Simplifications of the Derivations and Forms of Steady-State Equations for Non-Equilibrium Random Substrate-Modifier and Allosteric Enzyme Mechanisms

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The steady-state equations for 'random' enzymic mechanisms (ones with alternative routes for substrate and enzyme to form enzyme-substrate complexes) are non-Michaelian and very complicated when a quasi-equilibrium approximation cannot be used. General methods for simplifying their forms and derivations are given and applied to several single-substrate mechanisms of general or topical interest. The special simplifications resulting from partial ordering of reaction mechanism, from gross inequalities of rate constants, and from special relationships between catalytic and dissociation rate constants, are considered with reference to allosteric mechanisms. Some equations mentioned, but not given here, and more detailed working out of some of those given, have been deposited as Supplementary Publication SUP 50069 (18 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms given in *Biochem. J.* (1976) 153, 5.

The steady-state rate equations for model enzymic mechanisms with alternative pathways to enzyme-substrate complexes (e.g. those of Fig. 1) and catalytic constants high enough to render a quasi-equilibrium approximation inapplicable are non-Michaelian, complicated, and tedious to derive or manipulate. In this paper I discuss some general methods for simplifying their derivations and forms, and certain special assumptions that simplify equations for models whose general treatment remains too complicated.

The general simplification of form and derivation comes about by using the information contained in the principle of microscopic reversibility. Equations written without using this information are unnecessarily complicated because they are unnecessarily general; they would remain true even if the principle were not. The general method is explained below by its application to a specific model.

Theory

General method applied to scheme (a) of Fig. 1

This model, in which substrate X and 'inhibitor' Y combine with enzyme to form the complexes EX, EY and EXY, with only EX catalytically active, corresponds in quasi-equilibrium kinetics to a mixed partial competitive-pure non-competitive inhibition (in the non-equilibrium case it can also give rise to activation by Y).

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The present investigations concern the case where catalytic rate constants (k_{+5} in this case) are large enough for the enzyme species not to be near equilibrium with each other and with substrates and modifiers.

The steady-state velocity equation is derived in the commonly used reciprocal form.

Step (1), the equation relating the maximum velocity of which the system is capable, v_m , divided by velocity v , to concentrations of enzyme species is written, in this case:

$$\frac{v_m}{v} = 1 + \frac{[E] + [EY] + [EXY]}{[EX]} \quad (1)$$

which equals:

$$1 + \frac{'E' + 'EY' + 'EXY'}{'EX'} \quad (2)$$

where 'E' etc. denote the products of rate constants in trees directed towards E etc. according to the rules of King & Altman (1956) (see Supplementary Publication SUP 50069 for a definition of directed trees).

Step (2), instead of writing out 'E' etc. in full, we note that each such expression (except for 'EX') contains some products that contain the catalytic step k_{+5} , and others that do not. Now the sum of the terms not containing k_{+5} are those we would obtain if treating the equilibrium of Fig. 2.

Fig. 2. Equilibrium part of scheme (a) of Fig. 1

The expressions for 'E' etc. in scheme (a) of Fig. 1 written by the method of King & Altman (1956) are the same as those for the equilibrium illustrated here (denoted 'E'_{eq.} etc.) plus further terms involving k_{+5} (see eqn. 3 and the text.)

We then denote these terms corresponding to equilibrium 'E'_{eq.} etc. Then:

$$\left. \begin{aligned}
 'EX' &= 'EX'_{eq.} \\
 'E' &= 'E'_{eq.} + k_{+5}(k_{-2}k_{-4} + k_{-2}k_{-3} + k_{+4}k_{-3}x) \\
 'EY' &= 'EY'_{eq.} + k_{+5}(k_{+2}k_{-4} + k_{+2}k_{-3})y \\
 'EXY' &= 'EXY'_{eq.} + k_{+5}k_{+2}k_{+4}xy
 \end{aligned} \right\} \quad (3)$$

where x and y are concentrations of X and Y respectively. Thus eqn. (2) becomes:

$$\frac{v_m}{v} = 1 + \frac{'E'_{eq.} + 'EY'_{eq.} + 'EXY'_{eq.} + k_{+5}[k_{-2}(k_{-3} + k_{-4}) + k_{+4}k_{-3}x + k_{+2}(k_{-3} + k_{-4})y + k_{+2}k_{+4}xy]}{'EX'_{eq.}} \quad (4)$$

