape 14).

(b) Heart isoenzyme. As in Fig. 18(b), with pyruvate concentration varied:

- 1:1 $'V' = 1.9 \pm 0.05$; $'K_m' = 6051 \pm 121$
 2:2 $\alpha_1 = 9.4 \pm 0.09$; $\alpha_2 = 0.0002 \pm 0.0006$;
 $\beta_0 = 2.6 \pm 0.07$; $\beta_1 = 60 \pm 1$; $\beta_2 = 13.0 \pm 0.3$

The 3:3 fit is not a significant improvement and to within experimental error the rate equation is 1:2.

(c) Muscle isoenzyme. With NADH concentration fixed (0.1 mM) the pyruvate concentration range was 0.0005–50 mM and the enzyme concentration was 250 ng/ml. Best-fit parameters are now given.

As in Fig. 18(c), with pyruvate concentration varied:

- 1:1 $'V' = 0.07 \pm 0.001$; $'K_m' = 0.06 \pm 0.0001$
 2:2 $\alpha_1 = 14.5 \pm 0.15$; $\alpha_2 = 0.0002 \pm 0.06$;
 $\beta_0 = 16 \pm 0.4$; $\beta_1 = 127 \pm 2.7$; $\beta_2 = 5.7 \pm 0.3$
 3:3 $\alpha_1 = 35.5 \pm 10$; $\alpha_2 = 417 \pm 224$;
 $\alpha_3 = 0.2 \pm 0.17$; $\beta_0 = 36 \pm 10$;
 $\beta_1 = 845 \pm 366$; $\beta_2 = 3349 \pm 1763$;
 $\beta_3 = 165 \pm 86$

The 1:2 fit is acceptable and little improvement is given by the 2:3 curve.

Peroxidase (EC 1.11.1.7). The horseradish enzyme was used in 0.1 M-sodium/potassium phosphate buffer, (pH 6.0, at 23°C with an enzyme concentration of 50 ng/ml, ionic strength being maintained with KCl (0.1 M). 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) was used, reading at 414 nm as described by Childs & Bardsley (1975). With H₂O₂ concentration fixed (5.6 mM) the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) concentration range was 0.04–25 mM; with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) concentration fixed (10 mM) the H₂O₂ concentration range was 0.03–185 mM. The best-fit parameters are now recorded.

Fig. 18. *Lactate dehydrogenase* (computer-fitted kinetic data)

Details were as for Fig. 9. The best-fit curves were as follows. (a) NADH concentration (0.1 mM) (H_4 isoenzyme): 1:1; ----, 2:2; —, 3:3. (b) NAD⁺ concentration fixed (1 mM) (H_4 isoenzyme); 1:1; ----, 2:2; —, 3:3. (c) NADH concentration fixed (0.1 mM) (M_4 isoenzyme) 1:1; ----, 2:2; —, 3:3.

Fig. 19. Peroxidase (computer-fitted kinetic data)

Details were as for Fig. 9. The best-fit curves were as follows. (a) H_2O_2 concentration fixed (5.6 mM): 1:1; ----, 2:2; —, 3:3. (b) 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) concentration fixed (10 mM): 1:1; ----, 2:2; —, 3:3.

(a) 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) concentration varied:

- 1:1 $'V' = 0.07 \pm 0.0003$; $'K_m' = 1 \pm 0.005$
 2:2 $\alpha_1 = 3.3 \pm 0.006$; $\alpha_2 = 0.8 \pm 0.003$;
 $\beta_0 = 47 \pm 0.04$; $\beta_1 = 94 \pm 0.3$;
 $\beta_2 = 8.3 \pm 0.004$
 3:3 $\alpha_1 = 0.004 \pm 0.006$; $\alpha_2 = 1.6 \pm 0.6$;
 $\alpha_3 = 3 \pm 0.15$; $\beta_0 = 1 \pm 0.3$; $\beta_1 = 2.5 \pm 3.8$;
 $\beta_2 = 135 \pm 23$; $\beta_3 = 35 \pm 2.5$

The results displayed in Fig. 19(a) show a distinct improvement in going from 2:2 to 3:3, and the 4:4 fit was not substantially better. Hence, on these grounds, the minimum degree is 3:3 and the ϵ sign change pattern is +++++-+++, requiring shape 7(ii).

(b) H_2O_2 concentration varied

- 1:1 $'V' = 0.0002 \pm 0.00002$;
 $'K_m' = 0.07 \pm 0.0001$
 2:2 $\alpha_1 = 1386 \pm 5$; $\alpha_2 = 0.0002 \pm 0.003$;
 $\beta_0 = 289 \pm 4.7$; $\beta_1 = 9975 \pm 11.7$;
 $\beta_2 = 141 \pm 1$
 3:3 $\alpha_1 = 2.8 \pm 0.9$; $\alpha_2 = 5.9 \pm 0.8$;
 $\alpha_3 = 0.0002 \pm 0.01$; $\beta_0 = 0.28 \pm 0.13$;
 $\beta_1 = 26.5 \pm 7.3$; $\beta_2 = 37.8 \pm 3.6$;
 $\beta_3 = 0.6 \pm 0.06$

The data are shown in Fig. 19(a), and, as no further improvement occurred with degree 4:4 and α_3 was not significant, we conclude that the minimum degree is 2:3, requiring shape 14(i) with sign pattern +-----+++. We have claimed else-

where (Childs & Bardsley, 1975) that the degree is probably 4:4. This conclusion was based on a different approach involving the behaviour of asymptotes over a wide range of substrate concentrations and is not invalidated by the present findings. The computer fitting only indicates the minimum acceptable degree under one fixed set of experimental conditions.

Xanthine oxidase (EC 1.2.3.2). The cow's-milk enzyme was assayed at 24°C in 0.1 M-sodium/potassium phosphatase buffer, pH 7.6, containing 0.1 mM-EDTA. The range of xanthine concentrations used was 0.0008–1 mM in air and the enzyme concentration was 2 $\mu\text{g}/\text{ml}$, initial rates being measured at 293 nm. Best-fit parameters for the data displayed in Fig. 20 were:

- 1:1 $'V' = 0.009 \pm 0.00005$;
 $'K_m' = 0.0004 \pm 0.000009$
 2:2 $\alpha_1 = 0.2 \pm 0.001$; $\alpha_2 = 0.16 \pm 0.019$;
 $\beta_0 = 0.2 \pm 0.0005$; $\beta_1 = 12 \pm 0.25$;
 $\beta_2 = 52 \pm 3.4$

There was no substantial improvement with a 3:3 function, and hence we conclude that the rate equation for the enzyme is 2:2.

