

SYNAPTIC MODELING : COFACTOR MODIFICATIONS

to

MICHAELIS-MENTEN KINETICS

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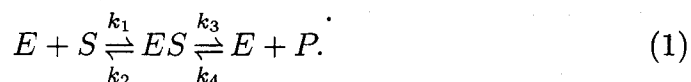
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Running head: Enzyme kinetic theory

In order to construct mathematical models for the dynamical operation of central nervous system synapses, accurate chemical kinetic theory is required for enzymatic reactions. The Michaelis-Menten formula for the rate of an enzymatic reaction is modified when there are cofactors involved. We distinguish the cases of essential and non-essential cofactors. In the case of an essential cofactor, the formula for the reaction rate is found to depend on the relative magnitudes of the concentrations of the primary enzyme and the cofactor, extending a result previously employed by various authors. The case of a non-essential cofactor is more complex and leads to reaction rate formulas which depend explicitly on the concentration of both the primary enzyme and the cofactor. The relative magnitudes of the concentrations of the latter two substances again determines the functional dependence of the reaction rate on these quantities. Simplified expressions are obtained under certain assumptions on a parameter describing the relative efficacies of the enzyme alone and the enzyme with cofactor. An example is given which arises from a mathematical model of a dopaminergic synapse, namely the conversion of tyrosine to DOPA via tyrosine hydroxylase and its biopterin cofactor.

1. Introduction

Let S be a substrate, E an enzyme and P be reaction product in the classical scheme



Then the Michaelis-Menten formula for the rate of reaction is

$$v = \frac{v_{max}}{1 + \frac{K_m}{(S)}}, \quad (2)$$

where v_{max} is the maximum possible rate,

$$K_m = \frac{k_2 + k_3}{k_1}, \quad (3)$$

and (S) denotes the concentration of S . (We will use round brackets throughout to denote concentration.) Derivation of formula (2) can be found, for example, in Segel(1976) and Westley(1969). A stochastic treatment of (1) involving a multi-dimensional birth and death process was formulated and solved by Heyde and Heyde(1969).

There are several biochemical schemes where the basic formula (2) does not apply. For example, if there is a competitive inhibitor I of the enzyme E according to the scheme



then the formula for the reaction rate becomes

$$v = \frac{v_{max}}{1 + \frac{K_m}{(S)} \left(1 + \frac{(I)}{K_I} \right)} \quad (5)$$

where

$$K_I = \frac{k_{2,I}}{k_{1,I}}. \quad (5A)$$

Formula (5) is also derived in the references cited above.

A study of reaction rates is important in neurochemistry and in particular for quantitative investigations of synaptic dynamics and transmission.

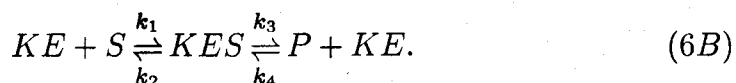
For example, complex mathematical models of dopaminergic synapses have been proposed and analyzed by Porenta and Riederer(1982), Justice, Nicolaysen and Michael(1988) and Nicolaysen,Ikeda,Justice and Neill(1988). These models are composed of systems of ordinary differential equations and incorporate modified Michaelis-Menten formulas due to competitive inhibitors and cofactors as well as various transport processes. However, it seems that further studies could be profitably made in the consideration of how cofactors alter the basic Michaelis-Menten theory. It is hoped to demonstrate that the expressions for the reaction rates depend on the relative concentrations of the cofactor and the primary enzyme and also on the reaction schemes underlying the mechanism of action of the cofactor.

We will examine two such reaction schemes and derive modified Michaelis-Menten formulas for various concentrations of enzyme and cofactor. In the last section we will illustrate the application of the results by considering aspects of dynamical models of dopaminergic synapses.

2. An Essential Cofactor

It is well known(Westley,1969)that the functioning of many enzymes requires the presence of small amounts of substances called cofactors. Cofactors are further classified as coenzymes or prosthetic groups. Here we adopt a classification into either (a) *an essential cofactor*, in which the primary enzyme does not function without the presence of the cofactor, with which it must first react to form an effective enzyme-cofactor complex ; or (b), *a non-essential cofactor*, without which the primary enzyme may function but whose functioning is enhanced when binding of cofactor and primary enzyme occurs to form a complex.

