Partition Analysis and the Concept of Net Rate Constants as Tools in Enzyme Kinetics†

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ABSTRACT: Analysis of partitioning is used to calculate net rate constants for chemical reaction or transfer of label, and these net rate constants are used to compute *V*, *V/K*, and rate expressions for isotope exchange and isotope partition studies. These methods are simple and save considerable time over the methods currently employed to derive kinetic equations for enzyme-catalyzed reactions.

Since the pioneering work of King and Altman (1956) enzyme kineticists have had the tools to derive steady-state rate equations for quite complex mechanisms, and these are easily simplified and rearranged to yield expressions for the *V*'s and the various Michaelis and inhibition constants (Cleland, 1963). It is often useful, however, to be able to write the expression for *V* or *V/K* down directly without deriving the full rate equation, particularly when one wishes to increase the number of intermediate steps, and thus the number of total rate constants in the mechanism. For example, the recent discovery of Northrop (1975) that the true value of the isotope effect on the bond-breaking step in an enzymatic reaction can be calculated from the observed deuterium and tritium isotope effects on *V/K*, and that the relationship between rate constants for steps before and after the bond breaking one can thus be deduced by analysis of the observed isotope effects on *V* and *V/K*, increases the need for a rapid method of directly writing down expressions for *V* and *V/K* for various possible mechanisms. It is the purpose of this paper to show how partition analysis and the concept of net rate constants can be used to accomplish this, as well as to provide kinetic expressions for other situations such as isotope partition and isotope exchange studies.

Theory

Enzymatic reactions consist of a sequence of interconversions between various enzyme forms, with the starting enzyme form being regenerated at the end of each catalytic cycle. Many of these steps are reversible, and unless K_{eq} is very large, only release of a product at zero concentration (as when measuring initial velocities) or addition of a substrate at infinite concentration (saturation) can be considered irreversible. If, however, for any step we substitute for the true rate constants in forward and reverse directions a "net rate constant" which is the rate constant that would produce the same flux through the step if it were irreversible, we will reduce the mechanism to one with consecutive irreversible steps. Thus mechanism 1 can be reduced to mechanism 2 by computing net rate constants (indicated by primes) by the method to be described below. The rate equation for such a modified mechanism is very easy to obtain, since the rate is equal to the concentration of any enzyme form times the net rate constant for its reaction. Since the net rate constant represents the "conductance" of any step, more enzyme piles up in those enzyme forms with the lowest net rate constants, and we can compute the fraction of enzyme in any form in mechanism 2 as follows:

$$E_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_3 \xrightarrow{k_5} E_1 \qquad (1)$$

$$E_1 \xrightarrow{k_1'} E_2 \xrightarrow{k_3'} E_3 \xrightarrow{k_5'} E_1 \qquad (2)$$

$$\frac{[E_1]}{[E_t]} = \frac{1/k_1'}{1/k_1' + 1/k_3' + 1/k_5'}$$

$$\frac{[E_2]}{[E_t]} = \frac{1/k_3'}{1/k_1' + 1/k_3' + 1/k_5'} \qquad (3)$$

$$\frac{[E_3]}{[E_t]} = \frac{1/k_5'}{1/k_1' + 1/k_3' + 1/k_5'}$$

Since the rate is the concentration of any enzyme form times its net rate constant, it is clear that the rate is the reciprocal of sums of reciprocals of the net rate constants times total enzyme concentration, or for mechanism 2:

$$v = [E_t]/(1/k_1' + 1/k_3' + 1/k_5') \qquad (4)$$

This method is general, and will derive the rate equation for any mechanism consisting of consecutive steps without branched pathways (see below for how to handle these).

Calculation of Net Rate Constants. For irreversible steps, the true rate constant and net rate constant are identical, but for reversible steps, the net rate constant is the true rate constant multiplied by the proportion of the enzyme form resulting from this step that partitions forward to yield products, as opposed to undergoing reversal. This proportion is given by the net rate constant of the following step, divided by the sum of the net rate constant for the following step and the true rate constant for reversal of the step being considered. Thus for the situation:

$$E_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_2 \xrightarrow{k_3'} E_3 \longrightarrow E_1 \qquad (5)$$

where k_1 and k_2 are true rate constants, but k_3' is the net rate constant for conversion of E_2 to E_3, the net rate constant for conversion of E_1 to E_2 is

$$k_1' = k_1\left(\frac{k_3'}{k_2 + k_3'}\right) \qquad (6)$$

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For bimolecular steps, the product of reactant concentration and bimolecular rate constant is used as the true rate constant.