Discussion

In the theoretical work of Part I of the present paper it was shown that relatively simple mechanisms can give highly complex rate equations. Hence there is every reason to expect that pseudo-steady-state data will betray complexity by leading

Fig. 20. *Xanthine oxidase (computer-fitted kinetic data)*
Details were as for Fig. 9. The best-fit curves were:
....., 1:1; —, 2:2.

to curvature, turning points or inflexions in double-reciprocal, Scatchard or Hanes plots. When enzyme action involves more than one site for the binding of substrate, then inclusion of catalytic steps leads to an increase in degree of the rate equation and the possibility of even more complex curves. Hence any experimental evidence for substrate inhibition or activation or curvature in double-reciprocal space may be consistent with third-degree or fourth-degree terms in the pseudo-steady-state rate equation. Velocity data over a wide range of substrate concentrations are needed to detect this, and, in general, even highly complex curves are consistent with relatively simple kinetic schemes.

The experimental results given in Part II of the present paper are typical of those we have also obtained over recent years with other enzymes, although the conclusions have not always been so rigorously established by computer fitting. They lead us to be suspicious of claims for linear double-reciprocal plots and to be equally sceptical about supposed 'kinky' plots with exaggerated irregularities. It is, of course, possible for enzyme mechanisms to lead to the Michaelis-Menten equation, and also there are relatively simple mechanisms that can give equations of great complexity and high degree. However, most enzymes have several substrates, allowing random pathways, or several sites, permitting site heterogeneity or perhaps sites for modifier or even non-specific substrate binding leading to co-operative effects. Hence the idea of an active site insulated from all effects elsewhere on the enzyme molecule seems to us to be an ideal simplified case, unlikely to be

encountered in practice. On these grounds we would not expect the Michaelis-Menten equation to be followed exactly. Equally, when graphs of high-degree rational functions are computed, they can give rise to multiple turning points and inflexions. Indeed, an $n:n$ rational function with positive coefficients can give up to a maximum of $n-1$ turning points in v -versus- $[S]$ or $(1/v)$ -versus- $(1/[S])$ space (Bardsley *et al.*, 1980), but these will tend to be so spaced geometrically that the possibility of detecting the features experimentally appears to be remote. Hence highly complex kinetic data, i.e. extremely wavy plots, seem to us to represent experimental error or enzyme aggregation rather than high-degree rational functions.

In general, it seems from our experience that statistical processing of steady-state data will require rate equations of degree 2:2, 3:3 or 4:4, and occasionally the last numerator term will be negligible, indicating dead-end substrate inhibition.

Let us see at this point what answers can be given to the questions posed at the beginning of the present paper in the light of the theoretical studies and experimental findings just discussed.

Answer to question 1. Even simple models can give rise to rate equations of high degree, and there seems no reason why such rate equations cannot give rise to all curve shapes open to rational functions of corresponding degree. For instance, the substrate-modifier mechanism can give at least 18 out of a possible 27 double-reciprocal plots. Hence we would expect double-reciprocal, Scatchard or Hanes plots to have inflexions (at the same $[S]$ values).

Answer to question 2. Some mechanisms involving only one substrate binding site per enzyme or perhaps only consisting of linear successions of steps give rate equations that are unchanged in form by the inclusion of catalytic steps. However, mechanisms involving several sites will, in general, increase in degree considerably as catalytic steps are included. For instance, the substrate-modifier mechanism increases linearly in degree with the number of catalytic steps. This increase in degree is not, in general, spurious. As an example, we have pointed out that the substrate-modifier mechanism increases from 3:3 to 4:4 on including catalytic steps, and this is a genuine increase in degree. The Sylvester resultants do not vanish, and highly complex curves can result.

Answer to question 3. Since our literature review was concluded (Hill *et al.*, 1977), the publication of examples of non-Michaelian kinetics has continued unabated and there are at present about 1000 examples. Undoubtedly some of these are artifacts, and increased attention to detail would reveal this, but equally many of these will stand the test of time. So, although the question cannot be answered

exactly, it seems unlikely that many of these examples will eventually turn out to be Michaelian. Hence we must await experimental examples of enzymes giving truly linear Scatchard plots over extended ranges of substrate concentration where computer fitting gives no evidence for non-linear terms in the rate equation before the relative numbers can be assessed.

Answer to question 4. As discussed above, we consider that most kinetic data can be adequately fitted by 2:2, 3:3 and 4:4 rational functions. However, this is quite consistent with rate equations of much higher degree than this. Non-linear regression is not a completely objective procedure, and the fit of data to equations involving more than three or four independent parameters is notoriously dependent on the program used, weighting assumed and so on. Hence we attach no value to the magnitude of the α_i, β_i constants given for 3:3 and 4:4 rational functions. They would, in any case, be highly complicated functions of the rate constants, and could not therefore be used to obtain rate-constant values. This will be clear from inspecting the expressions given in the Appendix. The fact that non-linear regression indicates minimum degree 3:3 with no improvement on proceeding to 4:4 merely puts a lower limit on the degree of the actual rate equation, which could be considerably higher.

Answer to question 5. Although a definitive answer cannot be given to question 5, we conclude by emphasizing one or two fairly obvious points. If any enzyme can be shown to follow Michaelis-Menten kinetics with the concentrations of all its substrates and effectors varied over a wide range of temperature, pH values and product concentrations, then there exist routine methods described in numerous text-books for obtaining the order of substrate addition, product release, magnitude of K_1 , K_m and V values, pK values of groups at the active site and so on. Hence there is a compelling temptation to regard experimental data as linear in double-reciprocal space by 'avoiding curvature near the $1/v$ axis' or 'remaining in the region where Michaelis-Menten kinetics is observed'. On the other hand, if curvature is observed, requiring non-linear terms in the rate equation, there is no corresponding routine procedure to apply.

