Derivation of a reversible Hill equation with modifiers affecting catalytic properties

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Abstract: – An existing generic enzyme rate equation, the reversible Hill equation, was generalized to account for modifiers affecting the catalytical properties of the enzyme as well as for the case of several substrates and products. The resulting generalized reversible Hill (GRH) equation has relatively few but operationally well-defined parameters. Its usefulness is demonstrated by fitting it to experimental data on mammalian muscle phosphofructokinase. The fit is superior to that of previous models to the same data. The rate equation derived is suitable for replacing more complicated rate equations when exact mechanisms are unknown and data is scarce or contradictory.

Keywords: – Metabolic Modeling, Enzyme Kinetics, Reversible Hill Equation, Operationally Well-defined parameters, Phosphofructokinase

1 Introduction

The fields of theoretical biology referred to as e.g. systems biology or metabolic modeling have during the recent years gained much attention. One common objective of this kind of research is to create large scale mathematical models of networks of enzyme catalyzed biochemical reactions. At the core of these models are rate equations for the enzymes considered in the model. A common problem is that the reaction mechanisms are not fully known for all enzymes and that the available quantitative data has large error margins. Enzymes whose activities are regulated by different modifiers (i.e. inhibitors or activators) and which deviate from Michaelis-Menten kinetics, are often particularly difficult to model.

In their paper [6], Hofmeyr and Cornish-Bowden (HCB) addressed this problem when deriving the Reversible Hill (RH) equation. This rate equation, although comparatively simple, successfully captures much commonly observed dynamics of complex and regulated enzyme catalyzed reactions. Its flexibility and relative simplicity makes it very attractive for use in metabolic models where the exact mechanism or mechanistic parameters of the enzyme under consideration are unknown or uncertain.

However, the RH equation derived by HCB only accounts for reactions with one substrate and one product. Also, the RH equation lacks the ability to account for modifiers affecting the catalytic properties (i.e. modifiers altering the limiting rate $V$). In this paper, we generalize the RH equation to take these effects into account, and also to the case with several substrates and products. We discuss the operational properties of the resulting equations, and demonstrate their usefulness with an
example where our generalized RH (GRH) equation is fitted to experimental data on mammalian muscle phosphofructokinase (PFK). The fit of the GRH equation is superior to earlier muscle PFK models.

The rate equations derived here are particularly useful for enzymes whose exact mechanism is poorly known, and those for which the existing quantitative data is scarce or contradictory; the former because Hill equations have often been found to capture the dynamics accurately [2, 6] and the latter because the equations contain relatively few parameters, many of which are operationally well-defined. The modeler may thus fairly easily control the behaviour of the enzyme model through its parameters and explore the parameter space according to experimental uncertainties.

2 Derivation of the GRH equation

Let us first consider an enzyme $E$ consisting of two subunits, each capable of binding a substrate molecule $S$ or product molecule $P$, as well as binding an allosteric modifier $X$. This is the same situation as that considered by HCB [6]. However, we will here allow the allosteric modifier to alter the catalytic properties of the enzyme as well as the binding of the substrate and product molecules. The methodology for deriving rate equations here will essentially be the same as in their paper, following the methodology outlined by Cha [1].

We write the total enzyme concentration $E_{tot}$ as

$$E_{tot} = E + ES_2 + ESP + EP_2 + EX_2 + EX_2S_2 + EX_2SP + EX_2P_2.$$  

(1)

We have here followed HCB [6] and neglected all intermediate steps of the binding of $S$, $P$, and $X$, i.e. we are assuming complete binding cooperativity. Now, from the reaction scheme (figure 1) it is clear that

$$\frac{E \cdot S \cdot S}{ES_2} = s_{0.5}^2, \quad \frac{E \cdot S \cdot P}{ESP} = s_{0.5}p_{0.5}, \quad \frac{E \cdot P \cdot P}{EP_2} = p_{0.5}^2.$$  