Step (3), but without needing to write 'E'_{eq.} etc. explicitly, we know the equilibrium ratios 'E'_{eq.}/'EX'_{eq.}, 'EY'_{eq.}/'EX'_{eq.}, and 'EXY'_{eq.}/'EX'_{eq.} to be respectively $k_{-1}/k_{+1}x$, $k_{-1}k_{+2}y/k_{+1}k_{-2}x$ and $k_{+3}y/k_{-3}$. (This is true by virtue of the principle of microscopic reversibility.) By using this and writing out 'EX'_{eq.}, eqn. (4) becomes:

$$\begin{aligned}
 v_m/v &= 1 + k_{-1}/k_{+1}x + k_{-1}k_{+2}y/k_{+1}k_{-2}x + k_{+3}y/k_{-3} \\
 &\quad + \frac{k_{+5}[k_{-2}(k_{-3} + k_{-4}) + k_{+4}k_{-3}x + k_{+2}(k_{-3} + k_{-4})y + k_{+2}k_{+4}xy]}{x[k_{+1}k_{-2}(k_{-3} + k_{-4}) + k_{+4}k_{-3}(k_{+1}x + k_{+2}y)]} \quad (5)
 \end{aligned}$$

When the method is already known, eqn. (5) can be written immediately on inspection of the mechanism. The last term can be slightly simplified and the equation written in a form that echoes a Briggs-Haldane (1925) expression (eqn. A1, Table 1).

This is a simpler and for many purposes more practical form than the 22-term expression obtained

by writing out all the King-Altman terms ('kappa products') individually, and is derived with a sparing of effort. Apparent homotropic co-operativity depends on the last term only, a fact that eases its analysis.

The general principle used here is the familiar one that when a scheme contains a cycle or a set of cycles, no matter how complicated, to which the principle of microscopic reversibility applies, then the ratio of the King-Altman expression of any two species in the cycles is equal to the ratio of the products of the constants for the steps along any pathway between the two species to the corresponding steps in the opposite direction.

Applications and extensions of the method in the treatment of other mechanisms

The principles just explained have been applied to the mechanisms of schemes (a)-(g) of Fig. 1. These all involve only one substrate, X , and either a reversible modifier, Y , or a change in configuration. All have some general or topical importance in enzyme-kinetic theory.

A separate discussion of most of these mechanisms is required because considering the properties of each scheme gives opportunities to economize effort and arrive at more compact expressions while avoiding algebraic manipulations than if the prescriptions above are followed rigidly. The further ideas used in

the simplifications, and their results, are outlined below, but for more detail of both see Supplementary Publication SUP 50069.

Approximations

Even with the simplifying approaches used here, general treatments and equations of the allosteric

schemes (h) and (i) (Fig. 1) remain too complicated. In the standard treatment their equations would contain several thousand King-Altman product terms. I have therefore discussed how tractable equations can be obtained when special assumptions other than the quasi-equilibrium assumption are made.

Table 1. Equations given by the schemes of Fig. 1

Scheme (a):

$$\frac{v_m}{v} = 1 + \frac{k_{-1}}{k_{+1}x} + \frac{k_{-1}k_{+2}y}{k_{+1}k_{-2}x} + \frac{k_{+3}y}{k_{-3}} + \frac{k_{+5}}{k_{+1}x} + \frac{k_{+5}k_{+2}y(k_{-3} + k_{-4} + k_{+4}x - k_{+4}k_{-3}/k_{+1})}{x[k_{+1}k_{-2}(k_{-3} + k_{-4}) + k_{+4}k_{-3}(k_{+1}x + k_{+2}y)]} \quad (A1)$$

Scheme (b):

$$\frac{v_m}{v} = 1 + \frac{k_{-4}}{k_{+4}x} + \frac{k_{-2}k_{-4}}{k_{+2}k_{+4}xy} + \frac{k_{-3}}{k_{+3}y} + \frac{k_{+6}}{k_{+4}x} + \frac{k_{+6}k_{-2}(k_{+3}y + k_{-1} + k_{+1}x - k_{+1}k_{+3}y/k_{+4})}{xy[k_{+4}k_{+2}(k_{+3}y + k_{-1}) + k_{+1}k_{+3}(k_{+4}x + k_{-2})]} \quad (A2)$$