To enlarge on this issue, let us consider the pseudo-linear sections of data such as that of Figs. 12, 17 and 18(b). It might be argued that application of linear regression to the low-substrate-concentration data would give an excellent fit and that the K_m and V values obtained would describe the properties of the active site with good accuracy, curvature at high concentrations being non-physiological or representing a progressive driving of reaction flux through alternative pathways. This

could be true in some instances, and may be vindicated in a few well-characterized enzymes by agreement about molecular mechanism obtained by alternative methods of approach. However, a number of points should be noted concerning this interpretation. First, all rational functions become asymptotically linear in transformed plots, and therefore, since there is reason to suppose that rational functions are appropriate models for a large proportion of enzymes under non-aggregating conditions, all enzyme kinetic data become linear at high and low substrate concentrations. Hence, by choice of substrate concentration range, all enzyme-kinetic data can be made to give apparently linear double-reciprocal plots. Secondly, the equation of these asymptotes will be the equations of the osculating hyperbolae (Bardsley & Waight, 1978), and hence at low substrate concentrations we would have for the rate equation:

$$v/[E]_0 = \sum_{i=1}^n \alpha_i [S]^i / \sum_{i=0}^n \beta_i [S]^i$$

the psuedo-kinetic constants:

$$K_m (\text{low } [S]) \approx -\alpha_1 \beta_0 / \psi_{11}^{20}$$

and

$$V (\text{low } [S]) \approx -\alpha_1^2 / \psi_{11}^{20}$$

and for the high concentration data we would get:

$$K_m (\text{high } [S]) \approx \phi_{n,n-1} / \alpha_n \beta_n$$

and

$$V (\text{high } [S]) \approx \alpha_n / \beta_n$$

Now it is sometimes true that even quite complex mechanisms give tolerably simple expressions for α_n / β_n , but, in general, the limiting slopes and intercepts of kinetic data will be enormously complicated expressions involving the rate constants. To illustrate this point, we ask the reader to calculate the appropriate expressions for eqn. 1(a) of the Appendix, which is, after all, the rate equation for an extremely simple mechanism. Where enzyme kinetic data are only gently curved over a wide range of substrate concentration, then the best-fit K_m and V values will be given by the parameters of the theoretical parent hyperbolae (Bardsley, 1977c,e), i.e.:

$$K_m (\text{overall}) \approx \alpha_n \beta_0 / \alpha_1 \beta_n$$

and

$$V (\text{overall}) \approx \alpha_n / \beta_n$$

although there are alternative expressions (Waight & Bardsley, 1977). Likewise these will be found to be complex expressions involving all of the independent rate constants. In other words, mathematical analysis does not substantiate the intuitive

feeling that the curve is essentially Michaelis–Menten kinetics for the active site, modified slightly by influences from minor pathways at high substrate concentrations. Hence we have no confidence that uncritical fitting of lines to selected ranges of non-linear data will give meaningful estimates of active-site parameters.

We have pointed out that complex mechanisms are consistent with almost any curve shapes, and this can make discrimination between rival mechanisms more difficult. A good example of this is the finding that the Monod–Wyman–Changeux steady-state model can give concave-down or inflected double-reciprocal plots, features that have sometimes been advanced as eliminating this particular mechanism. However, a more pressing need is to answer the question as to what new insight about molecular mechanisms is to be expected if the use of simplified models is abandoned. So far, there have been relatively few attempts to analyse kinetics in this way, but for alcohol dehydrogenase (Hanes *et al.*, 1972), peroxidase (Childs & Bardsley, 1975) and alkaline phosphatase (Waight *et al.*, 1977), analysis in terms of rational functions involving the use of results obtained by other workers led to the postulation of more complex reaction schemes than formerly envisaged. It may be that this is all that can be done at present in the realm of complex kinetics, i.e. adding final details about secondary catalytic pathways to schemes that have been established by conventional chemical techniques, but to condemn the theoretical and experimental study of complex kinetics on these grounds is to miss an important point. Faced with genuine non-artifact experimental evidence for deviant kinetics we may apply classical techniques that promise results of such power in the desperate hope that the results approximate to reality, or we may accept the non-linearities as evidence of more subtle processes involved in the catalysis. The first approach is convenient but the second more scientific.

Conclusion

The theoretical study of complex mechanisms reveals a bleak perspective. Calculation of the slopes and intercepts of limiting asymptotes to graphs given by such mechanisms reveals little hope of progress along the simple lines afforded by K_m and V of classical theory. Hence there is a potent force driving interpretation into the realms of equations derived by 'bold simplifying assumptions', as one anonymous referee puts it. However, the history of science affords many examples where the struggle with seemingly intractable problems has led to enrichment of theory, improved methodology and the eventual abandonment of simple

models. There seems no reason why this should not also apply in steady-state enzyme kinetics.

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Details of the rate equations, k_i values, α_i, β_i magnitudes and ϵ_i signs used in the text are given. Rate equations are all calculated for zero-product concentration and are presented in the form:

$$\frac{v}{[E_0]} = \sum_{i=1}^n \alpha_i [S]^i / \sum_{i=0}^n \beta_i [S]^i$$

Eqn. 1(a) (explicit form of the one-site/two-state version of the Monod-Wyman-Changeux model of Fig. 1a)

$$\alpha_1 = k_p[k_{+1}k_{-2}k_{-4}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-4}k_{+5}k_{-7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+6}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{-7} + k_{+1}k_{-2}k_{-4}k_{+5}k_q + k_{+2}k_{+3}k_{-4}k_{+5}k_q + k_{+2}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+1}k_{-2}k_{+4}k_{+6}k_{-7} + k_{-1}k_{+2}k_{+3}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{-7}] + k_q[k_{+1}k_{-2}k_{+4}k_{+5}k_{+6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{+7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+7} + k_{-1}k_{+2}k_{+3}k_{+6}k_p + k_{+2}k_{+3}k_{+5}k_{+6}k_p + k_{+1}k_{-2}k_{+4}k_{+6}k_p + k_{-1}k_{+2}k_{+3}k_{-5}k_{+6} + k_{-1}k_{+2}k_{+3}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{+4}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_p + k_{+2}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{+7}]$$

$$\alpha_2 = k_p[k_{+1}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_q] \\ + k_q[k_{+1}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+1}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+4}k_{+6}k_p + k_{+1}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+7}]$$

