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The reversible Hill equation: how to incorporate cooperative enzymes into metabolic models

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Abstract

Motivation: *Realistic simulation of the kinetic properties of metabolic pathways requires rate equations to be expressed in reversible form, because substrate and product elasticities are drastically different in reversible and irreversible reactions. This presents no special problem for reactions that follow reversible Michaelis–Menten kinetics, but for enzymes showing cooperative kinetics the full reversible rate equations are extremely complicated, and anyway in virtually all cases the full equations are unknown because sufficiently complete kinetic studies have not been carried out. There is a need, therefore, for approximate reversible equations that allow convenient simulation without violating thermodynamic constraints.*

Results: *We show how the irreversible Hill equation can be generalized to a reversible form, including effects of modifiers. The proposed equation leads to behaviour virtually indistinguishable from that predicted by a kinetic form of the Adair equation, despite the fact that the latter is a far more complicated equation. By contrast, a reversible form of the Monod–Wyman–Changeux equation that has sometimes been used leads to predictions for the effects of modifiers at high substrate concentration that differ qualitatively from those given by the Adair equation.*

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Introduction

In principle, enzymes with cooperative kinetics present no special problem for modelling the kinetic behaviour of metabolic pathways in the computer, because modern programs such as MetaModel (Cornish-Bowden and Hofmeyr, 1991), SCAMP (Sauro and Fell, 1991; Sauro, 1993) and Gepasi (Mendes, 1993) readily accept realistic reversible rate equations for the component enzymes. In practice, however, enzymes that show complex kinetic behaviour present serious difficulties, because hardly any enzymes exist for which an adequately complete rate equation has been determined experimentally. Even with the minority of enzymes for which extensive kinetic information exists, it is rare for this information to include much detail about the reverse reaction, or to include data for reasonably realistic physiological conditions, i.e. in the presence of all of the metabolites likely to affect the enzyme over a wide range of concentrations. One is normally forced to represent all but the simplest reactions by approximations to the actual rate equations.

The simplest reversible rate equation, for an enzyme catalysing an isomerization with Michaelis–Menten kinetics under all conditions and only competitive product inhibition, requires definition of four kinetic parameters: the forward and reverse limiting rates, and the forward and reverse Michaelis constants. Any complexity increases this number substantially: for example, the minimal reversible rate equation for a reaction that interconverts two substrates and two products by a compulsory-order ternary-complex mechanism contains two numerator terms and 11 denominator terms (Cornish-Bowden, 1995a), without allowing for any additional terms that might result from such plausible properties as binding of reactants to the wrong enzyme forms, etc. For an enzyme such as phosphofructokinase that shows cooperativity with respect to substrates and inhibitors, this complexity must inevitably increase very greatly.

It follows that even in the rare case where a reasonably complete rate equation is available, it may be very inconvenient to use it in its full form, because of the large number of constants that need to be defined, and probably also because of the computational burden that a complicated rate equation places on the modelling algorithm.

In practice, therefore, even quite sophisticated and modern models of metabolic systems commonly incorporate grossly oversimplified rate equations of some of the enzymes. For example, in their study of fermentation in suspended and immobilized yeast cells, Galazzo and Bailey (1992) treated several reactions as equilibria, and others, such as those catalysed by hexokinase and phosphofructokinase, as irreversible. Other workers, such as Thomas and Fell (1996), have used equations generalized from those of Monod *et al.* (1965) by analogy rather than by direct derivation. [Although Thomas and Fell (1996) regarded their equation as arbitrary, and described it explicitly as such, it can in fact be derived

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from the model of Popova and Sel'kov (1975) by assuming that substrate binds exclusively to the R form of the enzyme.] We choose these examples not because they were unusually skimpy but because, on the contrary, they were unusually thorough.

Although many cooperative enzymes have equilibrium constants sufficiently large for the contribution of the reverse reaction to be small, and perhaps negligible, we consider it dangerous to make a blanket assumption that all cooperative reactions can routinely be represented by irreversible rate equations. In general, it is dangerous to treat any reaction in a metabolic model as irreversible, apart from an exit reaction into a metabolic sink; some authors, such as Mendes *et al.* (1992), would not allow even this exception. Reversible and irreversible reactions behave in completely different ways, and affect the control properties of the system accordingly: most notably, substrate elasticities are always in the range 0–1 for reactions that follow irreversible Michaelis–Menten kinetics, whereas substrate elasticities in reactions close to equilibrium have *reciprocals* that are close to zero, and the elasticities themselves change sign at equilibrium via infinity, not via zero (see Cornish-Bowden, 1995b).

Popova and Sel'kov (1975, 1978) have generalized the model of Monod *et al.* (1965) to reversible one- and two-substrate reactions. However, the resulting equations are quite complicated, beyond the level that can be justified by experimental knowledge of the enzymes concerned. Thus, although we believe this to be a valuable step towards realistic treatment of reversible cooperative kinetics, we believe that a simpler approach, e.g. one based on the Hill equation, may be of more immediate practical use for computer modelling.

