Patterns of Apparent Co-operativity in a Simple Random Non-Equilibrium Enzyme–Substrate–Modifier Mechanism

COMPARISON WITH EQUILIBRIUM ALLOSTERIC MODELS

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It has often been claimed that random non-equilibrium mechanisms can result in apparent homotropic and heterotropic effects in steady-state kinetics of the kind more usually attributed to intersubunit allosteric interactions. However, it has never been shown whether any simple random mechanism could in fact give patterns of apparent interaction similar to those predicted by the well-known allosteric models. The patterns of apparent substrate co-operativity and affinity given by the steady-state of a standard simple random substrate–modifier mechanism in which catalytic velocity is proportional to substrate binding have been analysed mathematically and numerically. All patterns possible with this model are described. Some of them rather resemble those possible with standard allosteric models, in that there is a high-affinity and a low-affinity form at zero and infinite modifier concentrations (or vice versa) which show Michaelian behaviour, apparent co-operativity passing through a maximum or minimum at intermediate affinities. Unlike the allosteric models the family of curves is in principle not symmetrical. The random model can also give behaviour not possible with the standard allosteric models, such as higher substrate affinity at intermediate modifier concentrations than at either zero or infinite modifier, with concomitant negative apparent substrate co-operativity, or a single change of sign of apparent substrate co-operativity. The analysis uses recently discovered simplified forms of steady-state equations for random models.

‘Random’ mechanisms, such as the standard model of Fig. 1 for substrate–modifier interaction in a monomeric enzyme predict non-Michaelian steady-state kinetics. Non-Michaelian kinetics of a number of experimental systems have now been interpreted in more or less detail in terms of random mechanisms (Jensen & Trentini, 1970; Wong *et al.*, 1972; Hanes *et al.*, 1972; Rubsamen *et al.*, 1974; Meunier *et al.*, 1974; Pabst *et al.*, 1974; Mannervik *et al.*, 1974; Childs & Bardsley, 1975; Carlberg & Mannervik, 1975; Rossi *et al.*, 1975; Mannervik, 1976; Storer & Cornish-Bowden, 1977; Waight *et al.*, 1977; McMinn & Ottaway, 1977; Johansson & Pettersson, 1977; Monneuse-Doublet *et al.*, 1978).

A necessary condition for the kinetics to be appreciably non-Michaelian is that the decomposition of some enzyme–substrate complexes via catalysis and product dissociation must be sufficiently rapid compared with their non-catalytic decomposition, in other words the enzyme–substrate complexes must

not be near thermodynamic equilibrium with free enzyme and substrate.

Fig. 1. Enzyme–substrate–modifier mechanism studied in this paper

Abbreviations: E, enzyme; X, substrate; Y, modifier.

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Since interaction between substrate molecules bound to different sites on the enzyme molecule, whether the complexes are near to equilibrium or not, is also a principle that gives rise to non-Michaelian kinetics, there has been much speculation and discussion about whether the kinetic behaviour most usually ascribed to intersite ('allosteric') interactions could not equally well be explained by non-equilibrium random ('mnemonic') mechanisms without interactions between substrate sites [well-known papers suggesting this are Blum (1955), Rabin (1967), Ferdinand (1966) and Wyman (1975); for reviews and further references see Whitehead (1970), Fromm (1976) and Neet & Ainslie (1976)].

One object of the present paper is to examine a standard random model (Fig. 1) that arises naturally in any elementary consideration of substrate-modifier interactions, to see whether it can behave anything like a standard allosteric model, and if so in what conditions. It is often pointed out that random models can, like allosteric ones, give sigmoid curves of velocity against substrate concentration, as though that answered the question, but comparing two models in any branch of enzyme kinetics usually demands describing the families of curves (representing v as a function of concentrations of two substances, for example substrate X and modifier Y) that they predict. The random and the allosteric models have never been compared in this way. The nature of the curve families of some standard allosteric models is well known, but those of the random mechanisms is not known at all, so it has not been shown that the two types of mechanism can give similar behaviour. This gap in knowledge is undoubtedly caused by the discouraging complexity of the equations of the random models. Hence a second aim of this paper is just to show that the equations of models of complexity comparable with Fig. 1 can be analysed and to suggest, by example, how this analysis could be done in other cases. This task is usefully facilitated by using the simplified forms of equations for such mechanisms developed by Whitehead (1976).