$$\begin{aligned} \beta_0 = & k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-3}k_{-5}k_{-7} + k_{-1}k_{-2}k_{-5}k_{+6}k_{-7} + k_{-2}k_{-3}k_{+5}k_p k_q + k_{-2}k_{-3}k_{+5}k_{-6}k_p \\ & + k_{-1}k_{-2}k_{-4}k_{-7}k_p + k_{-1}k_{-2}k_{-4}k_{-5}k_{-7} + k_{-1}k_{-2}k_{-3}k_p k_q + k_{-1}k_{-2}k_{-4}k_p k_q + k_{-1}k_{-2}k_{+6}k_p k_q \\ & + k_{-1}k_{-2}k_{-3}k_{-6}k_p + k_{-2}k_{+5}k_{+6}k_p k_q + k_{-2}k_{-3}k_{+5}k_{-7}k_p + k_{-2}k_{+5}k_{+6}k_{-7}k_p + k_{-1}k_{-2}k_{-4}k_{-6}k_p \\ & + k_{-1}k_{-2}k_{-3}k_{-5}k_q + k_{-1}k_{-2}k_{-4}k_{-5}k_q + k_{-1}k_{-2}k_{-5}k_{+6}k_q + k_{-1}k_{-2}k_{-3}k_{-5}k_{-6} + k_{-2}k_{-4}k_{+5}k_p k_q \\ & + k_{-1}k_{-2}k_{-3}k_{-7}k_p + k_{-1}k_{-2}k_{+6}k_{-7}k_p + k_{-2}k_{-4}k_{+5}k_{-6}k_p + k_{-2}k_{-4}k_{+5}k_{-7}k_p + k_{-1}k_{-2}k_{-4}k_{-6}k_{+7} \\ & + k_{-1}k_{-2}k_{-3}k_{+7}k_q + k_{-1}k_{-2}k_{-4}k_{+7}k_q + k_{-1}k_{-2}k_{+6}k_{+7}k_q + k_{-1}k_{-2}k_{-3}k_{-6}k_{+7} + k_{-2}k_{+5}k_{+6}k_{+7}k_q \\ & + k_{-2}k_{-3}k_{+4}k_p k_q + k_{-2}k_{+4}k_{+6}k_p k_q + k_{-2}k_{-3}k_{+4}k_{-6}k_p + k_{-2}k_{-4}k_{+5}k_{+7}k_q \\ & + k_{-2}k_{-3}k_{+4}k_{-5}k_{-7} + k_{-2}k_{-3}k_{+4}k_{-7}k_p + k_{-2}k_{-3}k_{+4}k_{-5}k_q + k_{-2}k_{+4}k_{-5}k_{+6}k_q \\ & + k_{-2}k_{-3}k_{+4}k_{-5}k_{-6} + k_{-2}k_{-3}k_{+5}k_{+7}k_q + k_{-2}k_{-3}k_{+5}k_{-6}k_{+7} + k_{-2}k_{+4}k_{+6}k_{-7}k_p + k_{-1}k_{+2}k_{-4}k_{-5}k_{-7} \\ & + k_{+2}k_{-3}k_{+5}k_p k_q + k_{+2}k_{-3}k_{+5}k_{-6}k_p + k_{-1}k_{+2}k_{-3}k_{+5}k_{-7}k_p + k_{+2}k_{-3}k_{+5}k_{-7}k_p + k_{-1}k_{+2}k_{-4}k_{-6}k_p \\ & + k_{-1}k_{+2}k_{-5}k_{+6}k_{-7} + k_{-1}k_{+2}k_{-3}k_p k_q + k_{-1}k_{+2}k_{-4}k_p k_q + k_{-1}k_{+2}k_{+6}k_p k_q + k_{-1}k_{+2}k_{-3}k_{-6}k_p \\ & + k_{+2}k_{+5}k_{+6}k_p k_q + k_{-1}k_{+2}k_{-4}k_{-7}k_p + k_{+2}k_{-4}k_{+5}k_{-7}k_p + k_{-1}k_{+2}k_{-4}k_{-5}k_{-6} + k_{-1}k_{+2}k_{-3}k_{-5}k_{-7} \\ & + k_{-1}k_{+2}k_{-3}k_{-5}k_q + k_{-1}k_{+2}k_{-4}k_{-5}k_q + k_{-1}k_{+2}k_{-5}k_{+6}k_q + k_{-1}k_{+2}k_{-3}k_{-5}k_{-6} + k_{+2}k_{-4}k_{+5}k_p k_q \\ & + k_{-1}k_{+2}k_{+6}k_{-7}k_p + k_{+2}k_{-4}k_{+5}k_{-6}k_p + k_{+2}k_{+5}k_{+6}k_{-7}k_p + k_{-2}k_{-3}k_{-4}k_{+7}k_q + k_{-2}k_{+4}k_{+6}k_{+7}k_q \\ & + k_{-2}k_{-3}k_{+4}k_{-6}k_{+7} + k_{+2}k_{-3}k_{+4}k_{+7}k_q + k_{+2}k_{+4}k_{+6}k_{+7}k_q + k_{-1}k_{+2}k_{-4}k_{-6}k_{+7} + k_{-1}k_{+2}k_{-3}k_{+7}k_q \\ & + k_{-1}k_{+2}k_{-4}k_{+7}k_q + k_{-1}k_{+2}k_{+6}k_{+7}k_q + k_{-1}k_{+2}k_{-3}k_{-6}k_{+7} + k_{+2}k_{+5}k_{+6}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-7}k_p \\ & + k_{+2}k_{-3}k_{+4}k_p k_q + k_{+2}k_{+4}k_{+6}k_p k_q + k_{+2}k_{-3}k_{+4}k_{-6}k_p + k_{+2}k_{-4}k_{+5}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-5}k_{-7} \\ & + k_{+2}k_{+4}k_{+6}k_{-7}k_p + k_{+2}k_{-3}k_{+4}k_{-5}k_q + k_{+2}k_{+4}k_{-5}k_{+6}k_q + k_{+2}k_{-3}k_{+4}k_{-5}k_{-6} + k_{+2}k_{-3}k_{+5}k_{+7}k_q \\ & + k_{+2}k_{-3}k_{+5}k_{-6}k_{+7} + k_{+2}k_{-3}k_{+4}k_{-6}k_{+7} \end{aligned}$$

$$\beta_1 = k_{-1}k_{+3}k_{-4}k_{-5}k_q + k_{-1}k_{+3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{+3}k_{+6}k_{-7}k_p + k_{+2}k_{+3}k_{-5}k_{+6}k_{-7} + k_{+3}k_{+4}k_{+6}k_{-7}k_p \\ + k_{-1}k_{+3}k_{-5}k_{+6}k_{-7} + k_{-1}k_{+3}k_{-4}k_p k_q + k_{-1}k_{+3}k_{-4}k_{-6}k_p + k_{-1}k_{+3}k_{-4}k_{-5}k_{-7} + k_{-1}k_{+3}k_{-4}k_{+7}k_q \\ + k_{-1}k_{+3}k_{-4}k_{-6}k_{+7} + k_{+3}k_{+5}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{-5}k_{-7} + k_{+1}k_{-2}k_{-5}k_{+6}k_{-7} \\ + k_{+1}k_{-2}k_{-3}k_p k_q + k_{+1}k_{-2}k_{-4}k_p k_q + k_{+1}k_{-2}k_{+6}k_p k_q + k_{+1}k_{-2}k_{-3}k_{-6}k_p + k_{+1}k_{-2}k_{-4}k_{-5}k_{-7} \\ + k_{+1}k_{-2}k_{-3}k_{-5}k_q + k_{+1}k_{-2}k_{-4}k_{-5}k_q + k_{+1}k_{-2}k_{-5}k_{+6}k_q + k_{+1}k_{-2}k_{-3}k_{-5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{-7}k_p \\ + k_{+1}k_{-2}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-6}k_p + k_{+1}k_{-2}k_{-4}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-4}k_{+5}k_{-7} \\ + k_{+1}k_{-2}k_{+5}k_{+6}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{-7} \\ + k_{+1}k_{-2}k_{-4}k_{+5}k_q + k_{+3}k_{-4}k_{+5}k_{-7}k_p + k_{+3}k_{-4}k_{+5}k_p k_q + k_{+2}k_{+3}k_{-4}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-6}k_{+7} \\ + k_{+2}k_{+3}k_{-4}k_p k_q + k_{+2}k_{+3}k_{-4}k_{+5}k_q + k_{+1}k_{-4}k_{+5}k_{-7}k_q + k_{+1}k_{-3}k_{+4}k_{-5}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+7} \\ + k_{+2}k_{+3}k_{-4}k_{-5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{-5}k_{-7} + k_{+1}k_{-2}k_{-3}k_{+7}k_q + k_{+1}k_{-2}k_{-4}k_{+7}k_q + k_{+1}k_{-2}k_{+6}k_{+7}k_q$$