For irreversible reactions, it has long been known that the Hill equation often gives a remarkably good approximation to more realistic (and much more complex) models of cooperative behaviour, and many experimental cases of cooperativity have been characterized only in terms of the Hill equation. Indeed, during the period when there was the greatest interest in the mechanisms of kinetic cooperativity one of the theoretical challenges was to understand how an equation that lacked any mechanistic basis could describe so many experiments so precisely (Cornish-Bowden and Koshland, 1975). In general, the irreversible Hill equation requires just one extra parameter (the Hill exponent h) beyond what is required by the corresponding non-cooperative equation, and it provides a very attractive option for metabolic modelling, where the usual objetive is to represent the kinetic behaviour as precisely as possible without worrying particularly about the underlying mechanistic reason for the behaviour.

We are aware, of course, that the Hill equation must fail to give an accurate account of the behaviour at extreme

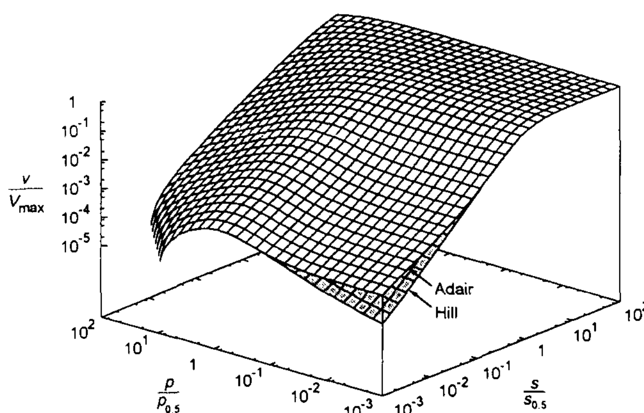

Fig. 1. A visual representation of the fit between the Adair equation and the generalized Hill equation derived in this paper. The two surfaces are essentially identical except in the region of low $s/s_{0.5}$ and $p/p_{0.5}$, where the difference is obvious in the figure. This difference is noticeable only when, as here, the equations are plotted in log–log space. In the region of low $s/s_{0.5}$ and high $p/p_{0.5}$, equilibrium is approached and the slope of the surface tends to infinity. The Adair equation is that of a reversible two-site model, equation (8) in the text, with $\beta = 0.01$ and $K_{eq} = 1000$. The parameters of the Hill equation, equation (12) in the text, are $h = 1.74$ (adjusted by hand to give a satisfactory visual fit) and $K_{eq} = 1000$.

concentrations, because the limiting slope of the Hill plot must be unity at both extremes (see Cornish-Bowden, 1995a). From the point of view of metabolic control analysis, the failure is more serious at low concentrations, because the substrate elasticity in an irreversible reaction must in reality approach unity at low concentrations, although the Hill equation predicts an elasticity that approaches h at low substrate concentrations (Figure 1). However, in practice, these faults are rendered unimportant by the fact that such reactions are usually studied over a central range, very few accurate measurements of enzyme reactions being made outside the middle 10–90% range of saturation where the Hill equation usually fits well. In metabolic simulations one may, of course, wish to study behaviour under more extreme conditions than those used for the kinetic studies of the isolated enzymes, but in this case one risks that the calculated behaviour will not reflect the true properties regardless of what equation is used, as with any extrapolation outside the range of measurement.

Unfortunately, however, no reversible form of the Hill equation has been described in the literature, as far as we are aware, and generalizing the usual irreversible equation to a reversible analogue is not trivial, because of thermodynamic constraints that must not be violated. In this paper, therefore, we describe a simple reversible analogue of the Hill equation that reduces to the ordinary equation when either the product or the substrate concentration is zero, and which incorporates a correct and consistent definition of equilibrium, i.e. a thermodynamic state that depends only on the substrate and product concentrations, and is independent of the kinetic properties of the catalyst.

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Basic theory

For any reaction that interconverts a substrate S and product P with concentrations s and p, respectively, the equilibrium constant K is defined as the ratio p/s at equilibrium, and the 'mass action ratio' Γ is defined as the same ratio p/s under any conditions, so that $\Gamma = K$ for a reaction at equilibrium. As a chemical reaction can only proceed towards equilibrium in the absence of external influences, the direction of reaction must be given by the sign of the function $(1 - \Gamma/K)$, and the rate equation ought to be expressible in the form:

$$v = \left(1 - \frac{\Gamma}{K}\right)\mathrm{Pos}(s, p, \ldots) \qquad (1)$$

where Pos is a non-negative function of the concentrations of S, P and any other species that affect the rate. If the rate equation is expressible in this form, the rate will be positive or zero if the reaction is on the substrate side of equilibrium, zero if it is at equilibrium, and negative or zero if it is on the product side of equilibrium.

