Alternative Perspectives of Enzyme Kinetic Modeling

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1. Introduction

The basis of enzyme kinetic modelling was established during the early 1900's when the work of Leonor Michaelis and Maud Menten produced a pseudo-steady state equation linking enzymatic catalytic rate to substrate concentration (Michaelis & Menten, 1913). Building from the Michaelis-Menten equation, other equations used to describe the effects of modifiers of enzymatic activity were developed based on their effect on the catalytic parameters of the Michaelis-Menten equation. Initially, inhibitors affecting the substrate affinity were deemed competitive and inhibitors affecting the reaction rate were labelled non-competitive (McElroy 1947). These equations have persisted as the basis for inhibition studies and can be found in most basic textbooks dealing with the subject of enzyme inhibition. Here the functionality of the competitive and non-competitive equations are examined to support the development of a unified equation for enzymatic activity modulation. From this, a modular approach to pseudo-steady state enzyme kinetic equation building is examined. Finally the assumption that these equations, which stem from the Michaelis-Menten equation, are truly pseudo-steady state is also examined.

2. Pseudo steady state enzyme kinetic

2.1 Michaelis-Menten kinetics

Conventional views on how to handle enzyme kinetic data have remained essentially the same for nearly a century following the proposal of the Michaelis-Menten equation (1913; Equation 1).

$$v = V_{max} \frac{[S]}{[S] + K_M} \tag{1}$$

The Michaelis-Menten equation was a large step forward in our ability to understand how biological systems control chemical processes. This equation linked the rate of enzymatic substrate catalysis to a mass action process relying on the fractional association between the substrate and the enzyme population. That is, the maximum conversion rate of substrate to product (V_{max}) could be directly related to the concentration of the enzyme ([E]) present and the catalytic rate at which individual enzymes converted substrate molecules to product (k_{cat}; Equation 2).

$$V_{max} = k_{cat}[E] \tag{2}$$

The second part of the equation describes the fractional association between the substrate and the enzyme population. Dependent on the Michaelis-Menten constant (K_M; Equation 3), this part of the Michaelis-Menten equation partitions the binding of substrate to the enzyme population relative to the Michaelis-Menten constant.

$$\frac{[S]}{[S] + K_M} \tag{3}$$

At substrate concentrations lower than the Michaelis-Menten constant, also known as the substrate affinity constant, less than half of the enzyme population would be expected to have substrate associated with it (Figure 1).
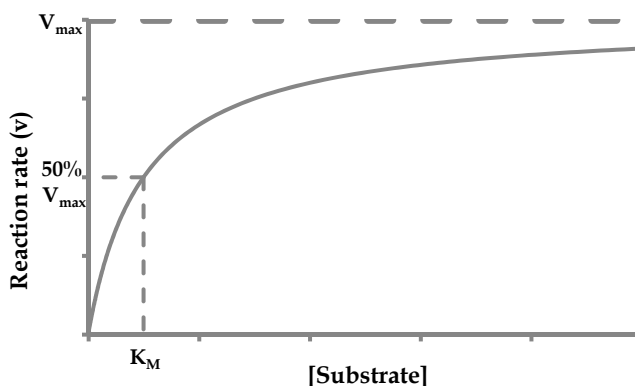

Fig. 1. Rectangular hyperbola plot of the Michaelis-Menten equation relating catalytic rate and substrate concentration.

At a concentration equal to the Michaelis-Menten constant, half of the enzyme population will have substrate associated with it. Therefore, the Michaelis-Menten constant itself is an inflection point. As substrate concentrations exceed the Michaelis-Menten constant, the fraction of the enzyme population interacting with substrate is pushed towards 100%. This term produces the characteristic rectangular hyperbolic profile associated with the Michaelis-Menten equation shown above.

2.2 Linearization of the Michaelis-Menten equation

The introduction of the reciprocal form of the Michaelis-Menten equation (Equation 4) in 1934 (Lineweaver & Burk) made the determination of the kinetic constants (K_M, V_{max}) of the Michaelis-Menten equation much simpler.

$$\frac{1}{v} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \tag{4}$$

The reciprocal form of the equation produced a straight line with intercept values on the Y axis of $1/V_{max}$ and on the X axis of $-1/K_M$ (Figure 2). This advancement in analysis of the Michaelis-Menten equation allowed for a simplified way of analyzing the effect of compounds that altered the catalytic activity of enzyme systems. Changes in enzymatic activity were observed to result from changes in the substrate affinity or maximum velocity (Lineweaver & Burk 1934) resulting in the definition of inhibitory equations based on their effects on the kinetic constants of the Michaelis-Menten equation.
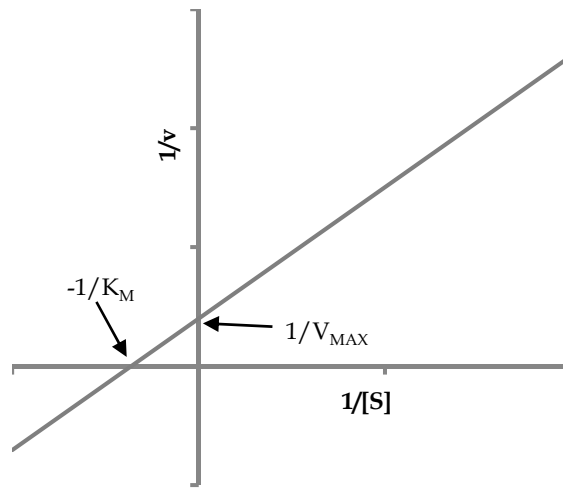

Fig. 2. Double reciprocal plot of the Michaelis-Menten equation indicating how the intercepts provide a simplified way of determining the kinetic constants of the equation.