$$\begin{aligned}
& + k_{+1}k_{-2}k_{-3}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+1}k_{-2}k_{+4}k_{-5}k_{-6} + k_{+1}k_{-2}k_{+4}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{-5}k_{+q} \\
& + k_{-1}k_{+2}k_{+3}k_{-5}k_{-6} + k_{+2}k_{+3}k_{+5}k_{-6}k_{+p} + k_{+2}k_{+3}k_{+5}k_{-7}k_{+p} + k_{+1}k_{-2}k_{+5}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+5}k_{+p}k_{+q} \\
& + k_{+1}k_{-2}k_{+4}k_{+p}k_{+q} + k_{-1}k_{+2}k_{+3}k_{-7}k_{+p} + k_{+1}k_{-2}k_{+4}k_{-6}k_{+p} + k_{-1}k_{+2}k_{+3}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{+p}k_{+q} \\
& + k_{-1}k_{+2}k_{+3}k_{-6}k_{+p} + k_{+1}k_{-2}k_{+4}k_{-5}k_{+q} + k_{+1}k_{-2}k_{+4}k_{-7}k_{+p} + k_{+1}k_{-3}k_{+4}k_{-5}k_{+q} + k_{+1}k_{+4}k_{+5}k_{+6}k_{+q} \\
& + k_{+1}k_{-3}k_{+4}k_{-5}k_{-6} + k_{+1}k_{-3}k_{+5}k_{+7}k_{+q} + k_{+1}k_{-3}k_{+5}k_{-6}k_{+7} + k_{+1}k_{-3}k_{+4}k_{+p}k_{+q} + k_{+1}k_{+4}k_{+6}k_{+p}k_{+q} \\
& + k_{+1}k_{-2}k_{+4}k_{-6}k_{+7} + k_{+1}k_{-3}k_{+4}k_{-6}k_{+p} + k_{+1}k_{+5}k_{+6}k_{+7}k_{+q} + k_{+1}k_{-3}k_{+4}k_{-7}k_{+p} + k_{+1}k_{-2}k_{+4}k_{+6}k_{-7} \\
& + k_{+2}k_{+3}k_{+4}k_{+6}k_{+p} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+q} + k_{+2}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+7}k_{+q} + k_{+2}k_{+3}k_{-4}k_{-6}k_{+7} \\
& + k_{-1}k_{+2}k_{+3}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+4}k_{-5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{+7} \\
& + k_{+1}k_{-2}k_{+5}k_{+7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+7} + k_{-1}k_{+2}k_{+3}k_{+6}k_{+p} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+p} + k_{+1}k_{+4}k_{-5}k_{+6}k_{+q} \\
& + k_{-1}k_{+2}k_{+3}k_{-5}k_{+6} + k_{+1}k_{-2}k_{+4}k_{+7}k_{+q} + k_{+2}k_{-3}k_{+4}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-5}k_{+q} + k_{+2}k_{+3}k_{+4}k_{-5}k_{-6} \\
& + k_{+2}k_{+3}k_{+5}k_{+7}k_{+q} + k_{+2}k_{+3}k_{+4}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{+7}k_{+q} + k_{-1}k_{+2}k_{+3}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-6}k_{+p} \\
& + k_{+2}k_{+3}k_{+4}k_{-7}k_{+p} + k_{+2}k_{+3}k_{+5}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+p}k_{+q} + k_{+1}k_{-3}k_{+4}k_{+7}k_{+q} + k_{+1}k_{+4}k_{+6}k_{+7}k_{+q} \\
& + k_{+1}k_{-3}k_{+4}k_{-6}k_{+7} + k_{-1}k_{+2}k_{+3}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{+4}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+7}k_{+q} \\
& + k_{+2}k_{+3}k_{+4}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+3}k_{-4}k_{+5}k_{-6}k_{+p} + k_{-1}k_{+3}k_{-4}k_{-7}k_{+p}
\end{aligned}$$

$$\begin{aligned}
\beta_2 = & k_{+1}k_{+3}k_{-5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+6}k_{-7}k_{+p} + k_{+1}k_{+3}k_{+4}k_{-5}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+p}k_{+q} + k_{+1}k_{+3}k_{-4}k_{-6}k_{+p} \\
& + k_{+1}k_{+3}k_{-4}k_{-5}k_{-7} + k_{+1}k_{+3}k_{+4}k_{+p}k_{+q} + k_{+1}k_{+3}k_{+4}k_{-6}k_{+p} + k_{+1}k_{+3}k_{+5}k_{-6}k_{+7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{-7} \\
& + k_{+1}k_{+3}k_{-4}k_{-5}k_{+q} + k_{+1}k_{+3}k_{-4}k_{-5}k_{-6} + k_{+1}k_{+3}k_{-4}k_{+5}k_{+q} + k_{+1}k_{+3}k_{-4}k_{-7}k_{+p} + k_{+1}k_{+3}k_{-4}k_{-6}k_{+7} \\
& + k_{+1}k_{+3}k_{+5}k_{+q} + k_{+1}k_{+3}k_{+4}k_{-6}k_{-7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+1}k_{+3}k_{-4}k_{+5}k_{+7} \\
& + k_{+1}k_{+3}k_{+4}k_{-7}k_{+p} + k_{+1}k_{+3}k_{+5}k_{+7}k_{+q} + k_{+1}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+p} + k_{+1}k_{+3}k_{-4}k_{+5}k_{+7} \\
& + k_{+1}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+7} + k_{+1}k_{+3}k_{-4}k_{+7}k_{+q} + k_{+1}k_{+3}k_{+4}k_{+7}k_{+q} + k_{+1}k_{+3}k_{+4}k_{-6}k_{+7}
\end{aligned}$$