For the comparison to make sense, the mechanisms compared must be restricted in some way, and the behaviour of the two classes must have something obvious in common. For this reason we have restricted the comparison to, on the one hand, the well-known allosteric models which assume that subunits may exist in two different conformations and that velocity is proportional to concentration of enzyme-bound substrate ('K-systems') and on the other hand the special case of the mechanism of Fig. 1 for which $k_{\text{cat. 1}} = k_{\text{cat. 2}}$, in which case velocity is also proportional to bound-substrate concentration.

Many of the formulae and conclusions are easily transferrable to other models, for instance one where conformational changes replace the modifier associa-

tion-dissociation steps of Fig. 1 (Blum, 1955; Wyman, 1975). If reversible catalytic steps and irreversible product dissociation steps:

are added to the model of Fig. 1 the form of the steady-state equation, and therefore our treatments and conclusions, are essentially unchanged. The 'allosteric building block' (Whitehead, 1976), a model in which all the forms of the enzyme appearing in Fig. 1 can undergo conformational change to forms E', E'X, E'Y and E'XY capable of reactions parallel to those of E, etc., is also described by exactly the same form of equation as is the scheme of Fig. 1, provided that the conformational changes are sufficiently rapid (Whitehead, 1976).

Results

The behaviour of a family of co-operative curves is conveniently represented (Wyman, 1967) by plotting the logarithm of half-saturating substrate concentration ($\log x_{\frac{1}{2}}$) against half-saturation 'Hill's coefficient' ($h_{\frac{1}{2}}$) defined by:

$$h_{\frac{1}{2}} = d \log [v/(V-v)] / d \log x = d \log [\bar{X}/(1-\bar{X})] / d \log x \quad (1)$$

at $\bar{X} = v/V = \frac{1}{2}$ (in the mechanism considered in this paper $\bar{X} = v/V$). The allosteric models considered give symmetric plots of the general form shown in Fig. 2(a). The main new conclusion reached here is that the random mechanism considered is compatible only with curves in this plot having the gross features of those in Figs. 2(b)–2(f).

This is concluded by piecing together the following facts.

(1) At zero or infinite modifier concentration, y (the starting and end points of the curves), $h = 1$.

A glance at Fig. 1 will show that under these two conditions the mechanism becomes a simple Briggs-Haldane (1925) mechanism, with the Briggs-Haldane constants:

$$(x_{\frac{1}{2}})_{y=0} = (k_{-1} + k_{\text{cat.}}) / k_{+1} \equiv K'_1$$

and

$$(x_{\frac{1}{2}})_{y=\infty} = (k_{-4} + k_{\text{cat.}}) / k_{+4} \equiv K'_4 \quad (2)$$

(since $k_{\text{cat. 1}}$ and $k_{\text{cat. 2}}$ are equal we replace them by a single symbol, $k_{\text{cat.}}$).

(2) Apparent substrate co-operativity can be positive at all y or negative at all y , or positive at low y changing to negative at high y . We shall refer to these patterns as X+, X− and X+− respectively. The formulae that determine the sign of co-operativity are eqns. (3) or (4) of Appendix 1, and they show that no other pattern is possible.

(3) Each of these three patterns of apparent co-operativity is consistent with y being either an 'activa-

tor' ($K'_1 > K'_4$) or an 'inhibitor' ($K'_1 < K'_4$). This can be seen from the formulae just mentioned.

These three facts tell us that when there is no extremum of $\bar{X}$ or v as a function of y (in other words

Fig. 2. Plot of h_+ (ordinates) against $\log x_+$ (abscissae) allowed by the mechanism of Fig. 1 (when $k_{cat.1} = k_{cat.2}$), compared with those of two-conformation K-system allosteric models