As a simple example, the reversible Michaelis–Menten equation may be written as:

$$v = \frac{\dfrac{V_f s}{s_{0.5}} - \dfrac{V_r p}{p_{0.5}}}{1 + \dfrac{s}{s_{0.5}} + \dfrac{p}{p_{0.5}}} = \frac{V_f \sigma - V_r \pi}{1 + \sigma + \pi} \qquad (2)$$

where V_f and V_r are the limiting rates in the forward and reverse directions, respectively, and $s_{0.5}$ and $p_{0.5}$ are the forward and reverse Michaelis constants, respectively. (We prefer these symbols to the more usual K_m in this paper partly to emphasize that the Michaelis constant is the concentration at which the rate is half maximal, but also to allow use of the same symbols later for non-Michaelis–Menten kinetics.) The reduced form at the right, which is useful for simplifying much of the algebra, is obtained by defining $\sigma = s/s_{0.5}$ and $\pi = p/p_{0.5}$ as the substrate and product concentrations scaled by their Michaelis constants. As the rate must be zero at equilibrium, it follows that:

$$K = \frac{V_f p_{0.5}}{V_r s_{0.5}} \qquad (3)$$

(i.e. the Haldane relationship), and that equation (2) can be rearranged into the form of equation (1):

$$v = \frac{\left(1 - \dfrac{\Gamma}{K}\right)\dfrac{V_f s}{s_{0.5}}}{1 + \dfrac{s}{s_{0.5}} + \dfrac{p}{p_{0.5}}} = \frac{\left(1 - \dfrac{\Gamma}{K}\right)V_f \sigma}{1 + \sigma + \pi} \qquad (4)$$

It is evident that the function Pos in equation (1) is the product of several positive quantities: a measure $V_f/s_{0.5}$ of the catalytic activity of the enzyme, the concentration s of the forward substrate, and a saturation function $1/(1 + s/s_{0.5} + p/p_{0.5})$, or more simply $1/(1 + \sigma + \pi)$, that expresses the fraction of enzyme molecules that have neither substrate nor product (nor, in the general case, any competing molecule) bound. Numerous reversible rate equations in enzyme kinetics have the same basic structure.

The Hill equation can be written for the irreversible case as follows:

$$v = \frac{V_f s^h}{s_{0.5}^h + s^h} = \frac{V_f \sigma^h}{1 + \sigma^h} \qquad (5)$$

in which V_f and $s_{0.5}$ have the same meanings as before, and h, the Hill coefficient, is an exponent that normally has a value in the range 1–4 for enzymes displaying positive cooperativity. Notice that $s_{0.5}$ is not a Michaelis constant, because the equation does not express Michaelis–Menten kinetics unless $h = 1$, and that it must be raised to the same power h as s if it is to be interpreted as a concentration. The corresponding Hill equation for the reverse reaction in the absence of substrate is clearly:

$$v = \frac{-V_r p^h}{p_{0.5}^h + p^h} = \frac{-V_r \pi^h}{1 + \pi^h} \qquad (6)$$

where the minus sign is introduced so as to maintain a consistent definition of v as the rate of conversion of S into P while avoiding a negative value for V_r. The challenge now is to write a general equation that reduces to equation (4) when $h = 1$, to equation (5) when $p = 0$, to the right-hand form of equation (6) when $s = 0$, and has the required form shown in equation (1).

If h has an arbitrary non-integral value (the usual experimental case), it is not possible to derive the Hill equation from a physical model [contrary to widespread belief, Hill (1910) explicitly disavowed any physical interpretation of the parameter now known as the Hill coefficient h, and regarded his equation as purely empirical]. However, if h is an integer and is equal to the number of interacting active sites of the enzyme, the Hill equation represents the limiting behaviour possible for models of interaction between active sites when the interaction is so strong that no intermediate states exist, i.e. the only species that need be considered are molecules with no sites occupied and molecules with all sites occupied. Although this property is less fundamental than the need to satisfy equation (1), it would be useful for any equation intended as a reversible form of the Hill equation to satisfy it as well as the thermodynamic requirements, and we shall show that the solution that we shall propose does have the same limiting meaning as the usual irreversible equation.

Two-site model of kinetic cooperativity

Scheme 1 shows a kinetic model for a two-site enzyme with the possibility of cooperativity. It is a kinetic version of the usual binding model (Adair, 1925) that leads to the Adair equation for equilibrium binding. The enzyme is assumed to

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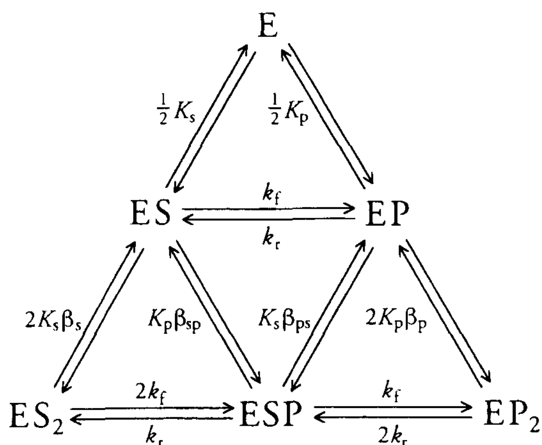
have two sites, each of which can accommodate a molecule of either substrate S or a product P, with K_s and K_p as the intrinsic dissociation constants for release of S and P from ES and EP, respectively. The factors β_s, β_{sp}, β_{ps} and β_p are interaction parameters that allow for the possibility that any binding affinity is altered by occupancy of the other site, e.g. β_{sp} is the factor by which the dissociation constant for P is increased by the presence of an S molecule in the other site. The factors 1/2 and 2 are statistical factors that take account of the fact that, for example, the species E has two sites at which S can bind, whereas the species ES has only one occupied site from which S can be released.