Scheme (c), fractional binding $\bar{X}$ of X is given by:

$$\frac{1}{\bar{X}} = 1 + \frac{k'_{-1}(1 + k_{+2}y/k_{-2})}{k_{+1}x(1 + k_{+3}y/k_{-3})} + \frac{k'_{+6}k_{+3}y\{(k_{-2} + k_{+2}y)[1 + k'_{-1}/(k_{-3} + k_{+3}y)] + k_{+1}x\}}{x[(1 + k_{+3}y/k_{-3})(k_{+1}k_{+4}k_{-3}x + k_{+2}k_{+4}k_{-3}y + k_{+1}k_{-2}k_{-3} + k_{+1}k'_{-4}k_{-2}) + k'_{+6}k_{+1}k_{-2}]} \quad (A3i)$$

when $k_{+5} = k_{+6}$ this is equal to v_m/v and to:

$$1 + \frac{(k_{-1} + k_{+5})(1 + k_{+2}y/k_{-2})}{k_{+1}x(1 + k_{+3}y/k_{-3})} + \frac{k_{+5}k_{+3}y(1 - k_{-4}/k_{-1})\{(k_{-2} + k_{+2}y)[1 + (k_{-1} + k_{+5})/(k_{-3} + k_{+3}y)] + k_{+1}x\}}{x[(1 + k_{+3}y/k_{-3})(k_{+1}k_{+4}k_{-3}x + k_{+2}k_{+4}k_{-3}y + k_{+1}k_{-2}k_{-3} + k_{+1}k_{-2}k_{-4}) + k_{+5}(k_{+2}k_{+4}y + k_{+1}k_{-2})]} \quad (A3ii)$$

Scheme (d):

$$\frac{v}{v_s} = \frac{k'_{+1}A_1x(1 + Ax)^3}{4k'_{+4}\left(1 + \sum_{i=1}^4 A_i x^i\right)} \quad (A4)$$

$$A_1 = 4k_{+1}/(k_{-1} + k'_{+1}),$$

$$A_2 = A_1[k_{+2}/(k_{-2} + k'_{+2}) + k_{+4}/2(k_{-4} + k'_{+4})]$$

$$A_3 = A_1k_{+2}k_{+3}/(k_{-2} + k'_{+2})(k_{-3} + k'_{+3}) = A_1k_{+4}k_{+5}/(k_{-4} + k'_{+4})(k_{-5} + k'_{+5})$$

$$A_4 = A_3k_{+6}/4(k_{-6} + k'_{+6})$$

$$\text{and } A = k_{+2}/(k_{-1} + k'_{+1})$$

Scheme (e):

$$\frac{v_m}{v} = 1 + \frac{(k_{-1}/k_{+1} + k_{-3}/k_{+3})}{x[1 + k/(k' + k_{+2})]} + \frac{k_{+2}(k_{+4} + k_{-4} + k_{+1}x)}{x[1 + (k' + k_{+2})/k](k_{+1}k_{+4} + k_{+3}k_{-4} + k_{+1}k_{+3}x)} \quad (A5)$$

Scheme (f):

$$\frac{v_m}{v} = 1 + \frac{k_{+3}k_{-4}/k_{-3}k_{+4}x + (k_{-1} + k_{+5})[1 + k_{+2}(k_{-4} + k_{+4}x + k_{-3})/k_{-3}k_{+4}x]/(k_{+2} + k_{+1}x)}{(1 + k_{+3}/k_{-3})} \quad (A6)$$

Scheme (h), when eqn. (14) holds and $k_{+2} = k_{-2} = k_{+8} = k_{-8} = 0$:

$$\frac{v}{E_T} = \frac{x[k_{+5}(1 + k_{+13}y/k_{-13}) + k_{+6}k_{+3}(1 + k_{+15}y/k_{-15})/k_{-3}]}{[x + (k_{-1} + k_{+5})/k_{+1}][1 + k_{+13}y/k_{-13}] + k_{+3}[x + (k_{-4} + k_{+6})/k_{+4}][1 + k_{+15}y/k_{-15})/k_{-3}]} \quad (A7)$$

