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Unlocking Complexity: The Versatility of Substrate Modulation Equations in Enzyme Analysis

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Abstract

Substrate modulation is the modification of an enzyme's activity either through inhibition or activation by its substrate. Substrate modulation plays key roles in the functioning of complex biological systems, such as the regulation of acetylcholine, where the two cholinesterases with opposite substrate regulatory mechanisms exist to control acetylcholine's concentration and distribution throughout the body. The importance of these systems is generally ignored in therapeutic development due to a lack of appropriate models for inhibitor interactions with enzymes that are modulated by their substrate. Here we will examine the most prevalent equation used to describe substrate modulation and by rearranging it we will demonstrate the ease with which it can be modified to model inhibitor or activator effects. In so doing we will establish an easy method for deriving the rearranged equation, providing an expandable framework for deriving equations with unlimited substrate modulation terms.

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Substrate inhibition and substrate activation are of major importance in the understanding of enzymatic regulation but are largely ignored by most researchers and drug developers. This is very evident in the development of cholinesterase inhibitors for Alzheimer's disease where drug characterization completely disregards the potential effects drugs may have on the substrate-modified forms of the enzymes. This is due in part to the lack of suitable equations for modeling inhibitor interactions with enzymes that experience substrate modulation.

Substrate modulation studies are generally fit to the equation proposed by Radic et al., in 1993 (Equation 1).

Eq 1

$$v = \frac{[S]}{k_M + [S]} V_{max} \left(\frac{1 + b\frac{[S]}{k_{SS}}}{1 + \frac{[S]}{k_{SS}}} \right)$$

This equation only describes changes in the enzyme's activity based on the binding of substrate to the enzyme's allosteric site. This equation does not describe the effect of inhibitors or activators even though it is based on the equation for nonessential activators proposed by Webb, in 1963 (Radic et al., 1993). While the derivation of this equation is not disclosed, both the substrate modulation equation (1) and the nonessential activator equation are based on the notion that the enzyme has a normal catalytic rate that is altered by the binding of an activator or a secondary substrate. This alteration is described with the constant *b* in equation 1, and results in a basic rate equation where the substrate is converted to a product based on catalysis by two enzyme forms, ES and ESS (Webb, 1963; equation 2)

Eq 2

$$v = k_{cat1}[ES] + bk_{cat1}[ESS]$$

To reveal the direct effect *b* has on the reaction rate of the substrate modulation equation (1) we can perform a simple rearrangement (equations 3 & 4)

Eq 3

$$v = \frac{[S]}{k_M + [S]} V_{max} \left(\frac{k_{SS} + b[S]}{k_{SS} + [S]} \right)$$

Eq 4

$$v = \frac{[S]}{k_M + [S]} V_{max} b \left(\frac{\frac{k_{SS}}{b} + [S]}{k_{SS} + [S]} \right)$$

Here, we end up with an equation (4) that defines the binding of the substrate to the enzyme's active site with the Michaelis Menten equation (Michaelis & Menten 1913; equation 5).

Eq 5

$$v = \frac{[S]}{k_M + [S]} V_{max}$$

While binding to the allosteric site in the substrate modulation equation (4) is now defined with an unusual-looking binding curve based on the allosteric site's binding constant (k_{ss}; equation 6).

Eq 6

$$\left(\frac{\frac{k_{SS}}{b}+[S]}{k_{SS}+[S]}\right)$$

In this rearrangement *b* directly multiplies the maximum velocity (equation 4) and is defined as the efficiency of hydrolysis for the allosterically bound enzyme (Radic et a., 1993). As such values for *b* are relative to the rate of the maximum velocity of the enzyme. Therefore, values less than one reduce activity, producing substrate inhibition, while values greater than one increase the activity, and are therefore used to describe substrate activation.

As stated above the binding curve for allosteric binding is unusual in that the numerator has a term where the allosteric binding constant is divided by the hydrolysis efficiency (equation 6). To understand what this term is we can expand the allosteric binding term (equations 7 & 8).