The catalytic reaction is represented by the horizontal $S \rightarrow P$ steps, and its reverse by the corresponding $S \leftarrow P$ steps, with rate constants k_f and k_r, respectively, which are modified by statistical factors in the same way as the dissociation constants. Note that the model assumes that binding steps are always at equilibrium: strictly speaking, such an assumption is no more justifiable than the corresponding one for simple non-cooperative kinetics, but it is nearly always made in treatments of cooperative kinetics because the alternative is to obtain rate equations that are too hopelessly complicated to be manageable.

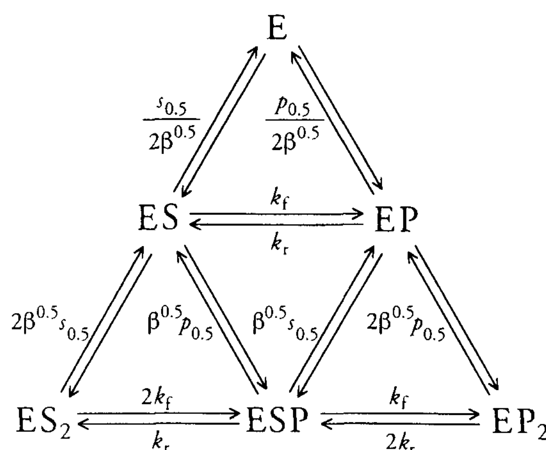
Considering just the binding of S at equilibrium, i.e. ignoring P and the catalytic reaction, the fraction y of sites with S bound is as follows:

$$y = \frac{[ES] + 2[ES_2]}{2([E] + [ES] + [ES_2])} = \frac{\dfrac{s}{K_s} + \dfrac{s^2}{K_s^2\beta_s}}{1 + \dfrac{2s}{K_s} + \dfrac{s^2}{K_s^2\beta_s}} \qquad (7)$$

It then follows from simple algebra that $y = 0.5$ when $s = K_s\beta_s^{0.5}$, i.e. that we can define $s_{0.5} = K_s\beta_s^{0.5}$ as the concentration of S for half-saturation. Similarly, $p_{0.5} = K_p\beta_s^{0.5}$ is the concentration of P for half-saturation of P in the absence of S. It is then convenient to scale the



Scheme 1. Kinetic model for a two-site enzyme.



Scheme 2. The model of Scheme 1 expressed in terms of half-saturation concentrations.

concentrations as before in terms of these half-saturation values, defining $\sigma = s/s_{0.5}$ and $\pi = p/p_{0.5}$.

Microscopic reversibility requires that the ratio p/s should be the same at equilibrium regardless of which of the four possible cycles $E \rightarrow ES \rightarrow EP \rightarrow E$, $ES \rightarrow ES_2 \rightarrow ESP \rightarrow ES$, $EP \rightarrow ESP \rightarrow EP_2 \rightarrow EP$ and $ESP \rightarrow ES \rightarrow EP \rightarrow ESP$ accomplishes the catalytic cycle. In terms of Scheme 1, this means that all of the β parameters must be equal, so that they can all be written without subscripts as β.

Taking account of these relationships, it follows that Scheme 1 can be redrawn as Scheme 2 without loss of generality. The rate v of conversion of S into P can then be expressed as follows:

$$\begin{aligned} v &= k_f([ES] + 2[ES_2] + [ESP]) - k_r([EP] + 2[EP_2] + [ESP]) \\ &= \frac{V_f(\beta^{0.5}\sigma + \sigma^2 + \sigma\pi) - V_r(\beta^{0.5}\pi + \sigma\pi + \pi^2)}{1 + 2\beta^{0.5}\sigma + \sigma^2 + 2\beta^{0.5}\pi + \pi^2 + 2\sigma\pi} \\ &= \frac{(V_f\sigma - V_r\pi)(\beta^{0.5} + \sigma + \pi)}{1 + (\sigma + \pi)(2\beta^{0.5} + \sigma + \pi)} \\ &= \frac{V_f\sigma\left(1 - \dfrac{\Gamma}{K}\right)(\beta^{0.5} + \sigma + \pi)}{1 + (\sigma + \pi)(2\beta^{0.5} + \sigma + \pi)} \end{aligned} \qquad (8)$$

where $V_f = 2k_f[E]_{total}$, $V_r = 2k_r[E]_{total}$, $\Gamma = p/s$, and $K = V_f p_{0.5}/V_r s_{0.5}$, as in equation (3).

