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A Universal Rate Equation for Systems Biology

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Abstract

Classical enzyme kinetics, as developed in the 20th century, had as a primary objective the elucidation of the mechanism of enzyme catalysis. In systems biology, however, the precise mechanism of an enzyme is less important; what is required is a description of the kinetics of enzymes that takes into account the systemic context in which each enzyme is found. In this paper we present the generalized reversible Hill equation as a universal rate equation for systems biology, in that it takes into account (i) the kinetic and regulatory properties of enzyme-catalysed reactions, (ii) the reversibility and thermodynamic consistency of all reactions, and (iii) the modification of enzyme activity by allosteric effectors. Setting the Hill coefficient to one yields a universal equation that can successfully mimic the behaviour of various detailed non-cooperative mechanistic models. Subsequently, it is shown that the bisubstrate Hill equation can account for substrate-modifier saturation, in agreement with experimental data from *Bacillus stearothermophilus* pyruvate kinase. In contrast, the classical Monod–Wyman–Changeux (MWC) equation cannot account for this effect. The proposed reversible Hill equations are all independent of underlying enzyme mechanism, are of great use in computational models and should lay the groundwork for a "new" enzyme kinetics for systems biology.

Introduction

One central aim of classical enzyme kinetics has been the determination of an enzyme's mechanism from initial rate studies with varying substrate and product concentrations [1, 2]. Kinetic equations have been derived for almost every conceivable mechanism, using (partial) equilibirum binding or steady-state kinetics, and there has been a continuing focus on experimental analyses that will be able to discriminate between these mechanisms [2]. The focus in enzyme kinetic analyses has thus been on the characterization of individual enzyme mechanisms, and many of the resulting kinetic equations (especially those for cooperative or multi-substrate reactions) are complex and contain numerous parameters.

In the post-genomic era the field of computational systems biology has received increasing prominence. Its aim is to build kinetic models of cellular pathways, with the individual pathway components (e.g. enzymes) quantitatively described by mathematical rate laws. As such, the overall behaviour of the pathway can then be calculated by the models, needing only the properties of the individual enzymes as input. As a consequence, the focus of enzyme kinetics has shifted. For a kinetic model, we require a kinetic rate law that that will describe the response of an enzyme to changes in substrate, product and modifier concentrations; however, for the overall pathway behaviour, the exact enzyme catalytic mechanism is unimportant as long as the enzyme rate as a function of substrate, product and modifier concentrations is adequately described. When building kinetic models from literature data, one is often faced with the problem that enzymes have not been characterized fully. For example, most often only K_m values for substrates are available and the exact mechanism or some of the other kinetic parameters such as K_i values have not been determined. This forces the modeller to make additional assumptions.

The model construction process would thus be greatly facilitated by a generic equation that contains fewer parameters and yet describes the kinetic behaviour of the enzyme adequately. In this paper, we present the reversible Hill equation as a candidate for fulfilling this task. Firstly, the uni-substrate Hill equation is generalized to an arbitrary number of substrates and products. Secondly, the non-cooperative version of the bi-substrate rate equation is shown to successfully describe the behaviour of two more complex detailed mechanistic models, i.e. ordered and ping-pong kinetics. Thirdly, the Hill and Monod–Wyman–Changeux (MWC) models are compared in terms of their description of allosteric modifier behaviour, with specific emphasis on whether the modifier effect saturates. Fourthly, experimental data for pyruvate kinase show modifier saturation, in agreement with the Hill model but not with the MWC model (Section 4). Finally, the implications of this work for computational systems biology are summarized.

A Generalized Reversible Hill Equation

The development of a universal rate equation for systems biology relies strongly on the foundations of the work of Hofmeyr and Cornish-Bowden [3], who generalized the Hill equation for cooperativity [4] to its reversible form. For a reaction $A \Leftrightarrow P$ this reads:

$$v = \frac{V_f \alpha \left(1 - \frac{\Gamma}{K_{eq}}\right)(\alpha + \pi)^{h-1}}{\frac{1+\mu^h}{1+\sigma^{2h}\mu^h} + (\alpha + \pi)^h} \quad (1)$$