2.3 Modes of inhibition

By far the most extensively documented form of interactions between modifiers of enzymatic activity and enzymes have been inhibitory, therefore, it is not surprising that the first mathematical models to be defined and accepted in the literature were the competitive (Equation 5) and non-competitive (Equation 6) modes of inhibition (Lineweaver & Burk 1934; McElroy 1947).

$$v = V_{max} \frac{[S]}{[S] + K_M \left(1 + \frac{[I]}{K_i}\right)} \tag{5}$$

$$v = V_{max} \frac{[S]}{([S] + K_M)\left(1 + \frac{[I]}{K_i}\right)} \tag{6}$$

The competitive inhibition (Equation 5) and non-competitive inhibition (Equation 6) equations model different inhibitory processes and are easily identified using Lineweaver-Burk double reciprocal plots (Figure 3). Competitive inhibition has been defined as a direct

competition between the substrate and the inhibitor molecule for the active site of the enzyme. As inhibitor concentration is increased, the enzyme's substrate affinity is decreased. However, due to the competitive nature of this interaction, decreases in catalytic activity can always be overcome with sufficient increases in substrate concentration. In contrast, non-competitive inhibition exclusively affects the catalytic velocity of the enzyme population. Shifts in the maximum velocity can be attributed to the inhibitor binding to the enzyme and shutting down its catalytic activity, such that the observed decrease in activity represents the percent of the enzyme population bound by inhibitor.
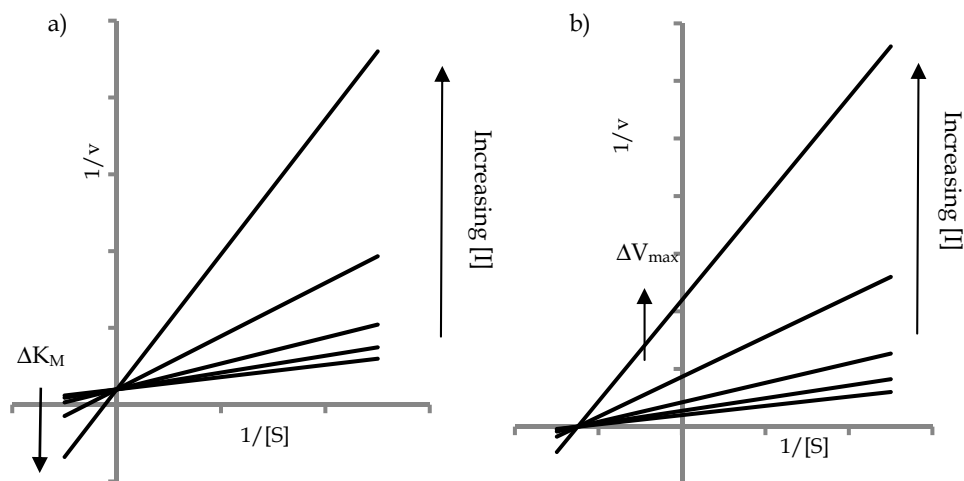

Fig. 3. Double reciprocal plots of a) competitive inhibition, where introduction of the inhibitor produces changes exclusively in the substrate affinity constant (K_M) and b) non-competitive inhibition, where inhibition is observed as a decrease in the maximum velocity of the enzyme catalyzed (V_{max}) reaction.

Both of the competitive and non-competitive inhibition equations can be derived from rate and conservation of mass equations like the Michaelis-Menten equation. The derivation of the competitive (Equation 5) and non-competitive (Equation 6) inhibition equation also results in a similar inhibitory term (Equation 7).

$$\left(1 + \frac{[I]}{K_i}\right) \tag{7}$$

This type of equation derivation, which segregates modes of inhibition based on inhibitory effect on kinetic constants of the Michaelis-Menten equation, has formed the basis for equation derivation in modern enzyme kinetics. However the use of the inhibitory term (Equation 7) found in the competitive (Equation 5) and non-competitive (Equation 6) inhibition equations may be regarded as an incomplete derivation that obscures the relationship between inhibitor binding and kinetic effect (Walsh et al., 2007).