Equation 6 includes the net rate constant for the following step, and thus it is clear that to calculate net rate constants one cannot start with the first step and move forwards through the mechanism in the direction of material flow, but must first start with an irreversible step for which the net rate constant equals the true rate constant.[1] One then moves back to the previous step and calculates the net rate constant for it by an equation analogous to eq 6. One then goes back to the step preceding this one, and so on until net rate constants are calculated for each step. By working backwards in this fashion, the net rate constants for the following steps are always known and the expression given by eq 6 can always be evaluated. For example, in the following sequence:

$$E_1 \underset{k_2}{\overset{k_1 A}{\rightleftharpoons}} E_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_3 \xrightarrow{k_5} E_1 \tag{7}$$

the net rate constants for the three steps, from right to left, are:

$$k_5, \quad \frac{k_3 k_5}{k_4 + k_5}, \text{ and } \frac{k_1 A(k_3 k_5/(k_4 + k_5))}{k_2 + (k_3 k_5/(k_4 + k_5))} \tag{8}$$

Calculation of V. To calculate V, one lets all substrate concentrations become infinite (or for apparent V, just the variable substrate concentration) and determines the net rate constants for all steps in the mechanism. As indicated by eq 4, V is then the reciprocal of the sum of reciprocals of the net rate constants, multiplied by enzyme concentration $[E_t]$. For example, in mechanism 7 saturation with A makes the net rate constant for the first step infinite, and thus:

$$V = \frac{[E_t]}{(1/k_5) + [(k_4 + k_5)/k_3 k_5]} \tag{9}$$

Calculation of V/K. V/K is the apparent first-order rate constant for reaction of enzyme and substrate to give products when the substrate is at very low concentration. Since it is the rate at very low substrate concentration divided by substrate concentration (that is, $V/K = \lim_{A \to 0}(V/[A])$), we can calculate V/K by determining the rate from eq 4 with net rate constants evaluated at very low substrate concentration, and then divide by substrate concentration. When we do this, however, we see that only those net rate constants having the variable substrate concentration as a factor will contribute to the result. This is because the substrate concentration is near zero, and thus the reciprocals of net rate constants which include this factor are much larger than the reciprocals of those which do not. As a result we can use a shortcut method for evaluating V/K by multiplying together the net rate constant for the step where the variable substrate combines and the concentration of the enzyme form with which it combines (this will be $[E_t]$, or if other substrates have previously combined, that proportion of E_t that is in the proper form to combine with variable substrate), and dividing by substrate concentration. In mechanism 7, for example, the net rate constant for addition of A is given by eq 8 as:

$$k_1' = \frac{k_1 k_3 k_5 [A]}{k_2(k_4 + k_5) + k_3 k_5} \tag{10}$$

and at low A all of the enzyme will be in the E_1 form, so that:

$$V/K = \frac{k_1 k_3 k_5 [E_t]}{k_2(k_4 + k_5) + k_3 k_5} \tag{11}$$

For the following mechanism:

$$E_1 \underset{k_2}{\overset{k_1 A}{\rightleftharpoons}} E_2 \underset{k_4}{\overset{k_3 B}{\rightleftharpoons}} E_3 \xrightarrow{k_5} E_1 \tag{12}$$

the net rate constant for addition of B is: $k_3[B]k_5/(k_4 + k_5)$, while at very low B if A is not saturating: $k_1[A][E_1] = k_2[E_2]$, so that:

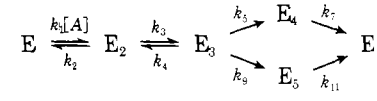
$$[E_2] = \frac{k_1[A][E_t]}{k_2 + k_1[A]} \tag{13}$$

The apparent value of V/K for B at finite A is then:

$$\text{apparent } V/K = \frac{k_1 k_3 k_5 [A][E_t]}{(k_2 + k_1[A])(k_4 + k_5)} \tag{14}$$

If A is saturating, $[E_2] = [E_t]$, and $V/K = k_3 k_5 [E_t]/(k_4 + k_5)$.