The numbers on the abscissae represent x_+ (not $\log x_+$). (a) Plots given by dimeric equilibrium allosteric models obeying the equation:

$$v/v_m = [L_1(1+x)\theta/(1+cx) + 2L_2(1+x)^2\theta^2/(1+cx)^2]/2[1 + L_1(1+x)\theta/(1+cx) + L_2(1+x)^2\theta^2/(1+cx)^2]$$

where x is the substrate concentration expressed in suitable units, L_1 and L_2 equilibrium constants for allosteric transitions in unliganded enzyme and θ a term representing the contribution of heterotropic effectors, $c=0.01$ in all cases; curve (A), Monod-Wyman-Changeux (1965) model ($L_1=0$); curve (B) Koshland-Nemethy-Filmer (1966) model with positive co-operativity; curve (C), same model with negative co-operativity. (b)-(l) Examples of each type of curve permitted by the mechanism of Fig. 1 when ($k_{cat.1}=k_{cat.2}$) computed from eqns. (5) and (1) using the rate constants reported in Table 1. Arrows indicate the direction of increase of modifier concentration (y).

when x_+ is between K'_1 and K'_4 at all y) curves of the types in Figs. 2(b)–2(g) are possible.

(4) A maximum of $\bar{X}$ or v as a function of y is possible, but not a minimum, so that the curve can go to the left of the interval (K'_1 , K'_4) as in Figs. 2(h)–2(m), but not to the right; this excursion is compatible only with $X-$ or $X+-$, but with the latter only when $K'_1 > K'_4$ as in Fig. 2(j). Numerical computations show that curves with leftwards excursions can have various slightly different forms, shown in Figs. 2(h)–2(l), including a loop.

(5) If substrate co-operativity changes sign, i.e. if the curve intersects the $h=1$ axis, it does so at a value of x_+ lying between K'_1 and K'_4 as in Fig. 2(j).

As a result of facts (4) and (5) above, apparent co-operativity is always negative in the region of a leftwards excursion.

The conditions for a maximum and impossibility of a minimum are shown by plausibility arguments below, and rigorous ones in Appendix 2. The incompatibility of maximum with the $X+$ pattern and with the $X+-$ pattern when $K'_1 < K'_4$, is shown by comparing these conditions with the formulae for the sign of apparent co-operativity.

Fact (5) above is proved by examining the value of the Michaelis constant obtained at that value of y that causes apparent substrate co-operativity to vanish (Appendix 3).

Plausibility arguments concerning maxima

As considerable calculation is required to arrive at the statements in fact (4) above, we may note that a plausible condition for an extremum is easily found. If K'_1 and K'_4 were equal, i.e.:

$$(k_{+1}k_{-4} - k_{+4}k_{-1}) + k_{\text{cat.}}(k_{+1} - k_{+4}) = 0 \quad (3)$$

then velocity at zero and infinite y would be equal. Except for special cases v varies with y and must therefore give an extremum when eqn. (3) holds, and also when the constants are varied by a sufficiently small amount from those satisfying eqn. (3). Eqn. (3) can only be satisfied when the two expressions in parentheses are of opposite signs. Hence a sufficient

condition for an extremum is that $(k_{+1}k_{-4} - k_{+4}k_{-1})$ and $(k_{+1} - k_{+4})$ be of opposite signs [this necessitates $(k_{+1} - k_{+4})$ and $(k_{-1} - k_{-4})$ be of the same sign], and $k_{\text{cat.}}$ be near enough to the value satisfying eqn. (3).

We can also attempt to reason intuitively whether the extremum will be a maximum or a minimum. Take again the case where eqn. (3) holds, i.e. $K'_1 = K'_4$. We can suppose (since the reasoning in the opposite case is symmetrical) that, in accord with the conditions for extremum $k_{+1} > k_{+4}$, $k_{-1} > k_{-4}$ and $k_{+1}/k_{-1} < k_{+4}/k_{-4}$, hence $k_{+2}/k_{-2} < k_{+3}/k_{-3}$. Imagine a steady state in which $k_{+3} = k_{-3} = 0$, then:

$$[\text{EXY}]/[\text{EX}] = k_{+2}/k_{-2}$$

and

$$[\text{enzyme-substrate complexes}]/[\text{free enzyme}] =$$

$$x/K'_1 = x/K'_4 \quad (4)$$

By the last inequality $[\text{EXY}]/[\text{EX}]$ is less than the equilibrium ratio k_{+3}/k_{-3} . Thus as k_{+3} and k_{-3} are increased from zero we shall expect a net flow of matter from EX to EXY, and a new steady state to be reached in which $[\text{EXY}]/[\text{EX}]$ is greater than in the previous one, and similarly $[\text{E}]/[\text{EY}]$ is greater than previously. That is, slower-dissociating enzyme-substrate complexes and faster-associating free enzyme forms are favoured, so enzyme-substrate complexes as a whole are favoured relative to the previous steady state where eqn. (4) holds. Thus enzyme-substrate complex concentration, and velocity, should be greater at intermediate y than that given by eqn. (4), i.e. than that at zero or infinite y , and the extremum a maximum. Such intuitive reasonings are admittedly no better than plausible when not confirmed by mathematical analysis. Appendix 2 confirms that the extremum is a maximum, and that the condition shown above to be sufficient for an extremum is also necessary.