where α is the concentration of substrate A scaled by its half-saturation constant $A_{0.5}$ ($\alpha = a / A_{0.5}$), π is the concentration of P scaled by $P_{0.5}$, Γ is the mass-action ratio, K_{eq} the equilibrium constant, h the Hill coefficient, μ the concentration of allosteric modifier M scaled by its half-saturation constant $M_{0.5}$, and σ is an interaction factor quantifying the extent to which binding of a modifier molecule affects substrate and product binding to the enzyme, thus leading to allosteric inhibition or activation. Apart from its ability to describe reversible reactions, this equation is significant in that it takes into account-and separates-the thermodynamic, kinetic and regulatory properties of the reaction [3]. One particularly useful aspect of this equation is the operational definition of its half-saturation constants. For example, at zero product and in the absence of modifier ($\pi = \mu = 0$), setting the concentration of A equal to its half-saturation constant ($\alpha = 1$), yields $v/V_f = 0.5$. The value of $A_{0.5}$ can thus easily be determined in an experiment as that concentration of substrate which yields half of the limiting (maximal) rate.

By following a similar approach as in [3], we have derived the reversible Hill equation for the two-substrate two-product (bi-bi) reaction $A_1 + A_2 \Leftrightarrow P_1 + P_2$ ([5]; Rohwer *et al.*, in preparation). For the case without allosteric modification this equation reads:

$$v = \frac{V_f \alpha_1 \alpha_2 \left(1 - \frac{\Gamma}{K_{eq}}\right)(\alpha_1 + \pi_1)^{h-1}(\alpha_2 + \pi_2)^{h-1}}{(1 + (\alpha_1 + \pi_1)^h)(1 + (\alpha_2 + \pi_2)^h)} \quad (2)$$

with the equation parameters and half-saturation constants defined as in Equation 1. By deriving the equation for the three-substrate three-product (ter–ter) case and extending the general pattern, it is possible to obtain a reversible Hill equation describing a reaction comprising an arbitrary number of substrate–product pairs [5]. For the reaction $A_1 + A_2 + ... + A_n \Leftrightarrow P_1 + P_2 + ... + P_n$ this equation reads:

$$v = V_f \prod_{i=1}^{n_s} \alpha_i \left(1 - \frac{\Gamma}{K_{eq}}\right) \prod_{i=1}^{n_s} \left(\frac{(\alpha_i + \pi_i)^{h-1}}{1 + (\alpha_i + \pi_i)^h}\right) \quad (3)$$

where n_s is the number of substrate-product pairs, and other parameters defined as in Equations 1 and 2. Moreover, it has been possible to obtain Hill equations for the one-substrate two-product (uni–bi) and bi–uni, as well as the bi–ter and ter–bi cases, which broadens the level of applicability of the reversible Hill equation. Equations 1 – 3 can all be transformed to their non-cooperative counterparts by setting the Hill coefficient h equal to

one. The derivations will not be shown here, but will be published in detail elsewhere (Rohwer *et al.*, in preparation); the reader is also referred to the Master's thesis of Hanekom, [5] which can be obtained from the authors of this paper on request.

Equations 1 – 3 thus constitute a set of reversible Hill equations that should be able to describe most, if not all, enzyme-catalysed reactions occurring in cellular pathways. All the relevant combinations of different numbers of substrates and products are covered, and by varying the Hill coefficient, the equation can describe reactions exhibiting positive, negative or no cooperativity. From this perspective, the equation indeed appears "universal" in terms of its applicability to cellular reactions. Yet, to be truly worthy of the label "universal", it is insufficient merely to be able to apply the equation to all reactions; the equation will also have to exhibit *realistic* kinetic properties. This will be addressed in the following sections in two different ways: first, we investigate the behaviour of the non-cooperative generalized equation and compare it to more detailed mechanistic models; and next, we compare the behaviour of the cooperative, allosterically inhibited equation to the MWC model and validate the results with experimental data from pyruvate kinase.

Non-Cooperative Bi-Substrate Kinetic Models

The kinetics of enzymes with two or more substrates have been studied in great detail. The field was pioneered by Cleland, who developed kinetic formulations for most conceivable mechanisms in the early 1960 s [6]. An important focus of this original work was to be able to derive the mechanism of an enzyme-catalysed reaction from kinetic studies, and any differences in kinetic behaviour between the various mechanistic models were thus exploited. The fact that reactions can proceed by (partial) equilibrium binding or steady-state kinetics, and that the mechanism can proceed via a ternary complex or substituted enzyme, leads to a multitude of possible formulations, which have been summarized comprehensively by Segel in the definitive textbook on the topic [2].