Net Rate Constants in Branched Pathways. For random mechanisms, or other mechanisms with alternate pathways, special care must be taken in computing net rate constants for the parallel pathways, and at the branch points. For example, in the following mechanism:



(15)

one might expect that the net rate constant for breakdown of E_3 would be $(k_5 + k_9)$, and for purposes of computing the net rate constant for the previous step this is correct. Thus:

$$k_3' = k_3(k_5 + k_9)/(k_4 + k_5 + k_9) \tag{16}$$

However, in calculating the rate, one must take into account k_7 and k_{11} as well as k_5 and k_9, and it is not correct to consider the conductance through the top path to be the reciprocal of the sums of reciprocals of k_5 and k_7, and that through the bottom path to be the reciprocal of sums of reciprocals of k_9 and k_{11}, and then to add these conductances. Such a procedure ignores the fact that the parallel paths must compete for the available enzyme, and thus that the flux through each will be less than if that path were the only one operative. The actual net rate constant for the conversion of E_3 to E_1 in mechanism 15 is (see below for the logic involved in deriving this expression):

$$k_{5,9}' = \frac{k_5 + k_9}{1 + (k_5/k_7) + (k_9/k_{11})} \tag{17}$$

Thus one would calculate V for mechanism 15 from the following equation (remembering that k_1' will be infinite, and thus its reciprocal will not contribute):

$$V = [E_t]/(1/k_3' + 1/k_{5,9}') \tag{18}$$

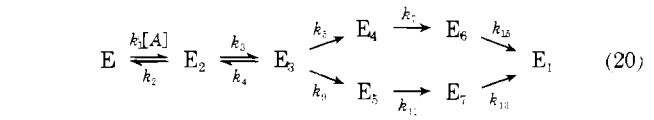
[1] If there is no irreversible step in the mechanism (that is, all products are present in finite concentration), but actual thermodynamic reversal can be ignored, the net rate constant for a given step is calculated by considering the previous step to be irreversible, and working back from there to the step being considered, calculating net rate constants for each step along the way. Only this final net rate constant is then valid; the intermediate ones are not. This process must be done separately for each step in the mechanism, and as a result the method described in this paper, while it works, does not save much time over conventional methods such as that of King and Altman (1956) in these cases.

In calculating V/K, however, one uses the net rate constant k_1', which is: $k_1[A]k_3'/(k_2 + k_3')$, so that:

$$V/K = \frac{k_1k_3(k_5 + k_9)[E_t]}{k_2(k_4 + k_5 + k_9) + k_3(k_5 + k_9)} \quad (19)$$

If $k_2 \gg k_3'$, the expression for V/K reduces to the ratio of $k_3'[E_t]$ and K_{ia} (the dissociation constant of A, which equals k_2/k_1), but V is not necessarily equal to $k_3'[E_t]$, nor K_a to K_{ia} in such a situation.

If the conversion of E_3 to E_4 is reversible (for instance if it involves release of product P present at finite concentration), then k_5 must be multiplied by a partition factor that represents the proportion of E_4 that reacts in the forward direction, and thus k_5 is replaced in eq 16, 17, and 19 by $k_5k_7/(k_7 + k_6[P])$, where k_6 is the rate constant for converting E_4 back to E_3. If the conversion of E_3 back to E_1 involves more than two steps in each path, as in mechanism 20, eq 16 is not changed, but the denominator of eq 17 includes k_5 multiplied times the reciprocals of all subsequent rate constants in that path (i.e., a k_5/k_{15} term is added), and k_9 multiplied times the reciprocals of the rate constants in the lower path (a k_9/k_{13} term is added).