(2)

Introducing the notation $\sigma = S/s_{0.5}, \pi = P/p_{0.5}$, we have

$$ES_2 = E\sigma^2, \quad ESP = 2E\sigma\pi, \quad EP_2 = E\pi^2.$$  

(3)

Furthermore, according to the reaction scheme

$$\frac{E \cdot X \cdot X}{EX_2} = x_{0.5}^2.$$  

(4)

Let the factor by which each equilibrium constant of each $EX$ complex is altered by the binding of one $X$ molecule equal $a$. Detailed balance at the pseudo-steady-state implies that $a$ also equals the factor by which each equilibrium constant of each binary enzyme-modifier complex $EX$ is altered by the binding of one $S$ molecule. Further, detailed balance requires that the influence of $P$ on the equilibrium constant of the $EX$ complex or vice versa is represented by the same factor $a$. For these reasons, we have

$$\frac{EX_2 \cdot S \cdot S}{EX_2S_2} = a^4s_{0.5}^2, \quad \frac{EX_2 \cdot S \cdot P}{EX_2SP} = a^4s_{0.5}p_{0.5}, \quad \frac{EX_2 \cdot P \cdot P}{EX_2P_2} = a^4p_{0.5}^2.$$  

(5)

Introducing $\xi = X/x_{0.5}$ and $\alpha = 1/a^4$, we now have

$$EX_2 = E\xi^2, \quad EX_2S_2 = E\alpha\xi^2\sigma, \quad EX_2SP = 2E\alpha\xi^2\sigma\pi, \quad EX_2P_2 = E\alpha\xi^2\pi.$$  

(6)

We may thus rewrite equation 1 as

$$E_{tot} = E(1 + \xi^2 + (\sigma + \pi)^2(1 + \alpha\xi^2)).$$  

(7)

According to the reaction scheme, the forward rate $j_1$ is

$$j_1 = k_1(2E\sigma^2 + 2E\sigma\pi) + \gamma k_1(2E\alpha\xi^2\sigma^2 + 2E(\alpha\xi^2\sigma\pi)).$$  

(8)
where $\gamma$ represents the factor with which the catalytic constants are altered by the modifier $X$, as seen in figure 1. In the original model, $\gamma = 1$ and the reversible Hill equation with an allosteric modifier is obtained. Here, we proceed to derive an expression accounting for the case when $\gamma \neq 1$. Equation 8 simplifies to

$$j_l = 2Ek_l(1 + \gamma\alpha\xi^2)(\sigma^2 + \sigma\pi). \quad (9)$$

Combining equations (7) and (9) and introducing $V_l = 2k_lE_{tot}$ gives

$$J_l = \frac{1 + \gamma\alpha\xi^2}{1 + \alpha\xi^2} V_l(\sigma^2 + \sigma\pi) \frac{(\sigma + \pi)^2 + \frac{1 + \xi^2}{1 + \alpha\xi^2}}{(\sigma + \pi)^2}. \quad (10)$$

In the same way, we obtain for the reversible reaction

$$j = \frac{1 + \gamma\alpha\xi^h}{1 + \alpha\xi^h} V_l(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi) \frac{(\sigma + \pi)^2 + \frac{1 + \xi^h}{1 + \alpha\xi^h}}{(\sigma + \pi)^2}. \quad (11)$$

where we have followed HCB and introduced $\Gamma = P/S$ and $K_{eq} = V_lP_{0.5}/V_lS_{0.5}$. The same reasoning as in their work leads to the expression for an arbitrary Hill coefficient $h$,

$$j = \frac{1 + \gamma\alpha\xi^h}{1 + \alpha\xi^h} V_l(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi)^{h-1} \frac{(\sigma + \pi)^2 + \frac{1 + \xi^h}{1 + \alpha\xi^h}}{(\sigma + \pi)^2}. \quad (12)$$

Here, we remark that if we let $V_l \rightarrow 0$ and $P_{0.5} \rightarrow \infty$, i.e., consider the irreversible case of equation 12, and further set $\gamma = 0$ and $h = 1$, we obtain

$$j = \frac{V_l\sigma}{\sigma(1 + \alpha\xi) + 1 + \xi}, \quad (13)$$

which should be recognized as the rate equation for linear mixed inhibition [2, 3]!