Eq 7

$$v=\frac{[S]}{k_M+[S]}V_{max}b\left(\frac{\frac{k_{SS}}{b}}{k_{SS}+[S]}+\frac{[S]}{k_{SS}+[S]}\right)$$

Eq 8

$$v=\frac{[S]}{k_M+[S]}V_{max}b\left(\frac{1}{b}\left(\frac{k_{SS}}{k_{SS}+[S]}\right)+\frac{[S]}{k_{SS}+[S]}\right)$$

With this expansion, we get an inverted binding curve where the allosteric binding constant is divided by itself and the substrate concentration (equation 9).

Eq 9

$$\left(\frac{k_{SS}}{k_{SS}+[S]}\right)$$

Rearranging this term, we will add zero to the numerator in the form of the substrate concentration minus itself (equation 10).

Eq 10

$$v=\frac{[S]}{k_M+[S]}V_{max}b\left(\frac{1}{b}\left(\frac{k_{SS}+[S]-[S]}{k_{SS}+[S]}\right)+\frac{[S]}{k_{SS}+[S]}\right)$$

We can now reduce this term to one minus the binding curve for the allosteric site (equation 11).

Eq 11

$$v=\frac{[S]}{k_M+[S]}V_{max}b\left(\frac{1}{b}\left(1-\frac{[S]}{k_{SS}+[S]}\right)+\frac{[S]}{k_{SS}+[S]}\right)$$

This form of the equation implies that changes in enzymatic activity result from a reduction in the regular enzyme activity by the fraction of $1/b$ to any change induced by the allosteric binding, so we can further simplify this by reintegrating b into the equation (equation 12).

Eq 12

$$v=\frac{[S]}{k_M+[S]}V_{max}\left(1-\frac{[S]}{k_{SS}+[S]}+b\frac{[S]}{k_{SS}+[S]}\right)$$

This can now be expanded to show the effect this term has on the V_{max} (equation 13).

Eq 13

$$v = \frac{[S]}{k_M + [S]}\left(V_{max} - V_{max}\frac{[S]}{k_{SS} + [S]} + bV_{max}\frac{[S]}{k_{SS} + [S]}\right)$$

This gets us to an expression that clearly shows the change induced by allosteric binding, where the catalytic rate produced by the enzyme is replaced by the rate of the allosterically bound enzyme based on substrate binding to the allosteric binding site (Walsh et al., 2007; Walsh 2012).

Cleaning up the equation we will replace the V_{max}, bV_{max}, and K_M with V_S, V_{SS}, and K_S respectively, to emphasize the form of the enzyme the terms relate to (equation 14).

Eq 14

$$v = V_S\frac{[S]}{[S] + K_S} - V_S\frac{[S]}{[S] + K_{SS}} + V_{SS}\frac{[S]}{[S] + K_{SS}}$$

While this form of the equation is mathematically equivalent to the form proposed by Radic et al., (1993; equation 1), there are several factors that make it superior for the analysis of substrate modulation. The equation in this form is more intuitive, clearly revealing the effect the allosteric binding curve has on the reaction rate (Figure 1).

Figure 1.