Numerical aspects

The curves of Fig. 2 have been computed with limited spans of values of rate constants of the same dimension (Table 1) in order to show that substrate

Table 1. Rate constants (arbitrary units) used in computations of curves of Fig. 2

Fig.	k_{-1}	k_{+1}	k_{-2}	k_{+2}	k_{-3}	k_{+3}	k_{-4}	k_{+4}	$k_{\text{cat.}}$	K'_1 [$=(k_{-1} + k_{\text{cat.}})/k_{+1}$]	K'_4 [$=(k_{-4} + k_{\text{cat.}})/k_{+4}$]
2(b)	4.514	31	3	16	94	1	73	1	70	2.40	143
2(c)	77	5	55	1.91	1	23	1	43	49	25.2	1.63
2(d)	45	71	14	24	22	28	1.71	2	85	1.83	43.34
2(e)	1	1	4	11	4	16.6	43	65	97	98	2.15
2(f)	103	1.85	7.59	0.70	49.0	2.23	957.7	8.46	78.5	98.1	122
2(g)	3262	5884	82.26	8.77	114.7	48.13	337.6	2398	170	0.583	0.212
2(h)	463	47	259	8	922	351	8	10	217	14.5	22.5
2(i)	4	40	34	692	193.6	228	828	479	118	3.05	1.97
2(j)	618.3	605.4	18.16	1.03	4.73	3.08	4.19	47.25	12.92	1.043	0.362
2(k)	429.3	249.5	15.71	11.69	43.23	281.5	1.39	7.09	11.1	1.765	1.765
2(l)	1	1	12	2	354	5	698	58.39	4	5	12.02

saturation curves can deviate from Michaelian, not just theoretically, but enough to be experimentally measurable, even with a conservative range of rate constants. Theoretically h for the mechanism of Fig. 1 can take values between 0 and 2. Values for h_+ over about half this range, i.e. from about 0.4 to 1.4 can be found with rate constants of the same dimension differing by a factor of less than 10^2 , though such extremes are uncommon when independent rate constants in this range are assigned randomly (by assigning a random number between 0 and 2 to logarithms of the rate constants). As the range from which rate constants can be chosen is enlarged, so the average and maximum deviations of h from 1 increase. With very large differences between rate constants hysteretic (Frieden, 1970; Fromm, 1976; Neet & Ainslie, 1976) as well as non-Michaelian steady-state behaviour can result.

It is very unusual to find constants which give the $X+ -$ pattern with both substantial positive and negative deviations from $h=1$, even with a large span of rate constants. In Figs. 2(f) and 2(g) the deviations on each side of the $h=1$ axis are very small. It is perhaps not surprising that the requirement that $h=1$ at some point on the curve leads to very small deviations from $h=1$ on at least one side of the curve. It is easier to find constants which give substantial deviations on both sides when a leftward excursion is also allowed, as in Fig. 2(j).

Discussion

Comparison with equilibrium allosteric models

It has often been suggested that random models might give behaviour similar to that of quasi-equilibrium allosteric models. The results summarized in Fig. 2 permit some detailed comparisons for restricted classes of models considered.

One can always invent a quasi-equilibrium model to give behaviour identical with that of any random model (this will require a greater number of sites than the random model simulated), though the converse may not be true. A comparison, to be interesting, must therefore be between restricted subclasses, whose behaviour is similar in at least some obvious respects. This is why we choose to compare only the case $k_{cat.1} = k_{cat.2}$ with some well-known allosteric models for K-systems. These have in common that there is no excess substrate inhibition, and velocity at substrate saturation is independent of modifier concentration.