Let K denote the cofactor, E the primary enzyme, S the substrate and P the end product. Then in the case where the cofactor is *essential* we have the following two reaction schemes,



Here the cofactor must form the complex KE with the primary enzyme in order to produce an effective enzyme complex KE . The latter forms an intermediate complex KES which gives rise to the product P . Note that the form of KE may be altered in progressing from left to right in (6B) but this does not affect the subsequent development.)

We follow the usual approach via steady states. From (6A), assuming at equilibrium the rates of synthesis and decomposition of KE are equal in magnitude, we have by standard reasoning (Segel,1976)

$$\frac{(K)(E)}{(KE)} = K_K, \quad (7)$$

where

$$K_K = \frac{k_{2,K}}{k_{1,K}}, \quad (8)$$

and from (6B),

$$\frac{(KE)(S)}{(KES)} = K_m, \quad (9)$$

where K_m is as before.

We now distinguish two cases. The first, case (i), is that where the cofactor concentration is less than that of the primary enzyme; that is $(K) < (E)$; in the second case, case (ii), we have $(K) > (E)$. The case $(K) = (E)$ can be obtained from either case (i) or (ii). One expects that in most circumstances the first case would be appropriate, but the second will be shown to be of interest also and may occur under certain conditions.

Case (i), $(K) \leq (E)$.

In this case we expect the maximal velocity of the reaction to be proportional to the total concentration of K because there may always be some E left over. Now, the total concentration of K is given by

$$(K_{tot}) = (K) + (KE) + (KES). \quad (10)$$

Invoking the usual arguments, the maximal velocity must be proportional to (K_{tot}) and the speed at less than maximal values must be proportional to

the concentration of the intermediate complex KES . Denoting the speed of reaction by v and the maximal speed by v_{max} , we have therefore

$$\frac{v_{max}}{v} = \frac{K_{tot}}{(KES)}, \quad (11)$$

or

$$\frac{v_{max}}{v} = 1 + \frac{(KE)}{(KES)} + \frac{(K)}{(KES)}. \quad (12)$$

Now the second term on the right is just $K_m/(S)$ from (9) and we have, also using (7), that

$$\frac{(K)}{(KES)} = \frac{K_m}{(S)} \cdot \frac{K_K}{(E)}. \quad (13)$$

Thus (12) becomes

$$\frac{v_{max}}{v} = 1 + \frac{K_m}{(S)} \left(1 + \frac{K_K}{(E)} \right), \quad (14)$$

Thus the reaction speed at substrate concentration (S) and enzyme concentration (E) is

$$v = \frac{v_{max}}{1 + \frac{K_m}{(S)} \left(1 + \frac{K_K}{(E)} \right)}. \quad (15)$$

This can be compared with the Michaelis-Menten formula (2) which it approaches as $(E) \rightarrow \infty$ for fixed (S) . We will now consider the alternative case for an essential cofactor.

Case (ii), $(K) \geq (E)$.

In the event that $(K) \geq (E)$, the maximal reaction rate must be determined by (E_{tot}) and we have

$$\frac{v_{max}}{v} = \frac{(E_{tot})}{(KES)}. \quad (16)$$

Now since

$$(E_{tot}) = (E) + (KE) + (KES), \quad (17)$$

(16) can be written

$$\frac{v_{max}}{v} = 1 + \frac{(KE)}{(KES)} + \frac{(E)}{(KES)}. \quad (18)$$

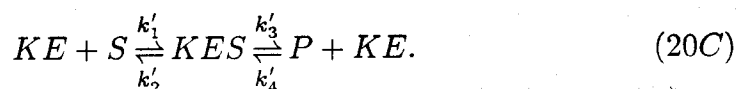
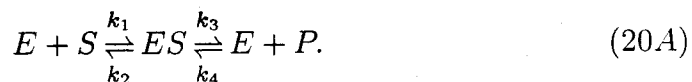
Thus, simplifying further gives, on inversion and rearrangement,

$$v = \frac{v_{max}}{1 + \frac{K_m}{(S)} \left(1 + \frac{K_K}{(K)} \right)}. \quad (19)$$

In the case $(K)=(E)$ we can use either (15) or (19). Equation (19) ($(K) \geq (E)$) is the same as that employed by Poreta and Riederer (1982) and Justice et al. (1988). We now turn to the situation in which the reaction proceeds with or without a cofactor.