Scheme (i), when all configurational transitions allowed are rapid:

$$\bar{X} = \frac{\sum_{i=1}^q ix^i \binom{q}{i} \frac{(1 + Lc^i)}{(1 + L)} \prod_{j=1}^i \frac{(k_{+1} + k_{+4}Lc^{j-1})}{[(k_{-1} + k_{+5}) + (k_{-4} + k_{+6})Lc^j]}}{q \left\{ 1 + \sum_{i=1}^q x^i \binom{q}{i} \frac{(1 + Lc^i)}{(1 + L)} \prod_{j=1}^i \frac{(k_{+1} + k_{+4}Lc^{j-1})}{[(k_{-1} + k_{+5}) + (k_{-4} + k_{+6})Lc^j]} \right\}} \quad (A8i)$$

$$\frac{v}{v_m} = \frac{\sum_{i=1}^q ix^i \binom{q}{i} \frac{(k_{+5} + k_{+6}Lc^i)}{(1 + L)} \prod_{j=1}^i \frac{(k_{+1} + k_{+4}Lc^{j-1})}{[(k_{-1} + k_{+5}) + (k_{-4} + k_{+6})Lc^j]}}{q \frac{(k_{+5} + k_{+6}Lc^q)}{(1 + Lc^q)} \left\{ 1 + \sum_{i=1}^q x^i \binom{q}{i} \frac{(1 + Lc^i)}{(1 + L)} \prod_{j=1}^i \frac{(k_{+1} + k_{+4}Lc^{j-1})}{[(k_{-1} + k_{+5}) + (k_{-4} + k_{+6})Lc^j]} \right\}} \quad (A8ii)$$

where $c = k_{+4}k_{-1}/k_{-4}k_{+1}$, L is the equilibrium constant: $L = [TX_0]/[RX_0]$

Fig. 3. Scheme giving relative concentrations of E, EX, EY, and EXY identical with those given by scheme (c) of Fig. 1

The rate constants k_{-1}' , k_{-4}' and k_{+6}' are defined so as to satisfy eqns. (6) and (7); the remaining rate constants are equal to the corresponding ones in scheme (c) of Fig. 1.

Scheme (b) of Fig. 1: obligatory activation

By symmetry with scheme (a), eqn. (A2) can be immediately written.

Scheme (c) of Fig. 1: both enzyme-substrate complexes catalytically active

If we mechanically applied the method illustrated above we should need to write 14 non-equilibrium King-Altman (1956) products. Instead, we use the fact that as far as the concentrations of enzyme species are concerned, scheme (c) (Fig. 1) is identical with that of Fig. 3 in which the newly defined constants k_{-1}' and k_{-4}' are subject to a formal law of microscopic reversibility:

$$k_{-1}'k_{-3}k_{+2}k_{+4} = k_{-4}'k_{-2}k_{+1}k_{+3} \quad (6)$$

and there is formally only one non-equilibrium catalytic constant, k_{+6}' , so we need to write only six 'non-equilibrium' terms. We define:

$$k_{-1}' = k_{-1} + k_{+5}, \quad k_{-4}' + k_{+6}' = k_{-4} + k_{+6} \quad (7)$$

so that the steady-state is identical with that in scheme (c) (Fig. 1), and eqns. (A3) and others (see Supplementary Publication SUP 50069) may be obtained, which replace equations containing 30 King-Altman products. Eqns. (6) and (7) together determine k_{+4}' and k_{+6}' :

$$k_{+6}' = k_{+6} - k_{+5}k_{-4}/k_{-1} \quad (8)$$

Generalized microscopic reversibility

Note that if in scheme (c) (Fig. 1)

$$k_{+6}/k_{+5} = k_{-4}/k_{-1} \quad (9)$$

from eqn. (8), $k_{+6}' = 0$. There is no formal non-equilibrium term and the overall form of kinetics is

Vol. 159

Fig. 4. Scheme in which the concentrations of the various enzyme species are identical with those in scheme (c) (Fig. 1) according to the principles of Volkenstein & Goldstein (1966)

Rate constants of value $(k_{-1}+k_{+5})$ and $(k_{-4}+k_{+6})$ are assigned to the processes $EX \rightarrow E$ and $EXY \rightarrow EY$.