### 2.1 Several modifiers

Let us now consider the case of two different modifiers which may bind to the enzyme. Two different cases arise: first, the modifiers may compete for the same site or, second, the modifiers bind to two separate sites. Both of these cases were discussed by HCB in the case of $\gamma = 1$. We will here state the general results for any $\gamma$. In the case of the modifiers competing for the same site, the denominator of the rate equation will consist of the terms $(1 + \xi^h + \xi^2 + (\sigma + \pi)^2(1 + \alpha\xi^h + \alpha\xi^2))$, and the numerator will consist of the terms $(1 + \gamma_1\alpha_1\xi^h + \gamma_2\alpha_2\xi^h)V_l\sigma(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi)^{h-1}$, which means that the rate equation becomes

$$j = \frac{1 + \gamma_1\alpha_1\xi^h + \gamma_2\alpha_2\xi^h}{1 + \alpha_1\xi^h + \alpha_2\xi^h} \frac{V_l\sigma(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi^h + \xi^2}{1 + \alpha_1\xi^h + \alpha_2\xi^h}}. \quad (14)$$

In the case of the modifiers binding to two different sites on the enzyme the situation is more complicated. We will here consider only the case when the modifiers bind to the enzyme independently of each other. Then, the rate equation is written

$$j = \frac{1 + \gamma_1\alpha_1\xi^h}{1 + \alpha_1\xi^h} \frac{1 + \gamma_2\alpha_2\xi^h}{1 + \alpha_2\xi^h} \frac{V_l\sigma(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi^h + \xi^2}{1 + \alpha_1\xi^h + \alpha_2\xi^h}} \frac{(1 + \xi^h + \xi^2)}{(1 + \alpha_1\xi^h + \alpha_2\xi^h)}. \quad (15)$$

We may generalize equations 14 and 15 to any number of modifiers, some of which may share the same site. Applying the same arguments used above we arrive at the following general rate equation:

$$j = \left(\prod_i \frac{1 + \gamma_{ij}\alpha_{ij}\xi_{ij}^h}{1 + \alpha_{ij}\xi_{ij}^h}\right) \times \frac{V_l\sigma(1 - \frac{\Gamma}{K_{eq}})(\sigma + \pi)^{h-1}}{(\sigma + \pi)^h + \frac{1 + \xi^h + \xi^2}{1 + \alpha_1\xi^h + \alpha_2\xi^h}} \frac{(1 + \xi^h + \xi^2)}{(1 + \alpha_1\xi^h + \alpha_2\xi^h)}. \quad (16)$$

where modifier site $i$ may be acted upon by several modifiers $X_{ij}$. 


2.2 Several substrates and products

We may generalize equation 16 further to let it encompass reactions with several substrates and products. We first consider the case of an enzyme consisting of two subunits catalysing the reaction of two substrates forming two products, when no modifiers are present. Then, we may as an ansatz write $E_{\text{tot}}$ as

$$
E_{\text{tot}} = E \left(1 + (\sigma_1 + \pi_1)^2 \right) \left(1 + (\sigma_2 + \pi_2)^2 \right).
$$

(17)

Here, the underlying assumption is that a $S_1$ and/or $P_1$ molecules may bind to their corresponding sites independently of whether $S_2$ and/or $P_2$ molecules are bound to their corresponding sites and vice versa. If we assume that the enzyme reaction proceeds only when both substrates or products are bound to the enzyme, we arrive at the following rate law:

$$
j = \frac{V_t \sigma_1 \sigma_2 \left(1 - \frac{\Gamma}{K_m} \right) (\sigma_1 \sigma_2 + \pi_1 \pi_2)}{(1 + (\sigma_1 + \pi_1)^2)(1 + (\sigma_2 + \pi_2)^2)}.
$$