In the competitive inhibition equation (Equation 5), the inhibitory term, as written, directly affects the Michaelis-Menten constant. This might be expected as competitive inhibition exclusively alters substrate affinity. In the non-competitive equation (Equation 6), the inhibitory term is inversely related to the maximum velocity (Equation 8).

$$\frac{V_{max}}{1 + \frac{[I]}{K_i}} \tag{8}$$

A rearrangement of this term (Equations 9-12) demonstrates the similarities of the inhibitory term and the term relating the fractional association between substrate and enzyme population (Equation 3).

$$\frac{V_{max}}{\frac{[I] + K_i}{K_i}} \tag{9}$$

$$\frac{V_{max}}{\frac{[I] + K_i}{[I] + K_i - [I]}} \tag{10}$$

$$\frac{\frac{V_{max}}{1}}{1 - \frac{[I]}{[I] + K_i}} \tag{11}$$

$$V_{max} - V_{max}\frac{[I]}{[I] + K_i} \tag{12}$$

Therefore the inhibitory term of the non-competitive inhibition equation directly equates shutting down of enzymatic activity with the fraction of the enzyme population bound by the inhibitor. This on off analog behaviour provides a simplistic way looking at enzymatic activity and limits the usefulness of this equation for describing anything other than complete inhibition of the enzyme upon inhibitor binding. However, the addition of a governor term (V_{max}-V_{max2}) changes the non-competitive term such that it can be used to account for changes in catalytic activity, other than complete inhibition (Equation 13; Walsh et al., 2007; Walsh et al., 2011a).

$$V_{max} - (V_{max} - V_{max2})\frac{[I]}{[I] + K_i} \tag{13}$$

This rearrangement, or insertion of a governor term, allows for the description of inhibitory effects ranging from just greater than 0% to 100% and has the potential to describe activation as well if the secondary maximum velocity is greater than the initial velocity. It is convenient to classify compounds with the potential to activate as well as inhibit as modifiers, denote here as X (Equation 14).

$$V_{max} - (V_{max} - V_{max2}) \frac{[X]}{[X] + K_x} \tag{14}$$

Even without the addition of the governor term to the non-competitive equation, this rearrangement (Equation 9-12) accounts for the rectangular hyperbolic change in maximum velocity produced by the non-competitive inhibition equation (Figure 4). This change is identical to the mass action binding observed between the substrate and the enzyme population in the Michaelis-Menten equation (Figure 1).
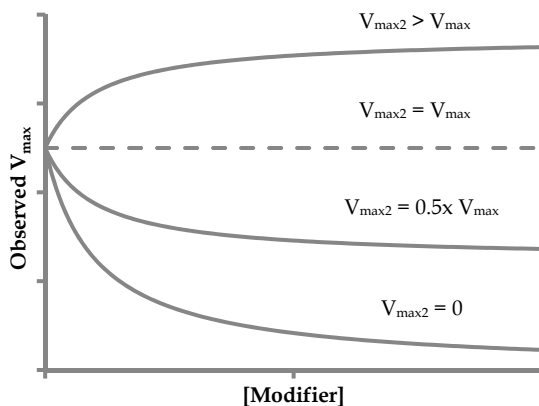

Fig. 4. Rectangular hyperbolic changes in the maximum velocity produced by modifiers. Here the mass action binding between the enzyme population and modifier results in the characteristic shape of the curve but the change in activity depends on the change induced by single binding events between the enzyme and the modifier. For example the four lines represent stimulation ($V_{max2} > V_{max}$), binding without catalytic effect ($V_{max2} = V_{max}$), partial inhibition ($V_{max2} = 0.5x V_{max}$) and complete inhibition ($V_{max2} = 0$) as would be observed with the classical non-competitive equation.

With the clear way non-competitive inhibition mimics the kinetics observed with the Michaelis-Menten equation, the manner in which competitive inhibitors affect enzyme activity becomes obscure. This can be demonstrated through the same rearrangement of the inhibitory term which directly affects the substrate affinity (Equations 15-19).

$$K_M \left(1 + \frac{[I]}{K_i}\right) \tag{15}$$

$$K_M \left(\frac{[I] + K_i}{K_i}\right) \tag{16}$$

$$K_M \left(\frac{[I] + K_i}{[I] + K_i - [I]}\right) \tag{17}$$

$$K_M \left(\frac{1}{1 - \frac{[I]}{[I] + K_i}} \right) \tag{18}$$

$$\frac{K_M}{1 - \frac{[I]}{[I] + K_i}} \tag{19}$$

As can be seen in equation 19, the inhibitory term in the competitive inhibition equation (Equation 5) actually describes a situation where the substrate affinity term is divided by the percent of the enzyme population free of the competitive inhibitor. This implies that modifiers that affect the substrate affinity exclusively produce a linear increase in the substrate affinity with increasing inhibitor concentration (Figure 5). However, as substrate binding specificity and affinity result from three point binding (Ogston, 1948), changes in the ability of an enzyme to bind substrate are more likely to result from inhibitor interactions that shift the enzyme's ability to do this away from its native state. Such perturbations would follow the same mass action mode of interaction as observed with non-competitive inhibition (Equation 6). These changes in substrate affinity would be finite and the overall observable effect would result from individual interactions between inhibitor and enzyme which would shift the binding affinity form that of the native enzyme (K_M) to an affinity produced by the inhibitor (K_{M2}) (Equation 20; Walsh et al., 2007; Walsh et al., 2011a).