The most widely discussed allosteric models (Monod *et al.*, 1965; Koshland *et al.*, 1966) suppose that the subunits of an oligomeric enzyme may assume two conformations, which can both bind substrate. A consequence of this supposition is an h_+ against $\log x_+$ curve with shapes like those of Fig.

2(a), with $h=1$ at its extremities (Rubin & Changeux, 1966; Wyman, 1967). (With allosteric models with more than two substrate sites and both positive and negative intersubunit co-operativity in the same enzyme molecule, more complicated types of curves with several extrema are theoretically possible.)

The behaviour of the random mechanism illustrated in Figs. 2(b)–(e) fairly resembles that of the allosteric models. It differs, however, in two ways.

(1) In the random model the entire curve of the diagram is traversed. In the allosteric models this may or may not happen (theoretically it never quite does) depending on the constants of the model.

Extreme examples where it does not happen are the limiting cases ('exclusive binding') in which binding to one of the conformations is so weak that the right-most limb of the curve is never reached, but only the maximum which is very flat (constant co-operativity at low affinity) and the still more specialized 'induced-fit' model (Koshland *et al.*, 1966) in which only this flat part is present (invariant co-operativity). In principle, the random model therefore cannot imitate the behaviour of the exclusive binding or induced-fit models.

(2) The curve in the symmetrical transition model (Monod *et al.*, 1965), and most variants of the sequential model (Koshland *et al.*, 1966) (those where the enzyme with all subunits in one conformation is fully symmetrical, with the same symmetry as when they are all in the other conformation), is symmetrical. The curve in the random model on the other hand is not in general symmetrical. In practice this asymmetry might not always be visible, as some curves in Fig. 2 demonstrate.

Figs. 2(f)–2(l) show behaviour allowed by the random model with other features besides asymmetry that are qualitatively impossible with any of the simple allosteric models (except that behaviour like Fig. 2(h) might be possible with a modifier that interacted both sterically and allosterically with the substrate). An equilibrium model giving anything like these types of behaviour would require there to be at least three conformations of the subunits with differing interactions with substrates. [An extremum as a function of modifier concentration is possible with the two-conformation equilibrium model with an appropriate arrangement of allosteric modifier sites; but the same curve is traversed back and forth; to every value of x_+ corresponds a single value of n_+ though perhaps more than one value of modifier concentration, and curves like Figs. 2(h)–2(l) do not result.]

In conclusion, therefore, we have stated and proved, for a particular model (a special case of the mechanism of Fig. 1) the possible patterns of behaviour of v as a function of two concentrations (substrate and modifier), i.e. those of Figs. 2(b)–2(l) and no others, and given the conditions for each pattern. This is the

first time this has been done for any random model. The mathematical calculations take advantage of the existence of somewhat simplified forms of the rate equations. Some of the patterns are quite similar to those predicted by simple quasi-equilibrium models, but most of them are clearly different from what the simple allosteric models allow. However, the conditions that must be satisfied to get curves of the types of Figs. 2(f)–2(l) are more stringent than for the simpler ones of Figs. 2(b)–2(e), so that these forms were only observed in about 10–15% of cases in the random-number simulation, and those with a detectable change in sign of apparent co-operativity were particularly rare.

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APPENDIX 1

Sign of Apparent Substrate Co-operativity

The steady-state equation for the mechanism of Fig. 1 (main paper; Whitehead & Egmond, 1979) when $k_{cat,1} = k_{cat,2}$, is a rather complicated ratio of two quadratics containing 30 different terms, but can be written (Whitehead, 1976) in the slightly more convenient form:

$$\frac{V_m}{v} = \frac{1}{\bar{X}} = 1 + \frac{k'_{-1}(1 + k_{+2}y/k_{-2})}{k_{+1}x(1 + k_{+3}y/k_{-3})} + \frac{k'_{cat,k_{+3}y\{(k_{-2} + k_{+2}y)[1 + k'_{-1}/(k_{-3} + k_{+3}y)] + k_{+1}x\}}}{x[(1 + k_{+3}y/k_{-3})(k_{+1}k_{+4}k_{-3}x + k_{+2}k_{+4}k_{-3}y + k_{+1}k_{-2}k_{-3} + k_{+1}k'_{-4}k_{-2}) + k'_{cat,k_{+1}k_{-2}}]} \quad (1)$$

where

$$k'_{-1} = (k_{-1} + k_{cat}), \quad k'_{-4} = k_{-4}(1 + k_{cat}/k_{-1}), \\ k'_{cat} = k_{cat}(1 - k_{-4}/k_{-1}) \quad (1a)$$

A convenience of this form is that only the last term, of form $(A+Bx)/x(C+Dx)$, contributes to apparent co-operativity. The sign of apparent co-operativity in a mechanism giving a two-degree equation (though not necessarily in others (Whitehead, 1978; Bardsley & Waight, 1978) has the same sign as second derivative in a double-reciprocal plot $d^2(V/v)/d(1/x)^2$, that is the same sign as $(AD-BC)$.