(18)

or, for an arbitrary Hill coefficient $h$,

$$
j = \frac{V_t \sigma_1 \sigma_2 \left(1 - \frac{\Gamma}{K_m} \right) (\sigma_1 \sigma_2 + \pi_1 \pi_2)^{h-1}}{(1 + (\sigma_1 + \pi_1)^h)(1 + (\sigma_2 + \pi_2)^h)}.
$$

(19)

We may generalize the equation further to account for an arbitrary number of substrate and product molecules, and we then write

$$
j = \frac{V_t \prod_i \sigma_i \left(1 - \frac{\Gamma}{K_m} \right) \left(\prod_i \sigma_i + \prod_j \pi_j\right)^{h-1}}{\prod_i \left(1 + (\sigma_i + \pi_i)^h\right)}.
$$

(20)

Unfortunately, there is no simple general expression if we include modifier effects since the denominator becomes impossible to factorize. However, in the special case when each modifier affects one substrate and product pair only, we may write

$$
j = \prod_k \left(\prod_i \sum_j \frac{1 + \gamma_{ki} \alpha_{kij} \xi_{ki}^h}{1 + \alpha_{ki} s_{kij}^h} \right) \times \frac{V_t \prod_k \sigma_k \left(1 - \frac{\Gamma}{K_{eq}} \right)}{\prod_k \sigma_k + \prod_l \pi_l} \times \frac{1}{h-1} \times \prod_j \left(1 + \frac{1 + \xi_{kj}^h}{1 + \alpha_{kj} s_{kj}^h} + (\sigma_k + \pi_k)^h\right).
$$

(21)

3 Operational meanings of the parameters

One appealing property of the Hill equations described above is that most of the parameters capture easily observed quantities. This is most easily elucidated if we look at the irreversible Hill equation with one substrate and one allosteric modifier (that is, with $K_{eq}$ and $\pi$ equal to zero):

$$
j = \frac{1 + \gamma \xi^h}{1 + \alpha \xi^h} \frac{V \sigma^h}{\sigma^h + \frac{1 + \xi^h}{1 + \alpha \xi^h}}.
$$

(22)

First, we note that when no modifier is present, that is, when $\xi = 0$, the equation simplifies to the traditional Hill equation. On the other hand, considering a situation where the modifier is present in great excess, one notices that $\lim_{\xi \to \infty} \frac{1 + \gamma \xi^h}{1 + \alpha \xi^h} = \gamma$ and that $\lim_{\xi \to \infty} \frac{1 + \xi^h}{1 + \alpha \xi^h} = 1/\alpha$. Thus, the modifier simply alters the effective limiting rate with a factor $\gamma$ and the effective half-saturation point with a factor $\alpha^{1/h}$. These two properties are usually directly observable from experimental plots. They may be varied independently in the GRH equation which thus in this sense is more versatile than the Monod-Wyman-Changeux (MWC) equation [8], where these properties, in addition to the effective Hill coefficient, are dependent of the model parameters in a non-trivial way [5].

The dissociation constants of the different enzyme-modifier complexes are unfortunately
much more difficult to estimate directly from experimental data. The traditional way of estimating this class of parameters is by means of double-reciprocal plots [2, 3]. However, this is not possible for the GRH equation. If we for instance calculate \( \sigma^h/j \), we obtain

\[
\frac{\sigma^h}{j} = \frac{\sigma^h}{V} \cdot V \cdot \frac{1 + \gamma \alpha \xi^h}{1 + \gamma \alpha \xi^h + V} \cdot \frac{1 + \xi^h}{1 + \xi^h + V}.
\]

(23)