$$v = V_{max} \frac{[S]}{[S] + K_M - (K_M - K_{M2}) \frac{[I]}{[I] + K_i}} \tag{20}$$

While true competitive inhibition may exist, the criteria for identifying an inhibitor as truly competitive needs to include a linear shift in substrate affinity resulting from increase in inhibitor concentration (Figure 5d; Walsh et al., 2007; Walsh et al., 2011a). This should be examined with global data fitting to confirm the inhibitory effect on substrate affinity.

3. Modular enzyme kinetic equation building

3.1 Unified modifier equation

By recognizing that changes in the substrate affinity and maximum velocity result from stoichiometric interactions between the modifier and the enzyme and that the effects of the modifier can be regulated with a governor term, a single equation for describing these effects can be generated (Equation 21; Walsh et al., 2007).

$$v = \frac{[S]}{[S] + K_{S1} - (K_{S1} - K_{S2}) \left(\frac{[X]}{[X] + K_{X1}} \right)} V_{S1} - (V_{S1} - V_{S2}) \left(\frac{[X]}{[X] + K_{X1}} \right) \tag{21}$$

Here the maximum velocity term has been abbreviated as V_{S1}, and the substrate affinity term K_{S1}, for simplicity. Of note, the modifier binding constant (K_{X1}) is the same in the term

modifying the substrate affinity and the term modifying the maximum velocity. This is in contrast to the mixed non-competitive equation (Equation 22) which has been used to describe similar dual effects on substrate affinity and maximum velocity but requires two separate inhibitor binding constants to accommodate the effects of a single inhibitor.

$$v = V_{max} \frac{[S]}{[S]\left(1 + \frac{[I]}{\alpha K_i}\right) + K_M \left(1 + \frac{[I]}{K_i}\right)} \tag{22}$$
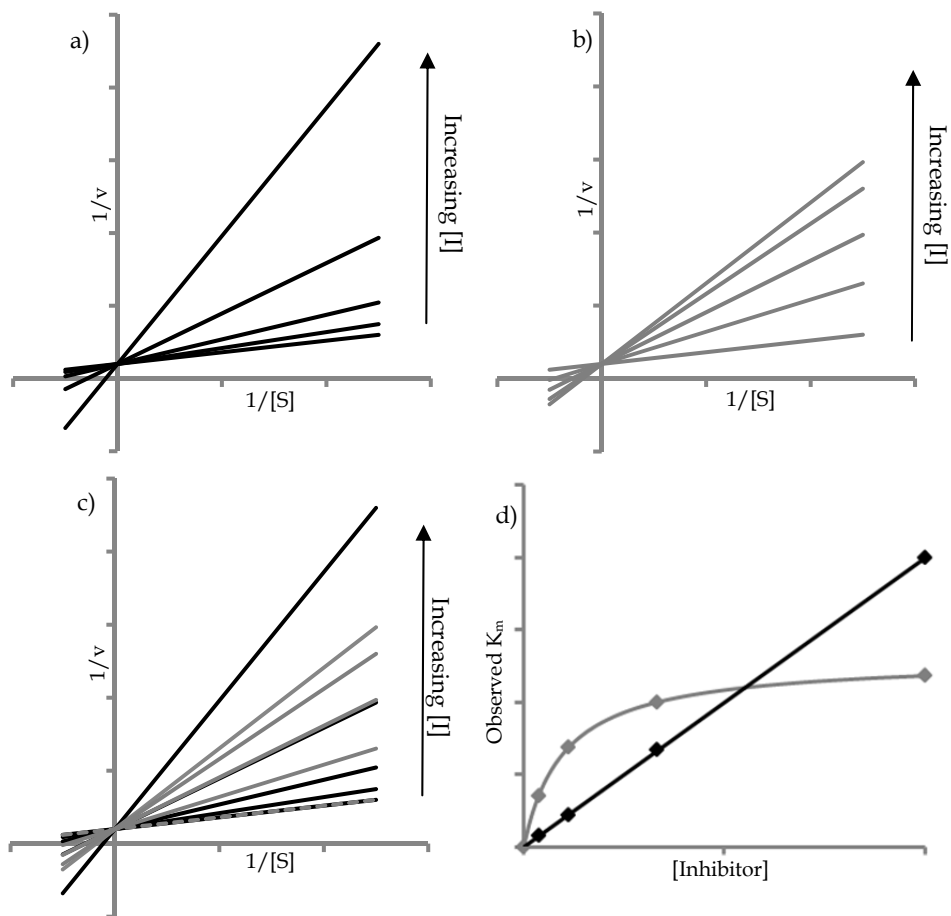

Fig. 5. Double reciprocal plots of the a) competitive inhibition equation (Equation 5), representing a continuous change in substrate affinity with increasing inhibitor concentration, b) Equation 20 representing a hyperbolic change from one substrate affinity to another as the inhibitor binds in a stoichiometric way with the enzyme, c) an overlay of the two plots and d) a plot of the shift in substrate affinity at different concentrations of the inhibitor.