Applying this to eqn. (1) apparent substrate co-operativity has the same sign as:

$$k'_{\text{cat.}}[k_{+1}k_{-3} + k'_{-4}k_{+1} + k'_{\text{cat.}}k_{+1} - k_{+4}k_{-3} - k_{+4}k'_{-1} + k_{+3}y(k_{+1} - k_{+4})] \quad (2)$$

or, substituting for k'_{-1} , k'_{-4} and $k'_{\text{cat.}}$, as:

$$(k_{-1} - k_{-4})[k_{+1}(k_{-1} - k_{-4}) + (k_{+4} - k_{+1})(k_{-1} + k_{-3} + k_{\text{cat.}} + k_{+3}y)] \quad (3)$$

or

$$(k_{-1} - k_{-4})[(k_{+4}k_{-1} - k_{+1}k_{-4}) + (k_{+4} - k_{+1})(k_{-3} + k_{+3}y + k_{\text{cat.}})] \quad (4)$$

A sufficient and necessary condition for any negative apparent co-operativity (either $X-$ or $X+-$) is that $(k_{-1} - k_{-4})$ and $(k_{+4} - k_{+1})$ be of opposite signs; for positive apparent co-operativity, all y ($X+$) it is both necessary and sufficient that they be of the same sign. The form (eqn. 3) makes it clear that co-operativity cannot be negative at low y and positive at high ($X+-$) for positive apparent co-operativity at high y requires $(k_{-1} - k_{-4})(k_{+4} - k_{+1}) > 0$ whereupon expression (3) is always positive.

Katsumata (1972) has obtained from the full unsimplified equation for this mechanism a formula which proves on examination identical, but it contains an unnecessary factor $(k_{-3} + k_{+3}y)$.

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APPENDIX 2

Extrema

Obviously when $k_{\text{cat. } 1} = k_{\text{cat. } 2}$ there can be no excess substrate inhibition, since velocity at substrate saturation is always $k_{\text{cat.}}[E_T]$, which is the maximum attainable in the system.

For the purpose of analysing maxima as a function of y it is convenient instead of using eqn. (1) (Appendix 1) to recall that it is derived (Whitehead, 1976) by using:

$$\frac{1}{\bar{X}} = 1 + \frac{\text{'EX}_{\text{eq.}}' \cdot (1 + k_{+2}y/k_{-2})k'_{-1}/k_{+1}x + k'_{\text{cat.}}[(k_{-2} + k_{+2}y)(k'_{-1} + k_{+3}y) + k_{+1}k_{+3}xy]}{\text{'EX}_{\text{eq.}}' \cdot (1 + k_{+3}y/k_{-3}) + k'_{\text{cat.}}k_{+1}k_{-2}x} \quad (1)$$

where 'EX_{eq.}' represents a series of King-Altman terms of form $(C_1 + C_2y)x$, where:

$$C_1 = k_{+1}k_{+4}k_{-3}x + k_{+1}k_{-2}k_{-3} + k_{+1}k_{-2}k'_{-4} \\ C_2 = k_{+2}k_{+4}k_{-3}$$

Thus:

$$\frac{1}{\bar{X}} = 1 + \frac{(C_1 + C_2y)(1 + k_{+2}y/k_{-2})K'_1 + k'_{\text{cat.}}[(k_{-2} + k_{+2}y)(k'_{-1} + k_{+3}y) + k_{+1}k_{+3}xy]}{x[(C_1 + C_2y)(1 + k_{+3}y/k_{-3}) + k'_{\text{cat.}}k_{+1}k_{-2}]} \quad (2)$$

where $K'_1 = (k_{-1} + k_{\text{cat.}})/k_{+1} = k'_{-1}/k_{+1}$. This may be written in the form:

$$\frac{V_m}{v} = \frac{1}{\bar{X}} = 1 + \frac{a_0 + a_1y + a_2y^2}{b_0 + b_1y + b_2y^2} \quad (3) \\ a_0 = C_1K'_1 + k'_{\text{cat.}}k_{-2}k'_{-1} \quad b_0 = a_0x/K'_1 \quad \text{etc.}$$