Obviously, this produces only linear terms in \( \xi^h \) if \( \gamma = 0 \), i.e. if the enzyme-modifier complex lacks catalytic activity. Thus, one has to resort to generic optimization procedures, as illustrated in our example below. However, if one only requires or is forced to seek a rough value of the parameter, for instance due to scarce or contradicting experimental data, one may note that when \( \xi = 1 \),

\[
\frac{j}{j^{(a)}} = \frac{\frac{1}{\alpha}}{\frac{1}{1+\alpha}} \cdot \frac{\sigma^h + \frac{1}{\alpha}}{\sigma^h + \frac{2}{1+\alpha}},
\]

(24)

where \( j^{(a)} \) is the limiting rate for a given value of \( \sigma \) obtained when great excess of modifier is present. This may give a rough approximation of \( x_{0.5} \) if the other quantities are already estimated. Of course, if applying a general form of the Hill equation, such as equation 21, all other substrates and modifiers must have been held constant during the experiment, and their effects have to be considered as incorporated into effective values of \( s_{0.5} \) and \( V \), when making these kind of estimations.

4 Application to phosphofructokinase

We will now demonstrate the usefulness of the GRH equation derived above. The subject of the example is the glycolytic enzyme phosphofructokinase (PFK), about which has been said “PFK may be an enzymologists favourite, but it is a modeler’s nightmare” [10]. Much of the difficulty lies in the fact that PFK is affected by a multitude of allosteric modifiers; mammalian muscle PFK, which we will be concerned with here, has well-characterized activating sites for adenine nucleotides and fructose bisphosphates, as well as for citrate and 3-P-glyceric acid, and an inhibitory site for ATP [7]. Muscle PFK has an important regulatory role not only in muscle but probably also in the pancreatic \( \beta \)-cell [11, 14], where it is thought to be involved in the generation of glycolytic oscillations.

In order to support theoretical investigations of this possibility, a good kinetic model of PFK is desirable. Smolen recognized this fact in a theoretical study of muscle glycolysis [9]. In that study, a specialized rate equation for PFK was derived, the independent subunit (IS) model, whose ability to fit experimental data was compared with that of the MWC model. This comparison clearly favoured the IS model, which was not entirely surprising since the applicability of the MWC model on muscle PFK kinetics had been questioned earlier as this enzyme exists in at least three different states in contrast to the two states of the MWC model [4]. Here, in turn, we show that the GRH equation gives a significantly better fit to the experimental data compared to the IS model.

Smolen’s study was based on the comparatively comprehensive dataset produced in a study by Tornheim and Lowenstein [12]. We will here use the same dataset, in particular the data points of figures 1–3 of that article, which were produced under identical laboratory conditions (only the different substrate and modifier concentrations were varied). This also allow us to compare the fit of the IS and GRH equations using the root mean square (RMS) measure, which Smolen calculated for the data points of these three figures. The experimental data are presented in figure 2 as different symbols representing different modifier concentrations as noted in the figure text.

Assuming that under the experimental conditions, PFK is saturated with ATP with regard to catalysis (half-saturation occurs at an ATP concentration of 20 \( \mu \)M [13]), well below the concentrations used in the experiments [12], we may write the PFK GRH equation as

\[
v = \frac{\prod_i \frac{1 + \gamma \alpha_i \xi_i^h}{1 + \alpha_i \xi_i^h}}{\sigma^h + \prod_i \frac{1 + \alpha_i \xi_i^h}{1 + \alpha_i \xi_i^h}} \cdot V \sigma^h,
\]

(25)
where $\sigma = F6P / s_{0.5}$, $\xi_i = [i] / x_i$ and where $i$ may represent FBP, AMP or ATP.