identical with that of a quasi-equilibrium scheme. This fact was noted by Botts & Morales (1955), Hearon *et al.* (1959) and Frieden (1964) and becomes obvious without needing algebraic development if we consider that for the purpose of deriving expressions for 'E' etc., scheme (c) is equivalent to that of Fig. 4, where the processes $EX \rightarrow E$, $EXY \rightarrow EY$ are characterized by single rate constants of value $(k_{-1}+k_{+5})$, $(k_{-4}+k_{+6})$ (Volkenstein & Goldstein, 1966). This scheme of Fig. 4 will behave like an equilibrium if the ratio of these Volkenstein-Goldstein rate constants is the same as that of the corresponding equilibrium rate constants k_{-1} and k_{-4} , i.e. eqn. (9). Then and only then do the rate constants of Fig. 4 formally obey the mathematical law that characterizes equilibria, that of microscopic reversibility, namely:

$$(k_{-1}+k_{+5})k_{-3}k_{+2}k_{+4} = (k_{-4}+k_{+6})k_{-2}k_{+1}k_{+3}$$

which is also equivalent to eqn. (9). We shall call such a condition 'generalized microscopic reversibility'.

An application of generalized microscopic reversibility to the oligomeric models of Ricard *et al.* (1974a) and Nari *et al.* (1974)

I suggested (Whitehead, 1970) that it might not be physically absurd that relations like eqn. (9) sometimes hold, at least approximately. A somewhat similar idea was used by Ricard *et al.* (1974a) and Nari *et al.* (1974) in their 'structural kinetic' models for possible steady-state kinetics of oligomeric allosteric enzymes. They make assumptions that result in every substrate dissociation of rate constant k_{-a} being paralleled by a catalytic pathway (see, e.g., scheme d of Fig. 1) of rate constant k_{+a}' such that:

$$k_{+a}'/k_{-a}' = k_{-a}/k_{-a} \quad (10)$$

for all α, β .

But this means that generalized microscopic reversibility applies round the square of their version

of the Koshland–Neméthy–Filmer (1966) ‘square’ model of interaction (scheme *d*, Fig. 1), which can therefore be treated like a quasi-equilibrium giving a fourth-degree generalized Adair equation:

$$\frac{v}{v_s} = \frac{\sum_{i=1}^4 i k'_i A_i x^i}{4k'_4 (1 + \sum_{i=1}^4 A_i x^i)} \quad (11)$$

where v_s is the velocity at saturation by X (not necessarily a maximum velocity), k'_i are rate constants for catalytic decomposition in the enzyme complex with i molecules of substrate (EX_i), and A_i are products of Briggs–Haldane constants (in the steady-state $[EX_i] = [EX_0] A_i x^i$). The A_i in terms of the constants of scheme (*d*) (Fig. 1) are given in Table 1 together with eqn. (A4), to which eqn. (11) simplifies because it is a consequence of the assumptions of the same workers, that $k_{+1}/k_{-(1-1)}$ is the same for all i values. Ricard *et al.* (1974a, eqn. 25) give instead a complicated fifth-degree equation.

The same assumptions can be used to simplify the fifth- and tenth-degree equations for dimer and tetramer models mentioned by Nari *et al.* (1974, eqns. 14 and 15) to forms similar to eqns. (11) and (A4) of the second and fourth degrees (in both substrate and inhibitor concentrations) respectively.

I differ from these workers in regarding relations like eqns. (9) and (10) as interesting hypotheses to consider, but not as necessary deductions from any firmly established physical principles.

Schemes (e) and (f) of Fig. 1; the monomeric models of Ricard et al. (1974b) and Rabin (1967)

It is perhaps curious that scheme (*e*) of Fig. 1 should have been proposed (Ricard *et al.*, 1974b; Meunier *et al.*, 1974) as a possible model for apparent co-operativity in a monomer by the same group that put forward the ideas of so-called ‘structural kinetics’ mentioned in the last section, since if formulated in accord with these ideas, which they seemed to think had universal validity, there could be no contribution of non-equilibrium to apparent co-operativity in this or any other configurational model, because generalized microscopic reversibility would always hold.