Ordered vs. ping-pong kinetics

A kinetic mechanism for a bi-substrate reaction that proceeds via a ternary complex with compulsory order binding differs substantially from a mechanism that proceeds via a substituted enzyme. In the former case (termed "ordered" mechanism from here on), both substrates are bound to the enzyme before catalysis occurs and the products are released. Moreover, the substrates cannot bind randomly but rather have to bind to the enzyme in a fixed sequence; likewise, the products are released in a fixed order. For the reaction $A + B \Leftrightarrow P + Q$ this can be symbolized as follows (*cf.* [1]):

$$E \Leftrightarrow EA \Leftrightarrow [EAB \cdot EPQ] \Leftrightarrow EQ \Leftrightarrow E \tag{4}$$

and leads to the following formulation of a rate equation [1, 2, 6]:

$$\frac{v}{V_\mathrm{f}} = \frac{\frac{ab}{K_\mathrm{iA}K_\mathrm{mB}}\left(1-\frac{pq/(ab)}{K_\mathrm{eq}}\right)}{1+\frac{a}{KiA}+\frac{K_\mathrm{mA}b}{K_\mathrm{iA}K_\mathrm{mB}}+\frac{K_\mathrm{mQ}p}{K_\mathrm{mP}K_\mathrm{iQ}}+\frac{q}{K_\mathrm{iQ}}+\frac{ab}{K_\mathrm{iA}K_\mathrm{mB}}+\frac{K_\mathrm{mQ}ap}{K_\mathrm{iA}K_\mathrm{mP}K_\mathrm{iQ}}+\frac{K_\mathrm{mA}bq}{K_\mathrm{iA}K_\mathrm{mB}K_\mathrm{iQ}} + \frac{pq}{K_\mathrm{mP}K_\mathrm{iQ}}+\frac{abp}{K_\mathrm{iA}K_\mathrm{mB}K_\mathrm{iP}}+\frac{bpq}{K_\mathrm{iB}K_\mathrm{mP}K_\mathrm{iQ}}} \quad (5)$$

By contrast, in the substituted enzyme mechanism (also termed “ping-pong” mechanism) the enzyme is modified after the first substrate molecule has bound and product molecule has been released. The modified enzyme then binds the second substrate, and upon catalysis and release of the second product, the original enzyme is returned. Mechanistically this is symbolized as follows, with *E*((prime)) denoting the modified enzyme:

$$E \Leftrightarrow [EA \cdot E'P] \Leftrightarrow E' \Leftrightarrow [E'B \cdot EQ] \Leftrightarrow E \quad (6)$$

and leads to the following rate equation formulation for the ping-pong mechanism [1,2,6]:

$$\frac{v}{V_\mathrm{f}} = \frac{\frac{ab}{K_\mathrm{iA}K_\mathrm{mB}}\left(1-\frac{pq/(ab)}{K_\mathrm{eq}}\right)}{\frac{a}{K_\mathrm{iA}}+\frac{K_\mathrm{mA}b}{K_\mathrm{iA}K_\mathrm{mB}}+\frac{p}{K_\mathrm{iP}}+\frac{K_\mathrm{mP}q}{K_\mathrm{iP}K_\mathrm{mQ}}+\frac{ab}{K_\mathrm{iA}K_\mathrm{mB}}+\frac{ap}{K_\mathrm{iA}K_\mathrm{iP}}+\frac{K_\mathrm{mA}bq}{K_\mathrm{iA}K_\mathrm{mB}K_\mathrm{iQ}}+\frac{pq}{K_\mathrm{iP}K_\mathrm{mQ}}} \quad (7)$$

Is the universal generic equation a good enough approximation?

The fact that ordered and ping-pong mechanisms and their associated rate equations are quite dissimilar, prompted us to investigate whether these differences result in markedly altered kinetic behaviour and, hence, are important from a systems biological perspective. Moreover, both Equations 5 and 7 contain numerous parameters (a K_i value, in addition to a K_m value, for each substrate and product), which have seldom all been determined in kinetic characterizations reported in the literature. When constructing kinetic models of pathways, this frequently leads to a lack of data, forcing the modeller to assume parameter values, particularly for K_i (for an example from our own work see [7]. In contrast to both the ordered and ping-pong models, the corresponding bi-substrate generic equation, which is derived from the reversible Hill equation (Equation 2) with $h = 1$, has fewer parameters, i.e. only a K_m value for each substrate and product and no K_i values, and reads as follows:

$$\frac{v}{V_\mathrm{f}} = \frac{\frac{ab}{K_\mathrm{A}K_\mathrm{B}}\left(1-\frac{pq/(ab)}{K_\mathrm{eq}}\right)}{\left(1+\frac{a}{K_\mathrm{A}}+\frac{q}{K_\mathrm{Q}}\right)\left(1+\frac{b}{K_\mathrm{B}}+\frac{p}{K_\mathrm{P}}\right)} \quad (8)$$