Eqn. 1(b) (implicit form of the one-site/two-state version of the Monod-Wyman-Changaux model of Fig. 1b)

$$\alpha_1 = k_p[k_{+1}k_{-2}(k_{-3} + k_q) + k_{-4}(k_{+1}k_{-2} + k_{+2}k_{+3}) + k_q[k_{+2}k_{+3}(k_{-1} + k_p) + k_{+4}(k_{+1}k_{-2} + k_{+2}k_{+3})]]$$

$$\alpha_2 = k_{+1}k_{+3}(k_{-4}k_p + k_{+4}k_q)$$

$$\beta_0 = (k_{+2} + k_{-2})[k_{+4}(k_{-3} + k_q) + k_{-4}(k_{-1} + k_p) + (k_{-3} + k_q)(k_{-1} + k_p)]$$

$$\beta_1 = k_{+3}(k_{+2} + k_{-4})(k_{-1} + k_p) + k_{+1}(k_{-2} + k_{+4}) \times (k_{-3} + k_q) + (k_{+1}k_{-2} + k_{+2}k_{+3})(k_{+4} + k_{-4})$$

$$\beta_2 = k_{+1}k_{+3}(k_{+4} + k_{-4})$$

Eqn. 1(c) [implicit form of the rate equation for the one-site/two-state version of the Monod-Wyman-Changaux model with the further assumptions that only free R and T forms interconvert as in the treatment of Dalziel (1968)]

$$\alpha_1 = k_p[k_{+1}k_{-2}k_{-4}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-4}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{+q} + k_{+1}k_{-2}k_{-3}k_{+5}k_{+q} + k_{+1}k_{-2}k_{-3}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{-7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+q}]$$

$$\alpha_2 = k_p[k_{+1}k_{-2}k_{+4}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+q} + k_{+2}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_{+q}] + k_q[k_{+1}k_{-2}k_{+4}k_{-5}k_{+6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{+7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+7} + k_{-1}k_{+2}k_{+3}k_{+6}k_{+p} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+p} + k_{+1}k_{-2}k_{+4}k_{+6}k_{+p} + k_{-1}k_{+2}k_{+3}k_{-5}k_{+6}]$$

$$\alpha_3 = k_p[k_{+1}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{-7}] + k_q[k_{+1}k_{+3}k_{+4}k_{-5}k_{+6} + k_{-1}k_{+2}k_{+3}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{+4}k_{+6}k_{+7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{+p} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+p} + k_{+1}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+7}]$$

$$\alpha_4 = k_q[k_{+2}k_{+3}k_{+4}k_{+6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+7}]$$

$$\beta_0 = k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-3}k_{-5}k_{-7} + k_{-1}k_{-2}k_{-3}k_{+5}k_{+6}k_{-7} + k_{-2}k_{-3}k_{+5}k_{+p}k_{+q} + k_{-2}k_{-3}k_{+5}k_{-6}k_{+p} + k_{-1}k_{-2}k_{-4}k_{-7}k_{+p} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-7} + k_{-1}k_{-2}k_{-3}k_{+p}k_{+q} + k_{-1}k_{-2}k_{-4}k_{+p}k_{+q} + k_{-1}k_{-2}k_{+6}k_{+p}k_{+q} + k_{-1}k_{-2}k_{-3}k_{-6}k_{+p} + k_{-2}k_{+5}k_{+6}k_{+p}k_{+q} + k_{-2}k_{-3}k_{+5}k_{-7}k_{+p} + k_{-2}k_{+5}k_{+6}k_{-7}k_{+p} + k_{-1}k_{-2}k_{-4}k_{-6}k_{+p} + k_{-1}k_{-2}k_{-3}k_{-5}k_{+q} + k_{-1}k_{-2}k_{-4}k_{-5}k_{+q} + k_{-1}k_{-2}k_{-3}k_{+6}k_{+q} + k_{-1}k_{-2}k_{-3}k_{-5}k_{-6} + k_{-2}k_{-4}k_{+5}k_{+p}k_{+q} + k_{-1}k_{-2}k_{-3}k_{-7}k_{+p} + k_{-1}k_{-2}k_{+6}k_{-7}k_{+p} + k_{-2}k_{-4}k_{+5}k_{-6}k_{+p} + k_{-2}k_{-4}k_{+5}k_{-7}k_{+p}$$

$$\alpha_1 = k_{+1}k_{-2}k_p(k_{-3} + k_q) + k_{+2}k_{+3}k_q(k_{-1} + k_p)$$

$$\beta_0 = (k_{+2} + k_{-2})(k_{-1} + k_p)(k_{-3} + k_q)$$

$$\beta_1 = k_{+1}k_{-2}(k_{-3} + k_q) + k_{+2}k_{+3}(k_{-1} + k_p)$$

Note that this may be obtained from eqn. 1(b) by setting $k_{+4} = k_{-4} = 0$, and to maintain analogy with the Monod *et al.* (1965) saturation function we may define:

$$V_1 = k_p, V_2 = k_q, K_1 = (k_{-1} + k_p)/k_{+1},$$

$$K_2 = (k_{-3} + k_q)/k_{+3}, L = k_{+2}/k_{-2}, C = K_1/K_2,$$

$$\alpha = S/K_1$$

whereupon the rate equation becomes:

$$\frac{v}{[E_0]} = \frac{(V_1 + V_2LC)\alpha}{(1 + \alpha) + L(1 + C\alpha)}$$

Eqn. 4(a) (explicit form of the homotropic substrate-modifier mechanism of Fig. 4a)