3. A non-essential cofactor

We here let both E and EK be effective in the enzymatic process but with different efficacies, that of EK being expected to be greater. efficacious. Thus we consider the trio,



(It is possible that the reactions



could also occur but we will not consider this here.)

The usual steady state considerations give

$$\frac{(E)}{(ES)} = \frac{K_m}{(S)}, \quad (21)$$

$$\frac{(KE)}{(KES)} = \frac{K'_m}{(S)}, \quad (22)$$

and

$$\frac{(K)(E)}{(KE)} = K_K, \quad (23)$$

where

$$K'_m = \frac{k'_2 + k'_3}{k'_1}, \quad (24)$$

$$K_K = \frac{k_{2,K}}{k_{1,K}}. \quad (25)$$

Utilizing the usual procedures we see that the reaction speed is

$$v = k_3(ES) + k'_3(KES), \quad (26)$$

so there remains to find the relation between the speed and the maximal speed. Again we will distinguish the cases $(K) \leq (E)$ and $(K) \geq (E)$.

Case (i). $(K) \leq (E)$

In the case where the concentration of the cofactor is less than that of the enzyme, there can be, in the condition of maximal reaction, left over E to produce P without K. Thus we have

$$v_{max} = k'_3(K_{tot}) + k_3((E_{tot}) - (K_{tot})). \quad (27)$$

Here we have the consistency check when $(K_{tot}) = 0$ which gives $v_{max} = k_3(E_{tot})$ as it is in the absence of a cofactor. Furthermore, with (E_{tot}) fixed,

$$\frac{dv_{max}}{d(K_{tot})} = k'_3 - k_3. \quad (28)$$

Thus the maximal velocity increases linearly with increasing (K_{tot}) - we always expect $k'_3 - k_3 > 0$ - from the value $k_3(E_{tot})$ when there is no cofactor present, to a maximum value which is greater. The greater the value of (E_{tot}) . We may now put

$$\frac{v_{max}}{v} = \frac{k'_3(K_{tot}) + k_3((E_{tot}) - (K_{tot}))}{k'_3(KES) + k_3(ES)}. \quad (29)$$

Let us define

$$T_1 = \frac{(k'_3 - k_3)[(K) + (KE) + (KES)]}{k'_3(KES) + k_3(ES)} \quad (30)$$

and

$$T_2 = \frac{k_3 [(E) + (ES) + (KE) + (KES)]}{k'_3(KES) + k_3(ES)}, \quad (31)$$

so that

$$\frac{v_{max}}{v} = T_1 + T_2. \quad (32)$$

To evaluate T_1 we substitute for (KES) to first obtain

$$T_1 = \frac{(k'_3 - k_3) \left[(K) + \frac{(K)(E)}{K_K} + \frac{(KE)(S)}{K_m} \right]}{\left(k'_3 \frac{(KE)(S)}{K_m} + k_3 \frac{(K)(E)}{K_K} \right)}. \quad (33)$$

On further substitution and simplification of the resulting expression and on treating T_2 similarly we finally obtain the following expression for the speed of reaction $v = v_{max}/(T_1 + T_2)$ in the case $(K) \leq (E)$:

$$v = \frac{v_{max}}{\frac{(k'_3 - k_3) \left[1 + \frac{(E)}{K_K} \left(1 + \frac{(S)}{K_m} \right) \right]}{\frac{(E)}{K_K} \left(k_3 + k'_3 \frac{(S)}{K_m} \right)} + \frac{k_3 \left[1 + \frac{(S)}{K_m} + \frac{(K)}{K_K} \left(1 + \frac{(S)}{K'_m} \right) \right]}{\left(k'_3 \frac{(K)(S)}{K_K K'_m} + k_3 \frac{(S)}{K_m} \right)}}. \quad (34)$$

Let us introduce the parameter

$$\alpha = \frac{k'_3}{k_3}, \quad (35)$$

which reflects the relative efficacies of KES and ES in producing the product P. We expect $\alpha > 1$.