We thus set out to investigate whether the kinetic behaviour of both the ordered and ping-pong mechanisms could be described equally well by the generic equation. To do this, data sets were generated with both the ordered and ping-pong mechanistic equations (Equations

5 and 7) by varying both substrates and both products independently over two orders of magnitude for three parameter sets (i.e. combinations of K_m and K_i values). The generic equation (Equation 8) was then fitted to both the ordered and the ping-pong data and goodness of fit assessed by the r^2 value [8]. As can be seen from the representative example in Fig. 1, the quality of fit was near perfect in some cases, and in all cases the r^2 value was grater than 0.94, indicating that the generic equation is capable of successfully mimicking the kinetic behaviour of both the ordered and the ping-pong mechanistic models.

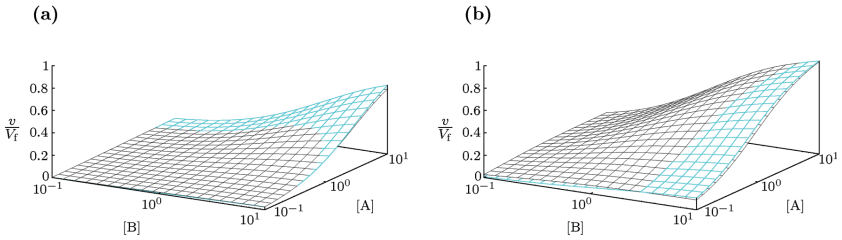

Figure 1. Examples of good fits of the generic rate equation to ordered and ping-pong model data. The generic bi–bi rate equation was fitted to data (a) from the ordered rate equation (Equation 5) with the following parameters: $K_{mA} = 3.3$, $K_{mB} = 3.3$, $K_{mP} = 0.83$, $K_{mQ} = 0.83$, $K_{iA} = 1.0$, $K_{iB} = 3.0$, $K_{iP} = 7.5$, $K_{iQ} = 10.0$; and (b) from the ping-pong rate equation (Equation 7) with the following parameters: $K_{mA} = 1.0$, $K_{mB} = 1.0$, $K_{mP} = 1.0$, $K_{mQ} = 10.0$, $K_{iA} = 1.0$, $K_{iB} = 1.0$, $K_{iP} = 1.0$, $K_{iQ} = 10.0$. K_{eq} was fixed at 10 in all cases. The original ordered and ping-pong data are indicated in black, the generic bi–bi fitted model in cyan. The generic equation was fitted on the complete data set where both substrates and both products were varied independently over two orders of magnitude. The plots show the fits at $p = q = 0.1$. Reproduced from [8] with permission from the Institution of Engineering and Technology.

While the fit was not always as good as in Fig. 1, it could be improved considerably by reducing the range of product concentrations included in the analysis. Since we varied both substrates and products over two orders of magnitude, this analysis really presents a "worst-case" scenario and in many cases of real-life kinetic models, the variation in substrates and products will be less.

This section has evaluated the performance of the universal rate equation in non-cooperative cases. In the next section, we investigate how the equation fares when dealing with cooperative and allosteric kinetics.

Cooperativity and Allosteric Modifier Saturation

Enzymes following normal Michaelian kinetics require an 81-fold increase in substrate concentration to "switch on" (increase their rate from 0.1 $V f$ to 0.9 $V f$). By comparison, enzymes that obey cooperative Hill kinetics only need a 9-fold increase in substrate concentration for the same effect (for a Hill coefficient of 2). Cooperative enzymes are thus

sensitive to small changes in substrate concentration and it is important that such cooperative enzymes be regulated with a high degree of precision [1]. Inhibition or activation by allosteric effectors is one mechanism that accomplishes such regulation, and it is a ubiquitous motif in metabolic pathways. It therefore becomes important to be able to describe the kinetics of such allosteric enzymes accurately in kinetic models.