$$\beta_1 = k_{-1}k_{+3}k_{-4}k_{-5}k_q + k_{-1}k_{+3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-3}k_{+7}k_q + k_{-1}k_{-2}k_{-4}k_{+7}k_q + k_{-1}k_{-2}k_{+6}k_{+7}k_q \\ + k_{-1}k_{-2}k_{-3}k_{-6}k_{+7} + k_{-2}k_{+5}k_{+6}k_{+7}k_q + k_{-1}k_{+3}k_{+6}k_{-7}k_p + k_{+3}k_{-4}k_{+5}k_{-6}k_p + k_{-1}k_{-2}k_{-4}k_{-6}k_{+7} \\ + k_{-1}k_{+3}k_{-5}k_{+6}k_{-7} + k_{-2}k_{-3}k_{+4}k_p k_q + k_{-2}k_{+4}k_{+6}k_p k_q + k_{-2}k_{-3}k_{+4}k_{-6}k_p + k_{-2}k_{-4}k_{+5}k_{+7}k_q \\ + k_{-2}k_{-3}k_{+4}k_{-5}k_{-7} + k_{-1}k_{+3}k_{-4}k_{-7}k_p + k_{-2}k_{-3}k_{+4}k_{-7}k_p + k_{-1}k_{+3}k_{-4}k_p k_q + k_{-1}k_{+3}k_{-4}k_{-6}k_p \\ + k_{-1}k_{+3}k_{-4}k_{-5}k_{-7} + k_{-2}k_{-3}k_{+4}k_{-5}k_q + k_{-2}k_{+4}k_{-5}k_{+6}k_q + k_{-2}k_{-3}k_{+4}k_{-5}k_{-6} + k_{-2}k_{-3}k_{+5}k_{+7}k_q \\ + k_{-2}k_{-3}k_{+5}k_{-6}k_{+7} + k_{-2}k_{+4}k_{+6}k_{-7}k_p + k_{+3}k_{+5}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{-5}k_{-7} \\ + k_{+1}k_{-2}k_{-5}k_{+6}k_{-7} + k_{+1}k_{-2}k_{-3}k_p k_q + k_{+1}k_{-2}k_{-4}k_p k_q + k_{+1}k_{-2}k_{+6}k_p k_q + k_{+1}k_{-2}k_{-3}k_{-6}k_p \\ + k_{+1}k_{-2}k_{-4}k_{-5}k_{-7} + k_{+1}k_{-2}k_{-3}k_{-5}k_q + k_{+1}k_{-2}k_{-4}k_{-5}k_q + k_{+1}k_{-2}k_{-5}k_{+6}k_q + k_{+1}k_{-2}k_{-3}k_{-5}k_{-6} \\ + k_{+1}k_{-2}k_{-3}k_{-6}k_p + k_{+1}k_{-2}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-6}k_p + k_{+1}k_{-2}k_{-4}k_{-7}k_p + k_{+1}k_{+2}k_{-4}k_{-5}k_{-7} \\ + k_{+2}k_{-3}k_{+5}k_p k_q + k_{+2}k_{-3}k_{+5}k_{-6}k_p + k_{-1}k_{+2}k_{-3}k_{-7}k_p + k_{+2}k_{-3}k_{+5}k_{-7}k_p + k_{-1}k_{+2}k_{-4}k_{-6}k_p \\ + k_{-1}k_{+2}k_{-5}k_{+6}k_{-7} + k_{-1}k_{+2}k_{-3}k_p k_q + k_{-1}k_{+2}k_{-4}k_p k_q + k_{-1}k_{+2}k_{+6}k_p k_q + k_{-1}k_{+2}k_{-3}k_{-6}k_p \\ + k_{+2}k_{+5}k_{+6}k_p k_q + k_{-1}k_{+2}k_{-4}k_{-7}k_p + k_{+2}k_{-4}k_{+5}k_{-7}k_p + k_{-1}k_{+2}k_{-4}k_{-5}k_{-6} + k_{-1}k_{+2}k_{-3}k_{-5}k_{-7} \\ + k_{-1}k_{+2}k_{-3}k_{-5}k_q + k_{-1}k_{+2}k_{-4}k_{-5}k_q + k_{-1}k_{+2}k_{-5}k_{+6}k_q + k_{-1}k_{+2}k_{-3}k_{-5}k_{-6} + k_{+2}k_{-4}k_{+5}k_p k_q \\ + k_{-1}k_{+2}k_{+6}k_{-7}k_p + k_{+2}k_{-4}k_{+5}k_{-6}k_p + k_{+2}k_{+5}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-4}k_{+5}k_{-7} \\ + k_{+1}k_{-2}k_{+5}k_{+6}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_q + k_{+1}k_{-2}k_{-3}k_{+5}k_{-6} + k_{+1}k_{-2}k_{-3}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{-7} \\ + k_{+1}k_{-2}k_{-4}k_{+5}k_q + k_{+3}k_{-4}k_{+5}k_{-7}k_p + k_{+3}k_{-4}k_{+5}k_p k_q$$

$$\beta_2 = k_{-2}k_{-3}k_{+4}k_{+7}k_q + k_{-2}k_{+4}k_{+6}k_{+7}k_q + k_{-2}k_{-3}k_{+4}k_{-6}k_{+7} + k_{+3}k_{+4}k_{+6}k_{-7}k_p + k_{-1}k_{+3}k_{-4}k_{+7}k_q \\ + k_{-1}k_{+3}k_{-4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{-4}k_{-5}k_q + k_{+1}k_{+3}k_{-4}k_{-5}k_{-6} + k_{+2}k_{+3}k_{-5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{-4}k_{-7}k_p \\ + k_{+2}k_{+3}k_{-4}k_{-7}k_p + k_{+1}k_{-2}k_{-4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{-5}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_p k_q + k_{+2}k_{+3}k_{-4}k_{-6}k_p \\ + k_{+1}k_{+3}k_{-4}k_p k_q + k_{+1}k_{+3}k_{-4}k_{-6}k_p + k_{+1}k_{+3}k_{-4}k_{-5}k_{-7} + k_{+2}k_{+3}k_{-4}k_{-5}k_q + k_{+2}k_{+3}k_{-4}k_{-5}k_{-6} \\ + k_{+2}k_{+3}k_{-4}k_{-5}k_{-7} + k_{+1}k_{-2}k_{-3}k_{+7}k_q + k_{+1}k_{-2}k_{-4}k_{+7}k_q + k_{+1}k_{-2}k_{+6}k_{+7}k_q + k_{+1}k_{-2}k_{-3}k_{-6}k_{+7} \\ + k_{+1}k_{+3}k_{+6}k_{-7}k_p + k_{+1}k_{-2}k_{+4}k_{-5}k_{-6} + k_{+1}k_{-2}k_{+4}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{-5}k_q + k_{-1}k_{+2}k_{+3}k_{-5}k_{-6} \\ + k_{+2}k_{+3}k_{+5}k_{-6}k_p + k_{+2}k_{+3}k_{+5}k_{-7}k_p + k_{+1}k_{-2}k_{+5}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+5}k_p k_q + k_{+1}k_{-2}k_{+4}k_p k_q \\ + k_{-1}k_{+2}k_{+3}k_{-7}k_p + k_{+1}k_{-2}k_{+4}k_{-6}k_p + k_{-1}k_{+2}k_{+3}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_p k_q + k_{-1}k_{+2}k_{+3}k_{-6}k_p \\ + k_{+1}k_{-2}k_{+4}k_{-5}k_q + k_{+1}k_{-2}k_{+4}k_{-6}k_p + k_{+1}k_{-3}k_{+4}k_{-5}k_q + k_{+1}k_{+4}k_{-5}k_{+6}k_q + k_{+1}k_{-3}k_{+4}k_{-5}k_{-6} \\ + k_{+1}k_{-3}k_{+5}k_{+7}k_q + k_{+1}k_{-2}k_{+5}k_{-6}k_{+7} + k_{-1}k_{+2}k_{-4}k_{-6}k_{+7} + k_{-1}k_{+2}k_{-3}k_{+7}k_q + k_{-1}k_{+2}k_{-4}k_{+7}k_q \\ + k_{-1}k_{+2}k_{+6}k_{+7}k_q + k_{-1}k_{+2}k_{-3}k_{-6}k_{+7} + k_{+2}k_{+5}k_{+6}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-7}k_p + k_{+1}k_{-4}k_{+5}k_{+7}k_q \\ + k_{+1}k_{-3}k_{+4}k_{-5}k_{-7} + k_{+2}k_{-3}k_{+4}k_p k_q + k_{+2}k_{+4}k_{+6}k_p k_q + k_{+2}k_{-3}k_{+4}k_{-6}k_p + k_{+2}k_{-4}k_{+5}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-5}k_{-7} \\ + k_{+2}k_{+4}k_{+6}k_{-7}k_p + k_{+1}k_{-3}k_{+4}k_p k_q + k_{+1}k_{+4}k_{+6}k_p k_q + k_{+1}k_{-3}k_{+4}k_{-6}k_p + k_{+1}k_{+5}k_{+6}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-5}k_q \\ + k_{+2}k_{+4}k_{-5}k_{+6}k_q + k_{+2}k_{-3}k_{+4}k_{-5}k_{-6} + k_{+2}k_{-3}k_{+5}k_{+7}k_q + k_{+2}k_{-3}k_{+5}k_{-6}k_{+7} + k_{+1}k_{-3}k_{+4}k_{-7}k_p \\ + k_{+1}k_{-2}k_{+4}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_q + k_{+2}k_{+3}k_{+5}k_{+6}k_{-7} + k_{+1}k_{+3}k_{-4}k_{+5}k_{-6} \\ + k_{+1}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+3}k_{+6}k_{-7} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{-7} + k_{+1}k_{-2}k_{+4}k_{-5}k_{+6} \\ + k_{+1}k_{-2}k_{-3}k_{+5}k_{+7} + k_{+1}k_{-2}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{-4}k_{+5}k_{+7} + k_{-1}k_{+2}k_{+3}k_{+6}k_p + k_{+2}k_{+3}k_{+5}k_{+6}k_p \\ + k_{+1}k_{-2}k_{+4}k_{+6}k_p + k_{+2}k_{+3}k_{-4}k_{+5}k_q + k_{-1}k_{+2}k_{+3}k_{-5}k_{+6}$$