$$v = \frac{v_{max}}{\frac{(\alpha - 1) \left[1 + \frac{(E)}{K_K} \left(1 + \frac{(S)}{K_m} \right) \right]}{\frac{(E)}{K_K} \left(1 + \alpha \frac{(S)}{K_m} \right)} + \frac{\left[1 + \frac{(S)}{K_m} + \frac{(K)}{K_K} \left(1 + \frac{(S)}{K'_m} \right) \right]}{(S) \left(\frac{1}{K_m} + \alpha \frac{(K)}{K_K K'_m} \right)}}. \quad (36)$$

Simplification of this formula is possible under the following assumptions:

$$\alpha \gg 1, \quad (37A)$$

$$\alpha \gg \frac{K_m}{(S)}, \quad (37B)$$

$$\alpha \gg \frac{K'_m K_K}{K_m (K)}. \quad (37C)$$

We then have

$$v \approx \frac{v_{max}}{1 + \frac{K_m}{(S)} \left(1 + \frac{K_K}{(E)}\right) + \frac{1}{\alpha} \left(1 + \frac{K'_m}{(S)} + \frac{K_K K'_m}{(K) K_m}\right)}, \quad (38)$$

or, if the second term in the denominator of this expression is also negligible compared to the first, then (38) reduces to

$$v \approx \frac{v_{max}}{1 + \frac{K_m}{(S)} \left(1 + \frac{K_K}{(E)}\right)}. \quad (39)$$

That is, it is approximated by (15) which is the formula for the case where the cofactor is essential. This provides a useful consistency check because $\alpha \gg 1$ implies that the enzyme operating by itself without the cofactor is very feeble compared to its acting with the cofactor. However, the condition $\alpha \gg 1$ is not the only requirement for the applicability of the approximation (38) or (39) because these expressions are also based on (37B) and (37C).

Case (ii). $(K) \geq (E)$

Although it is unlikely under usual conditions that a coenzyme would be in greater concentration than the primary enzyme, we include this case for completeness. The term $k_3((E_{tot}) - (K_{tot}))$ is then missing in (29) and we have

$$\frac{v_{max}}{v} = \frac{k'_3(K_{tot})}{k'_3(KES) + k_3(ES)}. \quad (40)$$

After some substitutions and simplifications the reaction speed is found to be

$$v = v_{max} \left[\frac{1}{1 + \frac{K'_m}{(S)} \left(1 + \frac{K_K}{(E)}\right)} + \frac{1}{\alpha \left[\frac{(K)}{K_K} \left(1 + \frac{K'_m}{(S)}\right) + \frac{(K) K'_m}{(E)(S)} \right]} \right]. \quad (41)$$

4. Discussion and Application at Dopaminergic Synapses

In Section 2, where we examined the case of an essential cofactor, we saw that expression for the reaction speed depends on whether the primary enzyme or the cofactor is in greater concentrations. Both expressions share, with formula (4) for the case of a competitive inhibitor, the feature that $\frac{K_m}{(S)}$ in the original Michaelis-Menten expression is multiplied by a factor of the form $1 + X$.

In the case of the competitive inhibitor I, $X = \frac{(I)}{K_I}$ so as $(I) \rightarrow \infty$ the reaction speed decreases and in the limiting case approaches $v = 0$.

For the case of an essential cofactor, case (i), with $(K) \leq (E)$ we see from (15), $X = \frac{K_K}{(E)}$ so that as $(E) \rightarrow \infty$ we have $X \rightarrow 0$ and v increases to eventually attain the usual Michaelis-Menten form. Similarly, when the cofactor is essential (case (ii)) and $(K) \geq (E)$, we see from (19) that $X = \frac{K_K}{(K)}$ so that $X \rightarrow 0$ as $(K) \rightarrow \infty$ and again in the limit the original Michaelis-Menten form is attained.

Let us now turn our attention to some of the neurochemistry of dopamine synapses, whose study motivated the present investigation. Mathematical models of such synapses have included the chemical kinetics of various reactions in the complex sequences of chemical and transport processes which occur at such synapses. In the synaptic terminal, tyrosine, S , is the substrate for the enzyme *tyrosine hydroxylase*, TH , in the production of *dihydroxyphenylalanine*, $DOPA$:-



However, according to Snyder(1976) the activity of TH is relatively feeble and a *biopterin* cofactor is required to make the reaction proceed rapidly enough. This reaction along with the cofactor forms one of the components of the mathematical models of dopaminergic synapses alluded to above. In addition, according to Weiner and Molinoff(1989), molecular oxygen is implicated in this reaction also, but it has not appeared in the aforementioned models. The reaction after (42) in the functional neurochemistry of the dopamine synapse is the conversion of $DOPA$ to *dopamine* by means of the enzyme *dopa-decarboxylase* - see Porenta and Riederer(1982) and

Justice et al (1988).