This need for two inhibitor constants and the problems associated with this notation can be attributed to the inverse way in which the inhibitory terms affect the kinetic constants of the Michaelis Menten equation, as outlines in the previous section.

In addition to the unification of binding constants and introduction of governor terms, the format of Equation 21 also represents an improvement over the competitive (Equation 5), non-competitive (Equation 6) and mixed non-competitive (equation 22) equations. This is due to the structure of the modifier term in that they can be expanded in a modular format to include additional modifier effects.

3.2 Modular substrate and modifier term expansion

Cholinesterases are the prime example of enzymes that have been found to be subject to substrate modulation. Specifically, acetylcholinesterase is known to experience substrate inhibition and butyrylcholinesterase is subject to substrate activation. To model these effects, equation 23 (Reiner & Simeon-Rudolf 2000) has been used.

$$v = \frac{V_{S1}[S]}{[S]+K_{S1}} \times \frac{1 + b[S]/K_{SS1}}{1 + [S]/K_{SS1}} \tag{23}$$

This equation expresses substrate inhibition or activation in the form of a ratio (*b*) with values greater than one indicative of activation and values lower than one indicative of inhibition. This equation, while being able to describe the effects of substrate modulation fairly well, lacks the ability for easy modification so an alternative equation based on a modular expansion of the Michaelis Menten equation was proposed (Equation 24; Walsh et al., 2007).

$$v = \frac{[S]}{[S]+K_{S1}}V_{S1} - \frac{[S]}{[S]+K_{SS1}}V_{S1} + \frac{[S]}{[S]+K_{SS1}}V_{SS1} \tag{24}$$

This equation is able to produce very similar results to those of equation 23 but is also easily segregated into its components, where the first term relates to reaction rates at lower substrate concentrations, the second term is a transition term that segregates the form of the enzyme present at lower concentrations from the form present at high concentrations and the last term describes the activity of the enzyme at higher substrate concentrations. An example of the expansion used in this equation is depicted in Figure 6, where a theoretical enzyme is affected initially by substrate activation and then by substrate inhibition.

The modular way in which this equation can be expanded (Equation 25) allows for easy integration of modifier terms similar to those found in equation 21 (Walsh et al., 2007).

$$v = \frac{[S]}{[S]+K_{S1}}V_{S1} - \frac{[S]}{[S]+K_{SS1}}V_{S1} + \frac{[S]}{[S]+K_{SS1}}V_{SS1} - \cdots \frac{[S]}{[S]+K_{Sn-1}}V_{Sn-1} - \frac{[S]}{[S]+K_{Sn}}V_{Sn-1} + \frac{[S]}{[S]+K_{Sn}}V_{Sn} \tag{25}$$

It also demonstrates how the modifier term can also be expanded in a similar modular fashion (Equation 26).

$$V_{S1} - (V_{S1} - V_{S2})\left(\frac{[X]}{[X] + K_{X1}}\right) + (V_{S1} - V_{S2})\left(\frac{[X]}{[X] + K_{X2}}\right) - (V_{S1} - V_{S3})\left(\frac{[X]}{[X] + K_{X2}}\right) \quad (26)$$

This term (Equation 26) is almost identical to equation 24 except that there is an initial catalytic velocity term (V_{S1}). This rate is altered by the high affinity modifier binding term (K_{X1}), shifting the velocity term (V_{S1}) to a new velocity term (V_{S2}). Upon binding of the second modifier molecule (K_{X2}) the velocity shift caused by the single binding event (V_{S2}) disappears and the new velocity term (V_{S3}) resulting from the presence of two modifiers bound to the enzyme replaces it. For example, the modifier homocysteine thiolactone was found to be stimulatory of the non-substrate activated form of human butyrylcholinesterase at lower concentrations but inhibited the enzyme at higher concentrations (Walsh et al., 2007).
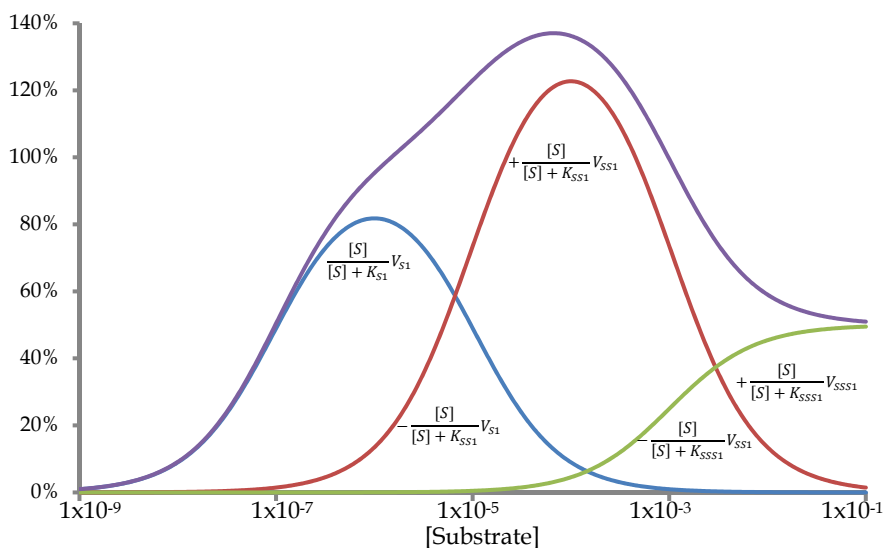