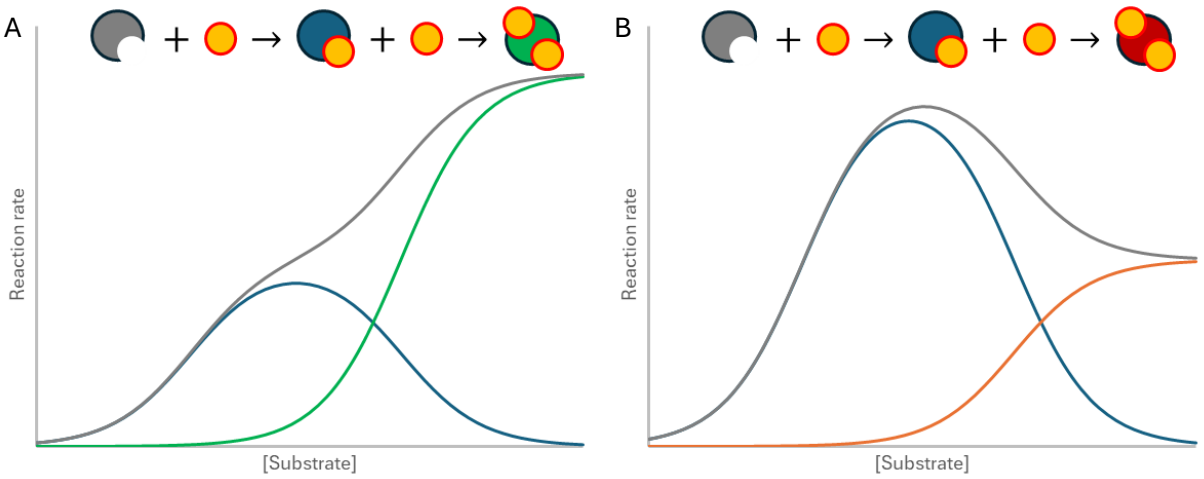

Fig 1. By clearly distinguishing between the binding and effect of the secondary substrate interaction, the effect of inhibitors or activators can be examined based on the specific enzymatic form. A) schematic of the effect of allosteric substrate activation, B) schematic of the effect of allosteric substrate inhibition.

In addition, the clear representation of the substrate binding curves allows natural modification of these curves for the study of inhibitor or activator effects on either form of the substrate-modulated enzyme (Walsh et al., 2007; Walsh 2012; Walsh 2024). By simply adding terms describing inhibition or activation of the enzyme form free from allosteric regulation (equations 15 & 16) in parallel with terms affecting the substrate-modulated form (equations 17 & 18) an intuitive equation describing modifier effects can be produced (Walsh 2012).

Eq 15

$$V_S - \Delta V_S \frac{[X]}{[X] + K_{xs}}$$

Eq 16

$$K_S - \Delta K_S \frac{[X]}{[X] + K_{xs}}$$

Eq 17

$$V_{SS} - \Delta V_{SS} \frac{[X]}{[X] + K_{xss}}$$

Eq 18

$$K_{SS} - \Delta K_{SS} \frac{[X]}{[X] + K_{xss}}$$

Also, this expanded form of the substrate modulation equation (14) provides a simple path for its derivation and the derivation of equations for multiple substrate interactions.

Derivation of an iteratively expandable substrate modulation equation

To derive the expanded substrate modulation equation, we need to define the rate equation of substrate hydrolysis very similarly to the rate used to generate the nonessential activator equation (Webb, 1963; equation 2). But in this case, we will replace the constant *b* and use a term for the change in catalytic activity instead (Δk_{cat1}; equation 19).

Eq 19

$$v = k_{cat1}[ES] - \Delta k_{cat1}[ESS]$$

This changes the comparative scalar value of *b* to an absolute change in the catalytic rate that we will continue to expand by adding in the catalytic rate for the allosterically regulated form of the enzyme (k_{cat2}). With this addition, we can now define the rate as a shift between the catalytic rates associated with each form of the enzyme (equation 20).

Eq 20

$$v = k_{cat1}[ES] - (k_{cat1} - k_{cat2})\,[ESS]$$

At this point deriving our rate equation further could result in confusion if we tried to derive it in the traditional fashion. When deriving the Michaelis Menten equation or equations for inhibitor and activator interactions, at this point we would introduce a conservation of mass equation defining the states the enzyme can be found in (Equation 21; Walsh 2024). This would introduce the total enzyme concentration into the mix allowing us to eventually define the maximum reaction rate (V_{max}) for our enzyme population.

Eq 21

$$[E]_{total} = [E] + [ES] + [ESS]$$

In this case, proceeding with the derivation based on the total enzyme population would be confusing. But if we think about this as the chemical binding equilibrium of substrate interacting with two separate binding sites, the derivation becomes much simpler. In this case, we can define

substrate binding to the active site as equation (22) and we can define binding to the allosteric binding site as equation (23).

Eq 22

$$[active\ site]_{total} = [E] + [ES]$$

Eq 23

$$[allosteric\ site]_{total} = [ES] + [ESS]$$

These equations can be equated to the total enzyme population with the assumption that the enzyme has one active site and one allosteric site. This allows us to essentially treat them independently in the derivation, akin to deriving two Michaelis Menten equations at the same time. From here we can define our chemical equilibriums for substrate binding to the active site (equation 24) and substrate binding to the allosteric site (equation 25).