Allosterically regulated cooperative enzyme reactions are usually modelled with irreversible MWC kinetics [9]. However, when Hofmeyr and Cornish-Bowden [3] derived the uni-substrate reversible Hill equation (Equation 1 above), they also demonstrated that this equation predicts substantially different allosteric inhibition kinetics, compared to the MWC equation. In particular, the Hill model shows modifier saturation in that at high substrate concentration the allosteric inhibitor ceases to have an effect, whereas the MWC equation does not show this saturation and the inhibitor always has an effect irrespective of the substrate concentration. Allosteric inhibitors in the MWC equation thus behave analogously to competitive inhibitors. The effect was, however, only demonstrated for the uni–uni case.

Since most enzyme-catalysed reactions in biochemical pathways have two or more substrates and products, we first set out to demonstrate that the difference between the Hill and MWC models with respect to allosteric modifier saturation also exists for the bi-substrate case. As the inhibitor saturation effect has to our knowledge not been demonstrated experimentally, we subsequently present data for the allosteric enzyme pyruvate kinase, which also show saturation of the allosteric modifier effect, thus lending support to the Hill equation and contrasting with the MWC model.

Modifier saturation in Hill vs. MWC

The bi-substrate Hill equation for the irreversible reaction $A + B \rightarrow P + Q$ with allosteric modifier M reads as follows [5]:

$$v = \frac{V_f \alpha^h \beta^h}{\left(\frac{1+\mu^h}{1+\sigma^{4h}\mu^h}\right) + \left(\frac{1+\sigma^{2h}\mu^h}{1+\sigma^{4h}\mu^h}\right)[\alpha^h + \beta^h] + \alpha^h\beta^h} \tag{9}$$

with $\beta = b/B_{0.5}$ and the other parameters defined as in Equations 1 and 2. The MWC equation for the same reaction is given by:

$$v = \frac{V_f \left(\frac{[A][B]}{K_{mA}K_{mB}}\right)\left(1+\frac{[A]}{K_{mA}}\right)^{n-1}\left(1+\frac{[B]}{K_{mB}}\right)^{n-1}}{\left(1+\frac{[A]}{K_{mA}}\right)^{n}\left(1+\frac{[B]}{K_{mB}}\right)^{n} + L_0\left(1+\frac{[I]}{K_i}\right)^{n}} \tag{10}$$

where $V f$ is the limiting enzyme rate, K_{mA} and K_{mB} are the intrinsic dissociation constants for substrates A and B from the R-form of the enzyme, [I] is the inhibitor concentration, K_i is the intrinsic dissociation constant for inhibitor I from the T-form of the enzyme, n is the

number of enzyme subunits and L_0 is the equilibrium ratio of L_0/R_0 in the absence of substrates and products. This equation was derived by simplifying the generalized MWC model of Popova and Sel'kov [10] along the assumptions of the original paper of Monod *et al.* [9]: (i) the reaction is irreversible, (ii) the T-form of the enzyme does not participate in catalysis, (iii) the inhibitor only binds to the T-form, and (iv) the substrates bind only to the R-form of the enzyme, which is catalytically active.

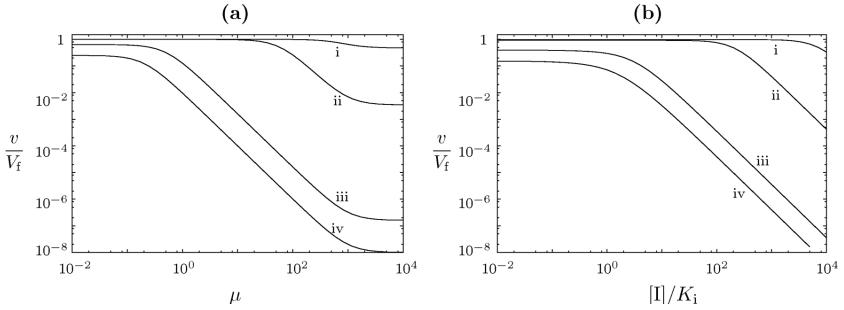

Figure 2. Enzyme activities of the Hill and MWC models as a function of inhibitor concentration at different substrate conditions. (a) Bi-substrate Hill equation (Equation 9) with $h = 2$ and $\sigma = 0.1$. (b) Bi-substrate MWC equation (Equation 10) with $n = 2$ and $L = 10$. Data are plotted in double logarithmic space. Substrates A and B were varied simultaneously; their scaled concentrations are i: 150, ii: 25, iii: 2 and iv: 1. Reproduced from [19] with permission from the Institution of Engineering and Technology.