Anyway, whenever a species, such as EP in scheme (*e*) (Fig. 1), is connected reversibly to only one other, a node compression can be effected. Scheme (*e*) of Fig. 1 can be shown to be equivalent to that of Fig. 5, where

$$[EP'] = [E_s X] + [EP] = [E_s X][1 + k/(k' + k_{+2})] \\ = [EP][1 + (k' + k_{+2})/k] \quad (12)$$

$$\left. \begin{aligned} k'_{-1} &= k_{-1}/[1 + k/(k' + k_{+2})] \\ k'_{-3} &= k_{-3}/[1 + k/(k' + k_{+2})] \\ k'_{+2} &= k_{+2}/[1 + (k' + k_{+2})/k] \end{aligned} \right\} \quad (13)$$

Formal microscopic reversibility holds in the ‘non-catalytic’ cycle of Fig. 5, hence the general method is easily applied to give eqn. (A5), which is distinctly simpler than that of Ricard *et al.* (1974b). Alternatively the same result can be economically obtained by a graph-theory argument given in Supplementary Publication SUP 50069.

Apparent substrate co-operativity has the same sign as $d^2(v_m/v)/d(1/x)^2$, to which only the last term of eqn. (A5) of form $(A+Bx)/x(C+Dx)$ contributes. It therefore has the same sign as $(AD-BC)$, so we easily reach the result of Ricard *et al.* (1974b) that apparent substrate co-operativity is positive or negative according as $k_{+3} \gtrless k_{+1}$.

Scheme (*f*) of Fig. 1 was proposed in a paper that was influential in stimulating interest in apparent co-operativity in non-equilibrium schemes (Rabin, 1967). By node compression we arrive at eqn. (A6).

A model with configurational transitions: scheme (g) of Fig. 1

The equation for this simple model for a monomeric enzyme (or an oligomeric one without interactions between subunits) which can exist in two different forms, R and T, must obviously be identical with that for scheme (*c*) (Fig. 1), except that the y values are now omitted. When $k_{+5} = k_{+6}$ (an allosteric ‘K-system’) we get eqn. (A3) without the y values. When $k_{+6} = 0$ or $k_{+5} = 0$ the right-hand side of eqns. (A1) and (A2) with the y values omitted equal respectively $[E_T]/[RX]$ and $[E_T]/[TX]$; to get v_m/v we must divide by $(1+k_{+3}/k_{-3})$ and $(1+k_{-3}/k_{+3})$ respectively.

Special assumptions

In the rest of this paper I consider the less general simplifications given by special assumptions, and their applications to the models of schemes (*h*) and (*i*) of Fig. 1. These assumptions are partial ordering of

Fig. 5. A scheme equivalent to scheme (*e*) of Fig. 1

EP' here represents $(E_s X + EP)$ in scheme (*e*), k'_{-1} , k'_{+2} and k'_{-3} are given by eqns. (13); the remaining rate constants are equal to the corresponding ones in scheme (*e*).

the reaction mechanism and gross inequalities of the rate constants.

Scheme (h) of Fig. 1: the allosteric building block, a model with configurational transitions and an allosteric effector

When an allosteric modifier Y is added to scheme (g) (Fig. 1) we generate the basic building block of common schemes of allosteric interaction in oligomeric enzymes, scheme (h) of Fig. 1.

The number of different rate constants can be decreased by ten, because by the concept of configuration, if X and Y are bound at separated sites it is reasonable to assume:

$$\left. \begin{aligned} k_{+1} = k_{+7}, k_{-1} = k_{-7}, k_{+4} = k_{+10}, k_{-4} = k_{-10} \\ k_{+13} = k_{+14}, k_{-13} = k_{-14}, k_{+15} = k_{+16}, \\ k_{-15} = k_{-16} \\ k_{+5} = k_{+11}, k_{+6} = k_{+12} \end{aligned} \right\} \quad (14)$$

Even with this and the methods already considered the steady-state rate equation remains dauntingly complicated. It is therefore useful to recognize the following special cases, where it simplifies.

If configurational transition is possible only for species with X bound ($TX \rightleftharpoons RX$ and $TX Y \rightleftharpoons RXY$ allowed, $k_{+2} = k_{-2} = k_{+8} = k_{-8} = 0$, a partial ordering of the reaction mechanism) then because of eqns. (14) the scheme contains no squares that violate generalized microscopic reversibility; $[R]/[T]$ and $[RY]/[TY]$ retain their equilibrium values, and an equation (A7; Table 1) of Michaelian overall form results. An equation of this form (but different in detail) results if configurational transition is possible for only the pairs without X bound or for only one pair.