Fig. 6. Modular partitioning of enzymatic states at different concentrations. The purple curve represents the observed enzymatic activity while the other three curves represent the relative contribution to the total activity by the different forms of the enzyme. Blue represents the enzyme found at low substrate concentration where there is no substrate modulation. The red line represents substrate activation produced by secondary substrate molecule binding to the enzyme and the green represents inhibition produced by a tertiary binding event. Reaction rate is reported as values relative to the maximum activity of the enzymatic form not subject to substrate modulation (blue).

The utility of this sort of modular equation building was further expanded in a paper examining the synergistic effects of multiple inhibitors on enzymatic activity (Walsh et al., 2011b). In this example, two inhibitors of human butyrylcholinesterase were examined for their inhibitory effect individually and in combination (Figure 7; Equation 27).
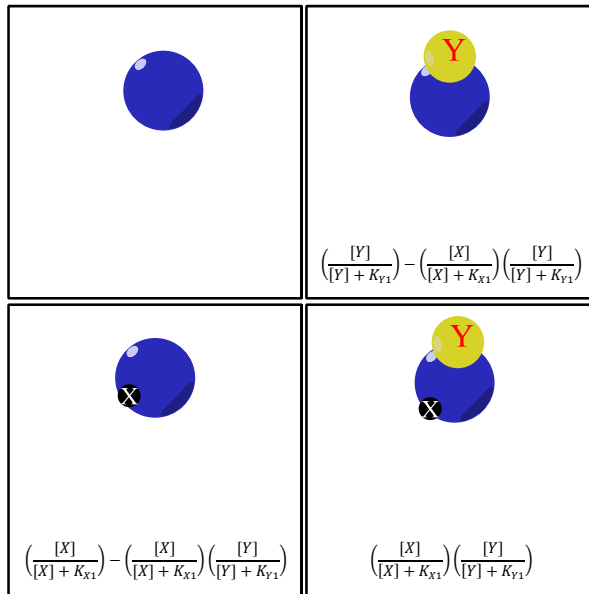
Fig. 7. Schematic representation of an enzyme bound by two inhibitors (X and Y), where the four quadrants represent the whole enzyme population divided into the inhibitor bound fractions that would be present if each inhibitors concentration were equal to its binding constant.

$$V_{S1} - (V_{S1} - V_{SX})\left(\left(\frac{[X]}{[X]+K_{X1}}\right) - \left(\frac{[X]}{[X]+K_{X1}}\right)\left(\frac{[Y]}{[Y]+K_{Y1}}\right)\right) -$$

$$(V_{S1} - V_{SY})\left(\left(\frac{[Y]}{[Y]+K_{Y1}}\right) - \left(\frac{[X]}{[X]+K_{X1}}\right)\left(\frac{[Y]}{[Y]+K_{Y1}}\right)\right) - (V_{S1} - V_{SXY})\left(\frac{[X]}{[X]+K_{X1}}\right)\left(\frac{[Y]}{[Y]+K_{Y1}}\right) \qquad (27)$$

Here the inhibitory effect on each catalytic constant was segregated into three effects, the effect that each inhibitor had individually and the effect the two inhibitors had together. Equation 27 uses the example of the non-substrate activated maximum velocity of the enzyme, however the same sort of term was applied to each of the enzymes catalytic constants (Equation 24). The first term denotes the effect the first inhibitor (X) has on the enzyme (V_{S1}-V_{SX}) multiplied by the percent of the enzyme population bound by the inhibitor but subtracting the percent of the population bound by both inhibitors (XY). The second term describes the same process but with the other inhibitor (V_{S1}-V_{SY}). The last term is the effect produced by both inhibitors binding at the same time (V_{S1}-V_{SXY}). Using this strategy, for kinetic equation generation, the effects of galantamine, an inhibitor which predominantly inhibits the non-substrate activated form of butyrylcholinesterase, and citalopram, an inhibitor of both the non-substrate activated form and the substrate activated forms of the enzyme, were modeled individually and together (Rockwood et al., 2011; Walsh et al., 2011b). The modeling of this system suggests a possible mechanism for the clinical benefit observed in the treatment of Alzheimer's disease when these drugs are prescribed together.