$$\beta_3 = k_{+2}k_{+3}k_{-4}k_{+7}k_q + k_{+2}k_{+3}k_{-4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{-4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{-5}k_q + k_{+1}k_{+3}k_{+4}k_{-5}k_{-6} \\ + k_{+1}k_{+3}k_{+5}k_{+7}k_q + k_{+2}k_{+3}k_{+4}k_{-5}k_q + k_{+2}k_{+3}k_{+4}k_{-5}k_{-6} + k_{+2}k_{+3}k_{+5}k_{+7}k_q + k_{+1}k_{+3}k_{+4}k_{-7}k_p \\ + k_{+1}k_{+3}k_{-2}k_{+4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{-5}k_{-7} + k_{-1}k_{+2}k_{+3}k_{+7}k_q + k_{-1}k_{+2}k_{+3}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-5}k_{-7} \\ + k_{+1}k_{+3}k_{+4}k_p k_q + k_{+1}k_{+3}k_{+4}k_{-6}k_p + k_{+1}k_{+3}k_{+5}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_p k_q + k_{+2}k_{+3}k_{+4}k_{-6}k_p \\ + k_{+2}k_{+3}k_{+4}k_{-7}k_p + k_{+2}k_{+3}k_{+5}k_{-6}k_{+7} + k_{+2}k_{-3}k_{+4}k_{+7}k_q + k_{+2}k_{+4}k_{+6}k_{+7}k_q + k_{+1}k_{-3}k_{+4}k_{+7}k_q \\ + k_{+1}k_{+4}k_{+6}k_{+7}k_q + k_{+1}k_{-3}k_{+4}k_{-6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{-7} + k_{+1}k_{+3}k_{+4}k_{-5}k_{+6} \\ + k_{-1}k_{+2}k_{+3}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+1}k_{-2}k_{+4}k_{+6}k_{+7} + k_{+1}k_{+3}k_{+5}k_{+6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_p \\ + k_{+1}k_{+3}k_{+4}k_{+6}k_p + k_{+1}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+2}k_{+3}k_{+4}k_{-5}k_{+6} + k_{+2}k_{+3}k_{-4}k_{+5}k_{+7} + k_{+1}k_{+3}k_{-4}k_{+7}k_q \\ + k_{+1}k_{-2}k_{+4}k_{+7}k_q + k_{+2}k_{-3}k_{+4}k_{-6}k_{+7}$$

$$\beta_4 = k_{+1}k_{+3}k_{+4}k_{+7}k_q + k_{+1}k_{+3}k_{+4}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+7}k_q + k_{+2}k_{+3}k_{+4}k_{-6}k_{+7} + k_{+2}k_{+3}k_{+4}k_{+6}k_{+7} + k_{+1}k_{+3}k_{+4}k_{+6}k_{+7}$$

Eqn. 4(b) (rate equation for the implicit form of the homotropic substrate-modifier mechanism illustrated in Fig. 4b)

$$\alpha_1 = k_{+1}k_{-2}k_p(k_{-3} + k_q + k_{-4})$$

$$\alpha_2 = k_{+3}k_{-4}(k_{+1} + k_{+2})k_p \\ + [k_{+1}k_{-2}k_{+4} + k_{+2}k_{+3}(k_{-1} + k_p)]k_q$$

$$\alpha_3 = k_{+3}k_{+4}(k_{+1} + k_{+2})k_q$$

$$\beta_0 = k_{-2}(k_{-1} + k_p)(k_{-3} + k_q + k_{-4})$$

$$\beta_1 = k_{+1}k_{-2}k_{-4} + k_{-2}(k_{+1} + k_{+4})(k_{-3} + k_q) \\ + k_{-4}(k_{+2} + k_{+3})(k_{-1} + k_p) \\ + k_{+2}(k_{-1} + k_p)(k_{-3} + k_q)$$

$$\beta_2 = (k_{+1} + k_{+2})[k_{+4}(k_{-3} + k_q) + k_{+3}k_{-4}] \\ + k_{+1}k_{-2}k_{+4} + k_{+2}k_{+3}(k_{-1} + k_p)$$

$$\beta_3 = k_{+3}k_{+4}(k_{+1} + k_{+2})$$

References

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