With regard to our second simplifying assumption, if the reactions of Y in schemes (a) (b) and (c) and the configurational transitions in scheme (g) of Fig. 1 are much faster than the reactions of X, the steady-state equations become Michaelian in x (see Supplementary Publication SUP 50069 and Segel, 1975), but still shows apparent homotropic interactions between Y, and likewise if the reactions of X are much faster than those of Y, curves of v against y , but not against x , become hyperbolic.

If all the configurational transitions of scheme (h) (Fig. 1) are allowed and sufficiently rapid, by applying the method of Cha (1968), the system is found (independently of eqns. 14) to give a steady-state equation identical with that for scheme (c) Fig. 1, including eqns. (A1), (A2) and (A3ii) for the special cases $k_{+6} = k_{+12} = 0$, $k_{+5} = k_{+11} = 0$ and $k_{+5} = k_{+6} = k_{+12} = k_{+11}$ respectively (see Supplementary Publication SUP 50069).

Finally, if the assumptions of Ricard *et al.* (1974a) held, we should have a series of Botts-Morales relationships: $k_{+5}/k_{-1} = k_{+6}/k_{-4} = k_{+11}/k_{-7} = k_{+12}/$

k_{-10} ; generalized microscopic reversibility (see above) would apply to all cycles and an equation of overall equilibrium form would result without need for any other special assumptions.

Oligomeric allosteric systems

Partial ordering of reaction mechanisms or gross inequalities of rate constants can simplify the enormously complicated equations for oligomeric allosteric models to quasi-equilibrium [Adair (1925) or generalized Adair (eqn. 11 above) of degree equal to the number of X-binding sites] form. The Michaelian forms for monomers, just considered, are the simplest case of this.

For example, if allosteric transitions are allowed for only unliganded or totally liganded oligomer in the Monod *et al.* (1965) model of scheme (i) (Fig. 1) the steady-state equations (see Supplementary Publication SUP 50069 and Dalziel, 1968) have the same overall form as in a quasi-equilibrium treatment. If the configurational transitions are all allowed and are sufficiently rapid, we get binding and velocity equations (eqn. A8, Table 1). In such cases the non-equilibrium processes affect successive apparent binding constants for X each to a different extent, so that apparent co-operativity is different from co-operativity at equilibrium.

The apparent co-operativity of CO binding to haemoglobin in the steady-state far from equilibrium (maintained through photodissociation) is no different from that at equilibrium (Brunori *et al.*, 1972). I have argued that this fact suggests an ordered mechanism of exclusive binding of CO to the haemoglobin subunits (Whitehead, 1974):

A different explanation by Szabo & Karplus (1973) works without a sequential mechanism being necessary by implicitly assuming generalized microscopic reversibility.

Discussion

I have shown above, by applications to some simple substrate-modifier and allosteric schemes, ways of using the principle of microscopic reversibility to simplify the forms and derivations of the steady-state equations for non-equilibrium 'random' mechanisms.

General mathematical analysis of the more complex allosteric models remains very difficult. I have therefore considered three assumptions likely to be significant in such models, which simplify their mathematics.

When certain configurational transitions are

forbidden (this is a partial ordering of the reaction mechanism), equations with an overall quasi-equilibrium form result. This also happens when Botts-Morales (1955) relations, which give rise to conditions that I have termed 'generalized microscopic reversibility', hold. When configurational transitions are sufficiently rapid, apparent homotropic interactions of substrate vanish in the 'square' mechanism of scheme (g) (Fig. 1) and its special subcases, whereas in the allosteric building block of scheme (h) of Fig. 1, the equation is greatly simplified, becoming identical with one for a 'square' model (e.g. schemes (a), (b) or (c) of Fig. 1); then, if reactions of one effector with the enzyme are much faster than those of the other, apparent homotropic interactions of the slowly reacting effector vanish. Possibilities of applying these assumptions to oligomeric allosteric models have been indicated.

The apparent homotropic interactions that are a feature of the steady-states of random non-equilibrium models have sometimes been thought important in biological control mechanisms [see Whitehead (1970) for review and references]. Simpler equations ease analysis of such effects, and have been exploited in analysis of apparent interactions in some schemes in this paper and also in random bisubstrate mechanisms (E. Whitehead, unpublished work).

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