In this model, β is a measure of the degree of cooperativity. If $\beta = 1$, there is no cooperativity, because a factor $(1 + \sigma + \pi)$ can then be cancelled between numerator and denominator, and equation (8) simplifies to the ordinary reversible Michaelis–Menten equation, equation (4). However, if $\beta < 1$, this implies destabilization of the binary complexes ES and EP with respect to the free enzyme E and the three ternary complexes, ES_2, ESP and EP_2, i.e. that the system shows positive cooperativity. The limiting degree of cooperativity possible with this model is obtained by

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inserting $\beta = 0$ in equation (8):

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)}{1 + (\sigma + \pi)^2} \tag{9}$$

Limiting cooperativity for any number of sites

As equation (9) refers to the case where the binary complexes ES and EP can be neglected, it can be obtained more simply and directly from a model in which these complexes are not written, as in Scheme 3: application to Scheme 3 of the logic used above to derive equation (8) leads directly to equation (9).

If there are more than two binding sites, both the schemes and the resulting equations are complicated if one tries to write them in full, as in Schemes 1 and 2, but in the abbreviated forms that ignore all states apart from the free enzyme and molecules with all binding sites occupied they remain quite simple. For example, a model for three sites with limiting cooperativity may be drawn as in Scheme 4, and this gives the following rate equation:

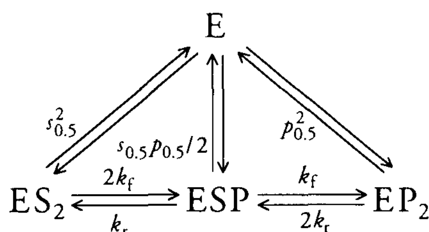
$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^2}{1 + (\sigma + \pi)^3} \tag{10}$$

More generally, the same logic can be used to obtain the following reversible rate equation for the limiting degree of cooperativity in an enzyme with n sites:

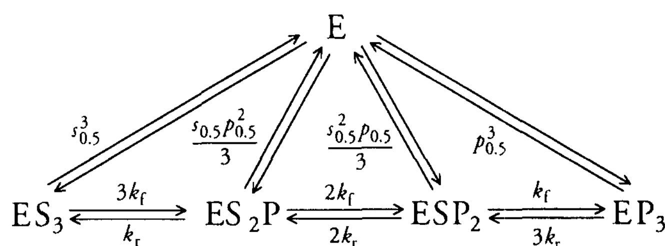
$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{n-1}}{1 + (\sigma + \pi)^n} \tag{11}$$

General form of the reversible Hill equation

The ordinary irreversible Hill equation, equation (5), can be obtained from equation (11) by putting $\pi = 0$ (and hence $\Gamma = 0$ also) and $n = h$. The assignment $n = h$ strictly applies only to the limiting case we have considered, in which intermediate states in the binding process are ignored. However, in the irreversible case there is abundant



Scheme 3. Limiting form of a model for a two-site enzyme. The Scheme differs from Schemes 1 and 2 in ignoring the complexes in an intermediate state of saturation, because in the limiting case of cooperativity such complexes make a negligible contribution.



Scheme 4. Kinetic model for a three-site enzyme, drawn with the same simplifying assumptions as in Scheme 3.

experimental evidence that equation (5) (with h not necessarily equal to n, and not necessarily an integer) gives a good description of the irreversible kinetics of numerous enzymes over the ranges of substrate concentration commonly studied. We may therefore regard the equation obtained from equation (11) by replacing n with h and avoiding any assumption about the values possible for h as the appropriate form of the Hill equation to use in reversible conditions:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{h-1}}{1 + (\sigma + \pi)^h} \tag{12}$$

Note that this equation has all of the properties defined as desirable at the outset: it has the form of equation (1), and is thus guaranteed to be thermodynamically consistent; setting $\pi = 0$ produces the normal irreversible form of the Hill equation, equation (5); in the case where $h = n$, it is the correct limiting equation to define the kinetics of an n-site enzyme with maximal cooperativity. As in the usual irreversible case, values of β intermediate between 0 and 1 lead to intermediate degrees of behaviour that can be approximated by non-integral values of h that are not equal to n.

As may be seen in Figure 1, surfaces calculated from equation (12) are virtually indistinguishable from ones calculated from the reversible Adair equation even in triple-logarithmic coordinates, except at very low σ and π values. In the region where the difference is easily visible on a logarithmic scale, the corresponding v/V values are extremely small, so that the difference would be virtually undetectable in any experimental application. It arises theoretically from the fact that equation (12), like the irreversible Hill equation, predicts a reactant elasticity of h in the limit at very low concentrations, whereas the true value is unity.

Modifier effects

Although most textbooks give the impression that substrate cooperativity is a widespread and important phenomenon, experimental examples of it nearly always occur as parts of examples of allosteric effects of modifiers. The question