Now, from eqn. (3):

$$\frac{\partial(1/\bar{X})}{\partial y} = \frac{(a_1b_0 - a_0b_1) + 2(a_2b_0 - a_0b_2)y + (a_2b_1 - a_1b_2)y^2}{(b_0 + b_1y + b_2y^2)^2} \quad (4)$$

The coefficients in the numerator of eqn. (4) are given in terms of rate constants and x below (eqns. 5). For $\partial(1/\bar{X})/\partial y = 0$ to have a positive root it is necessary and sufficient that one of the coefficients in the numerator be

of opposite sign to the other two [the coefficient of y^1 always has the same sign as that of y^0 or y^2 and eqn. (3) cannot give more than one extremum (Botts, 1955)]. It is easy to see from eqns. (5) that this requires $(k_{+1}k_{-4} - k_{+4}k_{-1})$ and $(k_{+1} - k_{+4})$ to be of opposite signs, and $k_{\text{cat.}}$ to be in a range that includes that given by $\gamma_1 = 0$ [i.e. eqn. (3) (main paper, Whitehead & Egmond, 1979)]. The condition given in the text of the main paper as sufficient for an extremum is thus also necessary.

$$\left. \begin{aligned} (a_2b_1 - a_1b_2) &= \gamma_2 k_{+3}^2 k_{+2} k_{-2} k_{-4} x / k_{-1} \\ (a_2b_0 - a_0b_2) &= \gamma_1 a_0 x k_{+2} k_{+3} / k_{-1}' \\ (a_1b_0 - a_0b_1) &= \gamma_0 a_0 x (k_{+1} k_{+3} x + k_{+2} k_{-1}' + k_{+3} k_{-2} k_{-1} / k_{-4}) \end{aligned} \right\} \quad (5)$$

where:

$$\begin{aligned} \gamma_2 &= (k_{+1}k_{-4} - k_{+4}k_{-1}) + k_{\text{cat.}} \frac{k_{-1}}{k_{-4}} (k_{+1} - k_{+4}) \\ \gamma_1 &= (k_{+1}k_{-4} - k_{+4}k_{-1}) + k_{\text{cat.}} (k_{+1} - k_{+4}) \\ \gamma_0 &= (k_{+1}k_{-4} - k_{+4}k_{-1}) + \frac{k_{\text{cat.}} (k_{+1}k_{+3}x + k_{+2}k_{-1}' + k_{+2}k_{-3})(k_{+1} - k_{+4})}{(k_{+1}k_{+3}x + k_{+2}k_{-1}' + k_{+2}k_{-3}k_{-1}/k_{-4})} \end{aligned}$$

The extremum must be a maximum. An extremum, we have just seen, requires $(k_{+1}k_{-4} - k_{+4}k_{-1})$ and $(k_{+1} - k_{+4})$ to be of opposite sign, whence $(k_{+1} - k_{+4})$ must be of the same sign as $(k_{-1} - k_{-4})$.

Comparing the coefficients of $(k_{+1} - k_{+4})$ in γ_2 and γ_0 respectively $k_{\text{cat.}} k_{-1}/k_{-4}$ and an expression of form $k_{\text{cat.}}(E + F)/(E + Fk_{-1}/k_{-4})$, E, F positive, it is easy to see that this implies algebraically, $\gamma_2 > \gamma_0$. Since γ_2 and γ_0 must be of opposite signs, the coefficient of y^2 is positive and that of y^0 negative, which implies a maximum in $\bar{X}$ as a function of y , by eqn. (4).