Since many parameters of the GRH equation have easily grasped meanings, it is fairly easy to make a starting guess from the experimental curves for further optimization. Thus, one expects $h$, $\alpha_{FBP}$, $\alpha_{AMP}$, $\gamma_{FBP}$, and $\gamma_{AMP}$ to be greater than one, while $\alpha_{ATP}$ and $\gamma_{ATP}$ should be less than one. An optimization in least squares sense yielded the parameter set presented in the text of figure 2. In the figure, the solid lines are the rates calculated according to equation 25 using the optimized parameter set. The RMS of the rate equation fit is 0.21 $\mu$M/min, which should be compared with the RMS of 0.38 $\mu$M/min for the fit of the IS rate equation to the same data as calculated by Smolen [9]. The PFK GRH equation thus provides a significant improvement over previous PFK models.

5 Discussion
We have extended the RH equation to account for modifier effects on catalytical activity. We consider the resulting GRH equation to be attractive when constructing models of regulated enzymes because of its relatively few parameters, their clear operational meaning and the flexibility of the equation. Moreover, Hill equations of this general type have produced very good approximations to more detailed models of cooperative enzyme kinetics [6], only failing in accuracy at very low substrate concentrations.

We exemplified the usefulness of the GRH equation by applying it to experimental data on muscle PFK. The new equation is more appropriate for describing the kinetics of this enzyme than previous models. Also, some further conclusions may be drawn from the data fitting. In particular, the experimental results presented by Tornheim & Lowenstein [12] were taken as evidence of FBP activation being dependent on AMP activation, clearly a heterotropic effect not captured by the GRH equation. The goodness of fit of the PFK GRH equation to the experimental data questions the need for this assumption. Further, $\gamma_{ATP} = 0$ at the optimum in least squares sense, which means that ATP appears to be a strong catalytical inhibitor of muscle PFK.

However, there are phenomena which the GRH equation is not able to capture. One example is modifiers affecting the effective Hill coefficient, a behaviour readily captured by the MWC equation [5]. Another limitation is that heterotropic effect of binding of one modifier affecting the binding of other modifiers are not accounted for.

References
Figure 1: The reaction scheme considered in this study. The different equilibrium constants are indicated, as well as the catalytic constants ($k_f$ and $k_r$). The factor $a$ represents altering of the equilibrium constants by the modifier $X$, while the factor $\gamma$ represents altering of the catalytic constants by the modifier. The intermediate binding steps (e.g. the ES complex) are neglected in order to obtain simple equations.
Figure 2: Experimental data of muscle PFK is indicated as follows: (a) □ AMP 50 µM, ○ AMP 20 µM, × AMP 1 µM. (b) △ FBP 0.3 µM AMP 20 µM, □ FBP 1.4 µM AMP 20 µM, ▽ FBP 7.9 µM AMP 20 µM, ◦ FBP 32 µM AMP 20 µM, ● FBP 84 µM AMP 20 µM, × FBP 32 µM AMP. (c) △ ATP 0.2 mM AMP 1 µM FBP 32 µM, ○ ATP 0.2 mM AMP 20 µM FBP 32 µM, ● ATP 0.5 mM AMP 20 µM FBP 32 µM, ▲ ATP 0.5 mM AMP 20 µM FBP 1.4 µM. Unless noted otherwise above or in the figures, concentrations were F6P 0.1 mM, ATP 0.5 mM, MgCl 8 mM. The reaction velocities \( v \) are in µM per minute. The solid lines are the corresponding theoretical curves calculated from equation (25). The optimized parameter set was \( s_{0.5} = 0.26 \) mM, \( x_{\text{FBP}} = 4.1 \) µM, \( x_{\text{AMP}} = 39 \) µM, \( x_{\text{ATP}} = 0.034 \) mM, \( h = 2.6 \), \( \alpha_{\text{FBP}} = 30 \), \( \alpha_{\text{AMP}} = 880 \), \( \alpha_{\text{ATP}} = 8.8 \times 10^{-5} \), \( \gamma_{\text{FBP}} = 1.4 \), \( \gamma_{\text{AMP}} = 1.3 \), \( \gamma_{\text{ATP}} = 0 \), \( V = 2.9 \) µM/min.