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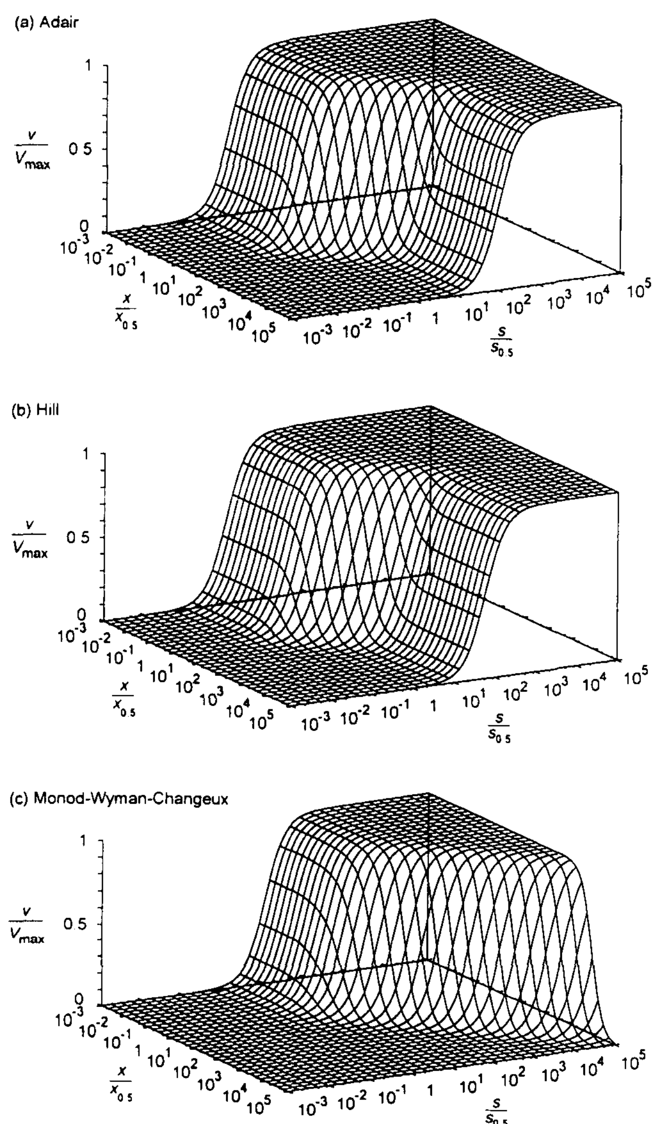
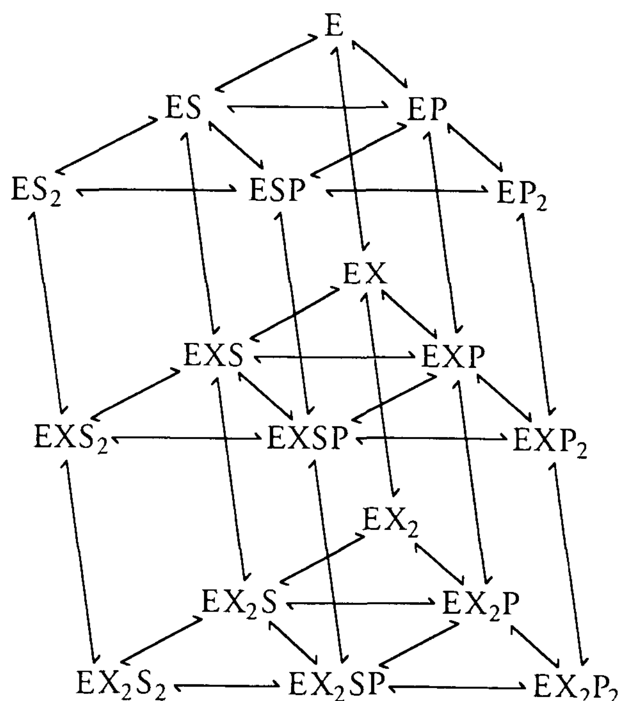


Fig. 2. A comparison of how various models account for substrate–modifier effects. In both (**a**) the Adair model and (**b**) the Hill model, the modifier (assumed for this illustration to be an inhibitor) ceases to have any effect at high substrate concentrations. In (**c**), the Monod–Wyman–Changeux model, this saturation of modifier effect is absent so that the modifier effect resembles that of a competitive inhibitor.

therefore arises whether substrate cooperativity is simply a side-effect of mechanisms for generating cooperativity in allosteric effects, and any physiological role that it may have is much less clear than is usually supposed. (The only example usually discussed in textbooks is that of haemoglobin, which is not an enzyme.) In contrast, cooperative effects of molecules not directly involved in the reaction, such as the end-product of the pathway in which a cooperative enzyme occurs, are very common and their physiological role is well understood (see, for example, Cornish-Bowden *et al.*, 1995). It follows, therefore, that any attempt to generalize the Hill equation to take account of reaction reversibility is seriously incomplete if it fails to consider effects of modifiers.

As we have mentioned, various workers, most notably Popova and Sel'kov (1975, 1978), have used the model of Monod *et al.* (1965) as the basis for dealing with reversible cooperative kinetics. However, there is an important respect in which even the irreversible form of this model fails to give a realistic account of modifier effects. As illustrated in Figure 2a, curves calculated from an Adair model for substrate–modifier interactions show saturation of the modifier effect, in the sense that at high substrate concentrations the modifier ceases to have any perceptible effect. This general property (independent of the numerical values used to calculate Figure 2a) is evident in Figures 5–11 and 5–12 of Atkinson (1977). Although the ranges of concentrations in which perceptible effects are found can be varied by varying the parameter that determines the strength of interactions between substrate and modifier binding (defined as α below), they are always finite for finite values of this parameter. However, when similar plots (Figure 2c) are made on the basis of the model of Monod *et al.* (1965), in the version [equation (4) therein] that incorporates modifier effects, the concentration ranges for perceptible effects are infinite, i.e. no matter how high the substrate concentration may be, the modifier can always produce an effect. This is, of course, a typical competitive effect, and derives from the assumption of exclusive binding of each ligand to one conformation, the assumption that is nearly always made in practical



Scheme 5. Kinetic model for an enzyme with two reactant sites and two modifier sites.