These two models (Equations 9 and 10) were then compared by plotting the reaction rate as a function of the concentration of allosteric inhibitor for different values of the substrate concentrations, which were increased together (Fig. 2). The results clearly demonstrate that the bi-substrate Hill model shows substate-modifier saturation in that increasing the modifier concentration above a certain threshold (here, $\mu \approx 10^3$) ceases to have an effect on the reaction rate. Moreover, the inhibitory effect is nullified at high substrate concentrations. The bi-substrate MWC model does not show this saturating effect, analogous to the uni-substrate case [3].

Experimental verification of modifier saturation in pyruvate kinase

Since the Hill and MWC models can be clearly distinguished using the effect of modifier saturation, we investigated experimentally whether this effect would be present in a bi-substrate cooperative enzyme. *Bacillus stearothermophilus* pyruvate kinase is a microbial cooperative enzyme that exhibits cooperativity towards its substrate phosphoenolpyruvate (PEP) [11]. The cooperative kinetics, structure and thermal stability of this enzyme have been studied in detail [11 – 13], and it has both allosteric activators and inhibitors. Moreover, it can be conveniently assayed with a simple spectrophotometric protocol [14]. Here, the kinetics of the allosteric inhibitor inorganic phosphate (P_i) were investigated (Fig. 3).

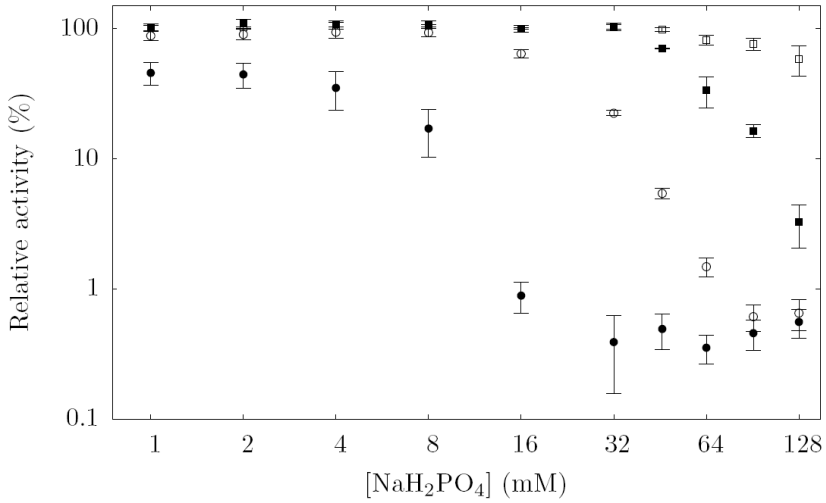

Figure 3. Relative pyruvate kinase activity as a function of inhibitor (NaH_2PO_4) concentration at increasing substrate concentrations. Data were normalized to the limiting rate measured at 20 mM PEP and 20 mM ADP in the absence of inhibitor. Note that data are presented in double-logarithmic space. The assay mixture contained equimolar concentrations of PEP and ADP: 1 mM (•), 4 mM (((odot))), 10 mM (((square))) and 20 mM (((osquare))). All data points are the average of 3 – 5 independent determinations ± SE. Experiments demonstrating the saturation of the inhibitory effect (4 mM substrates at ≥91 mM inhibitor; 1 mM substrates at ≥32 mM inhibitor) were all performed in five-fold. Reproduced from [19] with permission from the Institution of Engineering and Technology.

At high substrate concentrations, P_i could no longer inhibit the enzyme, even at high levels. In addition, modifier saturation is clearly visible when the substrates were present at 1 mM (P_i concentrations above 32 mM did not inhibit the enzyme further). At 4 mM substrate concentrations, saturation of the inhibitory effect was also visible for $[P_I]^3$91 mM. When comparing these results with the kinetic plots in Fig. 2, it is clear that the data are consistent with the Hill model but not with the classical MWC model.

Discussion and Conclusion

This paper has described a new universal rate equation for systems biology, which is based on the reversible Hill equation. The equation can be written for an arbitrary number of substrate–product pairs, as well as for uni–bi, bi–uni, bi–ter and ter–bi reactions. In addition, an arbitrary number of either independent or competing allosteric modifiers can be treated [5]. By varying the Hill coefficient through values ranging from less than one to greater than one, the equation can exhibit negative cooperativity, no cooperativity (i.e.