Eq 24

$$K_S = \frac{[E][S]}{[ES]}$$

Eq 25

$$K_{SS} = \frac{[ES][S]}{[ESS]}$$

Following this we can rearrange the chemical equilibriums to define binding to the active site in terms of the free enzyme concentration (equation 26) and binding to the allosteric site in terms of the enzyme-substrate complex (equation 27).

Eq 26

$$[E] = \frac{K_S[ES]}{[S]}$$

Eq 27

$$[ES] = \frac{K_{SS}[ESS]}{[S]}$$

We can now insert these into the equations we use for defining the binding sites (equations 22, 23) to produce equations (28 & 29)

Eq 28

$$[active\ site]_{total} = \frac{K_S[ES]}{[S]} + [ES]$$

Eq 29

$$[allosteric\ site]_{total} = \frac{K_{SS}[ESS]}{[S]} + [ESS]$$

Which we can then factor (equations 30 & 31)

Eq 30

$$[active\ site]_{total} = [ES]\left(\frac{K_S}{[S]} + 1\right)$$

Eq 31

$$[allosteric\ site]_{total} = [ESS]\left(\frac{K_{SS}}{[S]} + 1\right)$$

We can follow this up by rearranging to define the enzyme-substrate complexes (equations 32 & 33).

Eq 32

$$[ES] = \frac{[active\ site]_{total}}{\left(\frac{K_S}{[S]} + 1\right)}$$

Eq 33

$$[ESS] = \frac{[allosteric\ site]_{total}}{\left(\frac{K_{SS}}{[S]} + 1\right)}$$

This allows us to insert these equations into our rate equation (equation34).

Eq 34

$$v = k_{cat1}\frac{[active\ site]_{total}}{\left(\frac{K_S}{[S]} + 1\right)} - (k_{cat1} - k_{cat2})\frac{[allosteric\ site]_{total}}{\left(\frac{K_{SS}}{[S]} + 1\right)}$$

We can then multiply by the substrate concentration (equation 35).

Eq 35

$$v = k_{cat1}\frac{[active\ site]_{total}[S]}{[S] + K_S} - (k_{cat1} - k_{cat2})\frac{[allosteric\ site]_{total}[S]}{[S] + K_{SS}}$$

Now to get to our maximum reaction rates we must use the same assumption we used above, that the total enzyme population is equal to the total number of active sites and the total number of allosteric sites (equation 36).

Eq 36

$$[E]_{total} = [active\ site]_{total} = [allosteric\ site]_{total}$$

This allows us to get the maximum enzyme catalytic activity for the enzyme-substrate complex when we multiply the catalytic rate by the concentration of the active sites or the allosteric sites (equation 37).

Eq 37

$$v = V_S\frac{[S]}{[S] + K_S} - (V_S - V_{SS})\frac{[S]}{[S] + K_{SS}}$$

If we were to expand this further, we end up with equation (14), demonstrating the ease with which this equation can be derived. As is demonstrated above through the parallel derivation of binding to the active site and allosteric site, this approach can be used to derive equations for as many binding sites as required experimentally. For example, the substrate inhibition and substrate activation associated with cholinesterases can be modeled with the substrate modulation equation

(14) but γ-secretase requires a third binding interaction to account for substrate activation and inhibition affecting the same enzyme (Walsh, 2014).

Conclusions

Here we have shown that the commonly used equation for modeling substrate modulation (equation 1) can be easily rearranged into a form that clearly distinguishes active site binding from binding to the allosteric site (equation 14). This distinction between the substrate-modulated forms allows modeling of the effects of inhibitors or activators on these distinct forms. Distinguishing between the substrate interactions with their binding curves also simplifies the derivation of these equations resulting in a framework for modeling complex biological interactions. This framework for analysing biological interactions presents a unified standard way of comparing complex enzymatic mechanisms across biological systems. Such an approach has been missing from the literature due to the common practice of deriving new equations from scratch, using traditional assumptions, assumptions that are heavily flawed (Walsh 2024). Expanding on this approach should provide a better understanding of biological systems regulated by enzymatic activity.

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