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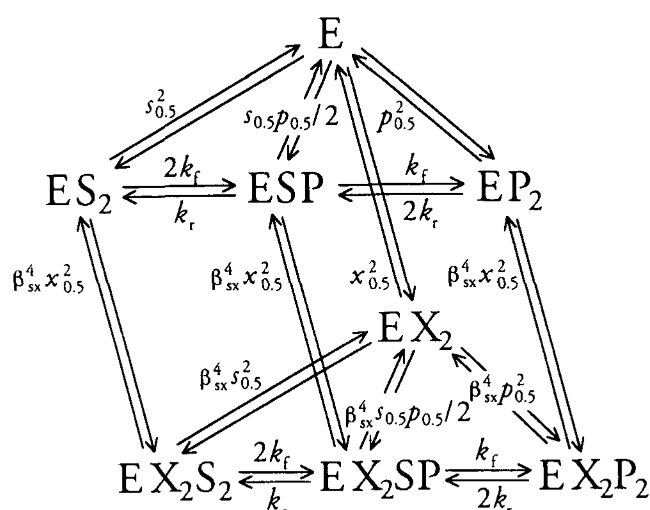

Scheme 6. Simplified kinetic model for an enzyme with two reactant sites and two modifier sites. This is drawn with the same simplifying assumptions as in Schemes 3 and 4.

applications of the model, and was indeed made by the original authors in deriving their equation (4). As far as we are aware, this consequence of the assumption of exclusive binding has not previously been pointed out. By contrast, the Hill equation generalized below to take account of modifier effects produces behaviour qualitatively indistinguishable from that obtained with the Adair model (Figure 2b).

Scheme 5 shows a model for an enzyme that has two sites at which a modifier X can bind in addition to the two sites that can each accommodate a molecule of substrate or a molecule of product. To avoid making the scheme too complicated to be understandable, the dissociation and rate constants are not shown. However, it is evident from inspection that the top layer (no X bound) is identical to Scheme 1, and that the parameters for the other two layers are the same as those for Scheme 1 with the inclusion of additional factors β_{xs} and β_{xp} to take account of the effects of the presence of X on the binding of S and P. The binary enzyme–modifier complex EX has a dissociation constant K_x, and the other vertical reactions in the scheme have the same dissociation constant K_x multiplied by additional factors β_{sx} and β_{px} to take account of the effects of the presence of S and P on the binding of X, and β_{xx} to take account of the effect of the presence of X on the binding of a second molecule of X.

The same arguments of microscopic reversibility that allowed us to conclude that all the different constants β in Scheme 1 were equal apply to Scheme 5, and show that there are just three classes of factor: those that take account of effects between substrate and product (the original β as in Scheme 1), those that take account of effects between modifier and substrate or product (which we now represent by a single symbol β_{sx}), and a single factor β_{xx} that takes account of effects between different modifier molecules. Most of these disappear if one considers the simpler case in which each pair of sites is filled in an all-or-none manner, and if one defines $s_{0.5}$ and $p_{0.5}$ as before, and $x_{0.5}$ correspondingly as $K_x \beta_{xx}^{0.5}$. This model can then be drawn as shown in Scheme 6, which is equivalent to adding a modifier layer to Scheme 3, and has an upper layer that is identical to Scheme 3.

The rate equation for this model, derived as before, is as follows:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)}{(\sigma + \pi)^2 + \left(\frac{1 + \xi^2}{1 + \frac{\xi^2}{\beta_{sx}^4}}\right)} \tag{13}$$

where ξ is the scaled modifier concentration, defined as $x/x_{0.5}$.

The explanation of the exponent 4 in equation (13) is that in the fully liganded complex the binding of each of the two molecules of X is affected by two reactant molecules; in the case of n sites, this would be n^2, because there would be n molecules of X bound, each affected by n reactant molecules.

Generalizing to the case where the number of sites is not necessarily two, and the cooperativity is not necessarily maximal, equation (13) becomes:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi^h}{1 + \alpha \xi^h}} \tag{14}$$

in which an interaction factor α replaces $1/\beta_{sx}^{h2}$: although the exponent h^2 make sense when h is an integer equal to the number of sites, it serves no purpose when modelling with non-integral Hill coefficients.

These equations apply equally well to allosteric inhibition and activation: if $\alpha < 1$, then X is an inhibitor; if $\alpha > 1$, then X is an activator; if $\alpha = 1$, then X has no effect on the rate.