Michaelian kinetics) or positive cooperativity. These features make the universal rate equation so generic and versatile that it should be possible, in principle, to use it for describing the kinetics of any enzyme-catalysed reaction.

The derivation of the generalised reversible Hill equation was based on the same assumptions as the uni-substrate case [3], i.e. (i) the limiting case of cooperativity (active sites are either empty or fully occupied, partially liganded enzyme species are not considered), (ii) random equilibrium binding of substrates, products and modifiers to the enzyme, also in the form of dead-end complexes, (iii) independently acting binding sites that do not influence each other, and finally (iv) generalization from the number of subunits (n) to the Hill coefficient (h), which can take on non-integer values (also less than one).

Although allosteric effects in the generalized reversible Hill equation presented in this paper only affect the binding strength of substrates or products through changing their apparent half-saturation constants (i.e. so-called "K-enzymes"), it should be pointed out that the reversible Hill equation has also been rewritten to include effects of allosteric modifiers on the catalytic properties of an enzyme (i.e. so-called "V-enzymes") [15, 16]. The details are not included here for lack of space; however, they contribute to the universality of the reversible Hill equation in its application to computational systems biology.

The non-cooperative formulation of the universal rate equation is capable of succesfully mimicking the kinetic behaviour of both the ordered and the ping-pong mechanistic models (Fig. 1). The equation for random bi–bi kinetics was not included in the analysis, since its derivation is based on equilibrium binding of substrates and products [2, 6] (the derivations of the ordered and ping-pong models are based on steady-state kinetics). The common ordered bi–bi mechanism is thus identical to our generic bi-substrate model barring the existence of the dead-end complexes, which should therefore lead to an even better correspondence than for the ordered and ping-pong mechanistic equations.

The cooperative version of the equation shows substrate–allosteric modifier saturation, in contrast to the irreversible MWC model, which does not (Fig. 2), and the validity of the reversible Hill model is corroborated by experimental data for the enzyme pyruvate kinase (Fig. 3), which also show modifier saturation. Together, these data provide *in silico* and *in vitro* evidence for validation of the universal equation.

The results are significant for two reasons: First, in general, generic equations based on the reversible Hill equation contain fewer parameters than mechanistic equations. As a result, fewer parameters need to be measured experimentally, which lessens the burden for experimental kinetic characterization. Moreover, the parameters can be determined directly because of the clear operational definition of the half-saturation constants (see Section 2). In contrast, MWC equations, for example, are mechanistic models that contain intrinsic metabolite dissociation constants, which cannot be determined directly in such an operational way, but only through fitting. Secondly, it is unnecessary to know the detailed mechanism of an enzyme in order to simulate its kinetics for modelling. For computational

systems biology, enzyme mechanism as such is less important but an accurate kinetic description in terms of quantification of the reaction rate as a function of substrates, products and effectors is crucial.

It should be emphasized that not all MWC equations are unable to account for modifier saturation; it is only the commonly used uni-substrate irreversible formulation [9] and its bi-substrate form (Equation 10) that have this limitation. In fact, we have shown that the generalized MWC model of Popova and Sel'kov [10, 17] gives near indistinguishable behaviour from the generalized reversible Hill equation, including allosteric inhibitor saturation [18]. The reason for this is that in the generalized MWC model [17], all species interacting with the enzyme (be it substrates, products or allosteric effectors) can in principle bind to both the T- and R-forms and both these enzyme forms are catalytically active (albeit to different extents), whereas in the original formulation of Monod, Wyman and Changeux [9], the restrictions outlined below Equation 10 were imposed. However, in experimental applications the original model has been used almost without exception, and the generalized form of Popova and Sel'kov has rarely been applied, which makes the distinction between Hill and MWC equations important.

Although mechanistic equations were derived for initial-rate kinetics (see e.g. [6]), the universal rate equation presented here is not limited to the analysis of initial rates. We have developed a new experimental method to obtain kinetic parameters through fitting of progress curve data obtained from time-course NMR spectroscopy (Hanekom *et al.*, in preparation). This is especially relevant for systems biology, since many of the high-throughput techniques of modern biology (transcriptomics, proteomics, metabolomics) generate such time-series data.

In conclusion, we propose that the universal rate equation presented in this paper should form the basis of a “new” enzyme kinetics for systems biology. It is simpler than mechanistic rate equations, can account for positive, negative or no cooperativity, is thermodynamically consistent and contains fewer parameters than mechanistic equations.

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