4. Pseudo steady state enzyme kinetic equations in time course modelling of substrate hydrolysis

4.1 First order kinetics

The decrease in substrate produced by enzymatic catalysis is exponential in nature and can be described by the first order chemical reactions (Equation 28).

$$A = A_o e^{-kt} \tag{28}$$

The equation describes the breakdown of substrate using the rate constant (k) and Euler's constant (e) to relate the decrease in substrate concentration ($A_o \rightarrow A$) with time (t). Euler's constant raised to the rate constant in this equation represents the fraction of the initial reactant present after the first time interval (k_1, Equation 29-32).

$$A = A_o e^{-k1} \tag{29}$$

$$\frac{A}{A_o} = e^{-k} \tag{30}$$

$$\frac{A}{A_o} = k_1 \tag{31}$$

$$A = A_o k_1{}^t \tag{32}$$

This notation is useful, for it now becomes trivial to define the reduction of substrate in terms of the fraction that is converted to product during each time period (1-k_2, Equation 33).

$$A = A_o (1 - k_2)^t \tag{33}$$

This results in a rate of reaction that is defined relative to the initial concentration of the reactant (Equation 34).

$$A = A_o \left(1 - \frac{v}{A_o}\right)^t \tag{34}$$

This equation can then be used to accommodate the Michaelis-Menten equation, as rates associated with the Michaelis-Menten equation exponentially decrease as substrate is catalyzed to product (Walsh et al., 2010; Equation 35).

$$A = A_o \left(1 - \frac{\frac{[S]}{[S] + K_{S1}} V_{S1}}{A_o}\right)^t \tag{35}$$

The approach was initially used to describe the inhibition of β-galactosidase by imidazole using a global kinetic data fitting approach (Walsh et al., 2010). The open structure of the expression when compared to integral forms of the Michaelis-Menten equation found in the literature allowed for the insertion of modifier terms in the same way outlined in the previous section (Walsh et al., 2010; Equation 36).

$$A = A_o \left(1 - \frac{\dfrac{[S]}{[S] + K_{S1} - (K_{S1} - K_{S2})\left(\dfrac{[X]}{[X] + K_{X1}}\right)} V_{S1} - (V_{S1} - V_{S2})\left(\dfrac{[X]}{[X] + K_{X1}}\right)}{A_o} \right)^t \quad (36)$$

Additionally, global fitting of this equation to the kinetic data was able to confirm that the inhibitor imidazole had an irreversible component to its inhibition of β-galactosidase (Kim et al., 2003) as the simple insertion of modifier terms into equation 35 was unable to describe the effect of the inhibitor on the enzyme. While the hydrolysis of substrate tended towards zero in the absence of imidazole, the introduction of the inhibitor stopped the enzymatic activity in a concentration dependent manner. Therefore it was reasoned that a certain fraction of the inhibitor bound enzyme population was inactivated by this process (Equation 37).

$$k_a \frac{[X]}{[X] + K_{X1}} \quad (37)$$

Therefore a ratio term, defining the ratio between the rate of enzyme irreversible inhibition and the rate of substrate hydrolysis was used to define the fraction of the substrate population that would be hydrolysed before complete enzyme inactivation occurred (Equation 38). An additional term defining the substrate that would persist after the enzyme was also defined (Equation 39). In these terms, equation 21 has been represented as v for simplicity.

$$A_o \left(\frac{\dfrac{v}{A_o}}{\dfrac{v}{A_o} + k_a \dfrac{[X]}{[X] + K_{X1}}} \right) \quad (38)$$

$$A_o \left(\frac{k_a \dfrac{[X]}{[X] + K_{X1}}}{\dfrac{v}{A_o} + k_a \dfrac{[X]}{[X] + K_{X1}}} \right) \quad (39)$$

Inserting these terms into equation 36 produced an equation that was able to model the decrease in substrate concentration with time and take into account the irreversible inhibitory effects of imidazole on β-galactosidase (Walsh et al., 2010; Equation 40).

$$A = A_o \left(\dfrac{\dfrac{v}{A_o}}{\dfrac{v}{A_o} + k_a \dfrac{[X]}{[X] + K_{X1}}} \right) \left(1 - \dfrac{v}{A_o \left(\dfrac{\dfrac{v}{A_o}}{\dfrac{v}{A_o} + k_a \dfrac{[X]}{[X] + K_{X1}}} \right)} \right)^t + A_o \left(\dfrac{k_a \dfrac{[X]}{[X] + K_{X1}}}{\dfrac{v}{A_o} + k_a \dfrac{[X]}{[X] + K_{X1}}} \right) \quad (40)$$