In the approaches adopted so far the formula used for the rate of production of DOPA has been of the form of (19)-

$$\frac{d(DOPA)}{dt} = \frac{v_{max}}{1 + \frac{K_m}{(TYR)} \left(1 + \frac{K_K}{(K)} \right)}, \quad (43)$$

where (K) is the concentration of the cofactor, biopterin. We note that the concentration of the cofactor was assumed to be an invariant in the above cited studies.

However, let us assume that the cofactor is in fact essential. Then since we expect $(K) < (TH)$ to apply, then, instead of (43) we should, according to the results of Section 2, employ the formula

$$\frac{d(DOPA)}{dt} = \frac{v_{max}}{1 + \frac{K_m}{(TYR)} \left(1 + \frac{K_K}{(TH)} \right)}. \quad (44)$$

However, the above remarks of Snyder(1976) imply that TH is effective by itself, albeit not so effective as with the cofactor. Thus we should perhaps employ the results for a nonessential cofactor. The rate of change of the $DOPA$ concentration should then be ,

$$v = \frac{v_{max}}{\frac{(\alpha-1) \left[1 + \frac{(TH)}{K_K} \left(1 + \frac{(TYR)}{K_m} \right) \right]}{\frac{(TH)}{K_K} \left(1 + \alpha \frac{(TYR)}{K_m} \right)} + \frac{\left[1 + \frac{(TYR)}{K_m} + \frac{(K)}{K_K} \left(1 + \frac{(TYR)}{K'_m} \right) \right]}{(TYR) \left(\frac{1}{K_m} + \alpha \frac{(K)}{K_K K'_m} \right)}}. \quad (45)$$

or, if conditions (37A-C) apply,

$$\frac{d(DOPA)}{dt} = \frac{v_{max}}{1 + \frac{K_m}{(TYR)} \left(1 + \frac{K_K}{(TH)} \right) + \frac{1}{\alpha} \left[1 + \frac{K'_m}{(TYR)} + \frac{K_K K'_m}{(K) K_m} \right]}. \quad (46)$$

Porenta and Riederer(1982) have also included the effects of two competitive inhibitors of tyrosine hydroxylase but these were ignored in the treatment of Justice et al. (1988). These and other factors have been incorporated into these authors' expressions for $\frac{d(DOPA)}{dt}$ so that their formulas

are more complex than the one we have considered here for the core reaction (42).

It is clear that if the equilibrium points of the system of nonlinear ordinary differential equations used to model a dopaminergic synapse are sought, then the solutions obtained will be substantially different if the system is based on the assumption that (44) rather than (43) is appropriate for the core reaction. This must be true because $1 + \frac{K_K}{(K)}$ is expected to be much larger than $1 + \frac{K_K}{(TH)}$. Similarly, if one employs (45) or (46), possibly modified by the existence of competitive inhibitors and other important components of a dynamical model of a synapse such as pumps, then one will obtain a quite different set of solutions for the equilibrium points from that obtained previously. It is hoped that a quantitative analysis will be reported in a forthcoming publication.

We remark that we have derived, in Section 2, for the simpler case of an essential cofactor, two formulas, namely (15) and (19) for the cases $(K) \leq (E)$ and $(K) \geq (E)$ respectively. Despite the fact that the first of these conditions is the more likely to hold, it seems that the formula for the opposing case has formed the basis of models designed to quantitatively study the dynamics of dopaminergic synapses. Finally, we point out that it is natural to try to ascertain the nature of the dependence of v on the values of (K) and (E) . However, the expression for v contains v_{max} which enters as if it is a constant whereas one expects v_{max} to depend on the amounts of enzyme and co-enzyme present. For example, if there is no cofactor present in the case where the cofactor is essential then v_{max} must be zero. This complication makes it difficult to determine the equilibrium points as functions of the concentrations of the various components.

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