Comparison of the conditions for a maximum just stated with the formulae (3 and 4 of Appendix 1) for the sign of apparent substrate co-operativity shows that such a maximum is compatible with the $X-$ pattern and either $K_1' > K_4'$ or $K_1' < K_4'$ [i.e. behaviour as in Figs. 2(i)–2(l) (main paper, Whitehead & Egmond, 1979)]. The maximum is compatible with the $X+-$ pattern only if $K_1' > K_4'$, so the behaviour shown in Fig. 2(j) is the only permitted one with an excursion out of the (K_1', K_4') range and a change in the sign of apparent substrate co-operativity. The proof that $X+-$ and a maximum are incompatible with $K_1' < K_4'$ follows.

Since for the $X+-$ pattern, from eqn. (4) (Appendix 1):

$$(k_{+1}k_{-4} - k_{+4}k_{-1}) + (k_{+1} - k_{+4})(k_{\text{cat.}} + k_{-3}) \quad (6)$$

must have the same sign as $(k_{+1}k_{-4} - k_{+4}k_{-1})$, γ_1 must certainly also have this sign. $K_1' < K_4'$ is identical with $\gamma_1 > 0$, therefore $(k_{+1}k_{-4} - k_{+4}k_{-1}) > 0$ and $(k_{+1} - k_{+4}) < 0$. The expression (6) must consequently be positive; this can be written:

$$\gamma_1 > k_{-3}(k_{+4} - k_{+1}) \quad (6)$$

Now there can only be a maximum if $\gamma_0 < 0$; this condition can be written, expressing γ_0 in terms of γ_1 by eqn. (5):

$$\gamma_0 = \gamma_1 + k_{\text{cat.}}(k_{+1} - k_{+4})k_{+2}k_{-3}(1 - k_{-1}/k_{-4}) < 0 \quad (7)$$

Inserting eqn. (6) into the expression in eqn. (7) we find the latter to be greater than:

$$k_{-3}(k_{+4} - k_{+1})k_{-1}/k_{-4}$$

We have already seen that $(k_{+4} - k_{+1})$ must be positive, and therefore the inequality in (7) cannot be satisfied. Hence there can be no maximum when apparent co-operativity changes sign and $K_1' < K_4'$.

Combining the facts that: (i) $X+$ is incompatible with an excursion, (ii) $X+-$ is compatible with an excursion only when $K_4' < K_1'$, but then since apparent co-operativity changes sign where $K_1' > x_1 > K_4'$ (Appendix 3), i.e. before the region of the excursion is reached, we see, by exclusion of other possibilities, that apparent co-operativity is always negative in the excursion range.

References

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APPENDIX 3

Proof that at the Modifier Concentration where Apparent Substrate Co-operativity Changes Sign (Vanishes) the Michaelis Constant lies between those at Zero and Infinite y Concentration (K'_1 and K'_4)

This particular value of y is that which gives:

$$(AD - BC) = 0 \quad (1)$$

A, B, C, D being the coefficients defined in Appendix 1 and the square-bracketed expression in eqn. (3) or (4) (Appendix 1) is zero. Under this condition the last term of eqn. (1) (Appendix 1):

$$(A + Bx)/x(C + Dx) = B/Dx = \frac{k_{\text{cat.}}(1 - k_{-4}/k_{-1})k_{+1}k_{+3}y}{(1 + k_{+3}y/k_{-3})k_{+1}k_{+4}k_{-3}x}$$

Eqn. (1) (Appendix 1) then reduces to the Michaelis equation, with Michaelis constant equal to:

$$\begin{aligned} & \frac{(k_{-1} + k_{\text{cat.}})(1 + k_{+2}y/k_{-2}) + k_{\text{cat.}}(1 - k_{-4}/k_{-1})k_{+1}k_{+3}y/k_{+4}k_{-3}}{k_{+1}(1 + k_{+3}y/k_{-3})} \\ &= \frac{K'_1 + K'_4 k_{+3}y/k_{-3}}{(1 + k_{+3}y/k_{-3})} \end{aligned} \quad (2)$$

which, it is easily seen, must lie between K'_1 and K'_4 , the Briggs-Haldane constants defined in eqn. (2) of the main paper. In terms of rate constants only, this Michaelis constant is equal to:

$$K'_4 + k_{-3}(1/k_{+4} - 1/k_{+1})$$

This is calculated by using eqn. (2) and equating the square-bracketed expression in formula (4) (Appendix 1) to zero.

Interestingly, formula (2) for K_m is the same as that which always holds in the case of a non-random mechanism when $k_{-2} = k_{+2} = 0$.