5. Conclusions

5.1 Modular enzyme kinetic equations

The modular method of equation generation discussed here does not necessarily require derivation from initial conservation of mass and rate equations that were used in the generation of classical pseudo steady state enzyme kinetic equations. Rather, by clearly distinguishing between the mass action binding terms and the governor terms, which describe the kinetic effect of modifiers, a general method to characterize the effect of inhibitors and activators on enzymatic activity is suggested (Equation 21). The structure of this modified version of the Michaelis Menten equation allows for its modular expansion to describe multiple substrate binding interactions (Equation 24), multiple modifier binding interactions (Equation 26) and the effects of more than one modifier binding to the same enzyme (Equation 27). The modular way in which these equations can be expanded to describe the bulk kinetic properties associated with enzyme kinetic modeling suggests that they may neglect processes such as modifier and substrate binding order. However there are several possibilities which may result from such processes. For example, an inhibitor may bind to an enzyme only in the absence of the substrate or only in its presence, in both of these instances the inhibition would most likely manifest as a rectangular hyperbolic change in the catalytic constants influencing enzymatic activity. Alternatively if the inhibitor binds both forms of the enzyme, the affinity for each form may be quite different resulting in a term similar to that proposed with equation 26. While there are undoubtedly many more possibilities, as have been outlined in texts such as Enzyme Kinetics by Segel (1993), the derivation of these equations have neglected the division between mass binding and modifier effect proposed here.

This distinction between mass binding and modifier effect combined with the modular equation construction described herein represents a new way of addressing enzyme kinetic modelling which permits the simple adaptation of kinetic models for data analysis. This allows for a simplified comparative global data fitting to discriminate between competing kinetic models using nonlinear regression. A helpful guide to nonlinear data fitting in excel has recently been published in Nature Protocols (Kemmer & Keller, 2010).

5.2 Pseudo-steady state equations in time course modeling

Integral forms of the Michaelis Menten equation have been proposed for use in time course analysis for many years, with more complex mathematical models appearing with time (Russell & Drane, 1992; Goudar et al., 1999). Integral forms of the Michaelis Menten equation however have been found to be limited in their usefulness for time course models

which has spurred further research (Liao et al., 2005). Integral forms of the Michaelis Menten equation also predominately model the Michaelis Menten equation and do not deal with modifier interactions. This may be in part due to the problems associated with pseudo-steady state modifier equations, such as lack of governor terms on the effects of modifiers in enzyme systems, as outlined in the first section of this chapter. To attempt to address these issues a new way of using pseudo-steady state equations in time course modeling has been proposed (Walsh et al., 2010). The proposed methodology inserts the pseudo-steady state equations directly into the exponential decay equation (Equation 35) allowing for the same degree of equation flexibility outlined with the methods for modular expansion of pseudo-steady state equations described in section 3.2.

The direct use of so called pseudo-steady state equations in exponential equations relies on several assumptions. Primarily, the development of pseudo-steady state equations has been based on experimental data generated in closed systems. That is, even if preformed in conditions where the rate of substrate hydrolysis is taken as linear or is linearized through the use of tangential slope lines, the observed rates are actually exponentially decreasing. Additionally, single substrate enzymes, which are not subject to conditions that would alter their catalytic activity, such as substrate or product modulation, as catalysts follow first order kinetics in closed systems (Equation 35). Due to this, time course modeling has the advantage of being able to identify a variety of kinetic situations, such as strong substrate activation or inhibition, for which initial rate analysis is not optimal (Shushanyan et.al., 2011). This sort of modeling can also be used to detect the influence of irreversible inhibition as deviation of the exponential curve away from the predicted initial exponential rate in substrate hydrolysis are more apparent with time course models than models using initial rates. For example, in our initial examination of the inhibition of β-galactosidase with imidazole with initial rates the irreversible inhibition of β-galactosidase was not apparent (Walsh et al., 2007), however it became quite apparent using time course models (Walsh et al., 2010).

The ease with which this method allows the integration of pseudo-steady state and time course kinetic equations holds the promise of making time course kinetic modeling a more prominent part of modifier kinetic analysis. Additionally, the modular compilation of kinetic components outlined in this chapter and their application to time course modeling suggest this form of modeling may be particularly useful for in-depth characterization of enzymatically regulated pathways which is directly applicable to systems biology.

6. References

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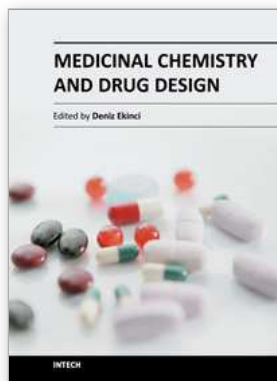
Medicinal Chemistry and Drug Design

Edited by Prof. Deniz Ekinci

Over the recent years, medicinal chemistry has become responsible for explaining interactions of chemical molecules processes such that many scientists in the life sciences from agronomy to medicine are engaged in medicinal research. This book contains an overview focusing on the research area of enzyme inhibitors, molecular aspects of drug metabolism, organic synthesis, prodrug synthesis, in silico studies and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in medicinal chemistry and drug design. Particular emphasis is devoted to both theoretical and experimental aspect of modern drug design. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in associated areas. The textbook is written by international scientists with expertise in chemistry, protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some medicinal approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of medicinal chemistry and drug design.

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