We have assumed here that the Hill coefficient for modifier effects must be equal to that for substrate and product effects. However, although this is certainly true if the equation is derived from a model with all-or-none binding to a protein with n sites, when h is integral and equal to n, it is less clear when the Hill coefficient is allowed to be non-integral (necessary for fitting many experimental examples).

Two modifiers

Equation (14) can readily be generalized further to cover the case where two modifiers X_1 and X_2 (which can be both inhibitors, both activators, or one of each) compete for a

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single site, with mutually exclusive binding, as follows:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi_1^h + \xi_2^h}{1 + \alpha_1 \xi_1^h + \alpha_2 \xi_2^h}} \quad (15)$$

where α_1 and α_2 are factors similar to α in equation (14) that take account of the effects of the presence of S and P on the binding of X_1 and X_2, respectively.

The equation is more complicated if X_1 and X_2 bind at separate sites, with the possibility of both being bound simultaneously:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi_1^h + \xi_2^h + \alpha_{12} \xi_1^h \xi_2^h}{1 + \alpha_1 \xi_1^h + \alpha_2 \xi_2^h + \alpha_1 \alpha_2 \alpha_{12} \xi_1^h \xi_2^h}} \quad (16)$$

where α_{12} is a factor that takes account of the effects of X_1 and X_2 on the binding of each other. If one is willing to assume that the binding of X_1 and X_2 is independent, i.e. that $\alpha_{12} = 1$, then this simplifies considerably:

$$v = \frac{V_f \sigma \left(1 - \frac{\Gamma}{K}\right)(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \left(\frac{1 + \xi_1^h}{1 + \alpha_1 \xi_1^h}\right)\left(\frac{1 + \xi_2^h}{1 + \alpha_2 \xi_2^h}\right)} \quad (17)$$

This simplification may, of course, be excessive for some enzymes in some circumstances, and we have examined possibilities for deriving more general equations. Unfortunately, however, the complexity increases very rapidly when one deviates from the simple cases assumed, and we believe that until the necessity for such equations has been demonstrated it will be more useful to stay with manageably simple ones. Similar comments apply to most of the cases we have considered.

Discussion

To justify the use of equations of the type proposed in this paper, we need to show that the type of behaviour that they generate is similar enough to that obtainable from physically meaningful models to substitute for it in computer models. Even equations based on the model originally proposed by Adair (1925) for haemoglobin are arguably unrealistic, as this is fundamentally a model for equilibrium binding that can only be generalized to the kinetic case by making assumptions that may well be false in many instances. However, attempts to treat kinetic cooperativity and subunit interactions without such assumptions (e.g. Ricard *et al.*, 1990) produce equations that are too complicated to be practically usable. Kinetic versions of the Adair model are the most realistic general cooperative equations that have been used in practice to study experimental data. Despite the existence of versions of the Monod–Wyman–Changeux equation that allow different values of the limiting activity for the R and T forms, these have been little used, nearly all authors preferring to stay with the 'perfect-*K*' form of the model; indeed, in the original description of the model (Monod *et al.*, 1965), the only kinetic equation given was of the 'perfect-*K*' type. In the 'perfect-*K*' form, the Monod, Wyman and Changeux model is itself a kinetic form of the Adair equation, though the way it is usually written has confused some authors into thinking it is not of Adair form. [A discussion of the relationship between the Adair equation and the classical models of cooperativity may be found on pp. 221–233 of Cornish-Bowden (1995a)]. We believe, therefore, that the reversible kinetic Adair equation provides the most appropriate basis for studying the behaviour of the reversible Hill equation that we propose.

The comparison illustrated in Figure 1 suggests that in practice the reversible Hill equation provides an excellent approximation to the kinetic Adair equation, and thus that it satisfies this test. Moreover, as illustrated in Figure 2, it gives qualitatively (and very nearly quantitatively) correct modifier behaviour over the whole range of concentrations, whereas the reversible Monod–Wyman–Changeux equation does not.

Nonetheless, we do not wish to imply that the reversible Hill equation is always to be preferred to the reversible Monod–Wyman–Changeux equation. On the contrary, there are cases where the latter equation can model observed behaviour that is impossible with the former. For example, the equations of Popova and Sel'kov can accommodate cooperativity in both kinetic and binding behaviour, whereas our equations consider only binding cooperativity. We have already noted that the Hill equation gives incorrect behaviour at very low substrate concentrations, but the Monod–Wyman–Changeux equation accounts accurately for this region. On the other hand, the well-known incapacity of the irreversible Monod–Wyman–Changeux equation to account for negative cooperativity applies equally to its reversible forms, whereas the Hill equations, both reversible and irreversible, allow negative cooperativity.

In the more usual cases, the large number of parameters in the reversible Monod–Wyman–Changeux equation means that one will inevitably have to guess some of their values. For example, equation (14) implies the existence of six parameters with clear operational meanings (degree of cooperativity, concentration for half-saturation, etc.), even if their mechanistic meanings may be obscure. The corresponding Monod–Wyman–Changeux equation for a 'perfect-*K*' enzyme with substrate, product and one modifier contains 13 parameters, all of which have clear mechanistic interpretations but which do not relate easily to observable properties.

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