(20)

One can rationalize the form of these equations as follows. In mechanism 20 the apparent net rate constant for flow from E_3 to E_1 through the top path in the absence of the bottom one would be given by:[2]

$$\text{app } k_{5,7,15}' = 1/(1/k_5 + 1/k_7 + 1/k_{15}) \quad (21)$$

where $1/k_5$ represents the amount of enzyme in E_3, $1/k_7$ the enzyme in E_4, and $1/k_{15}$ the amount in E_6. In the presence of the lower path, however, one must allow for enzyme piled up as E_5 and E_7. The concentration of these two forms depends on the relative flow through the two paths, which depends in turn on the ratio of k_9/k_5. Thus we must add $(k_9/k_5)(1/k_{11} + 1/k_{13})$ to the denominator of eq 21 to get:

$$\text{app } k_{5,7,15}' = \frac{1}{1/k_5 + 1/k_7 + 1/k_{15} + (k_9/k_5)(1/k_{11} + 1/k_{13})} = \frac{k_5}{1 + k_5/k_7 + k_5/k_{15} + k_9/k_{11} + k_9/k_{13}} \quad (22)$$

Similarly, the apparent net rate constant for flow through the bottom path is:

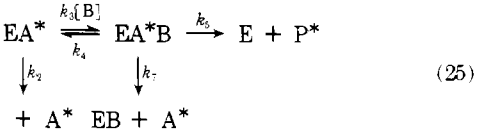
$$\text{app } k_{9,11,13}' = \frac{1}{1/k_9 + 1/k_{11} + 1/k_{13} + (k_5/k_9)(1/k_7 + 1/k_{15})} = \frac{k_9}{1 + k_9/k_{11} + k_9/k_{13} + k_5/k_7 + k_5/k_{15}} \quad (23)$$

Addition of these two expressions which have the same denominator gives:

$$k_{5,9}' = \frac{k_5 + k_9}{1 + k_5/k_7 + k_5/k_{15} + k_9/k_{11} + k_9/k_{13}} \quad (24)$$

[2] We are applying the same logic used in eq 4, where $V/[E_t]$ was the apparent net rate constant for one catalytic cycle (from E_1 back to E_1 in mechanism 2). The method described in this paper is basically one of determining a net rate constant for the entire mechanism which can be multiplied times $[E_t]$ to give the rate.

Isotope Partition Experiments. Rose et al. (1974) have recently introduced an isotope partition technique applicable to random mechanisms, or the first substrates in ordered cases, in which enzyme and labeled substrate are incubated together so that all of the enzyme is bound as a binary complex, and this solution is then diluted into a larger volume of solution containing a large excess of unlabeled substrate, plus variable amounts of the other substrate. Reaction is stopped after several seconds and the amount of radioactive product is determined. From a reciprocal plot of labeled product vs. concentration of the other substrate in the diluting solution, one can calculate the rates of dissociation of the labeled substrate from the binary and ternary complexes relative to $V/[E_t]$. In mechanism 25, one is interested in what proportion of EA* reacts with B to give P*, and what proportion dissociates A*, which then is diluted by the unlabeled A in the diluting solution.



(25)

The net rate constant for conversion of EA* to EA*B is given by:

$$k_3' = k_3[B](k_5 + k_7)/(k_4 + k_5 + k_7) \quad (26)$$

and the proportion of EA* giving P* is then the product of the proportion of EA* going to EA*B (which is $k_3'/(k_2 + k_3')$), and the proportion of EA*B giving P* (which is $k_5/(k_5 + k_7)$):

$$[P^*]/[E_t] = \left(\frac{k_3[B](k_5 + k_7)}{(k_2(k_4 + k_5 + k_7) + k_3[B](k_5 + k_7)}\right)\left(\frac{k_5}{k_5 + k_7}\right) = \frac{k_3k_5[B]}{k_2(k_4 + k_5 + k_7) + k_3(k_5 + k_7)[B]} \quad (27)$$

In reciprocal form:

$$[E_t]/[P^*] = \left(\frac{k_5 + k_7}{k_5}\right)\left(1 + \frac{k_2(k_4 + k_5 + k_7)}{k_3(k_5 + k_7)}\left(\frac{1}{[B]}\right)\right) = \left(\frac{[E_t]}{[P^*_{max}]}\right)\left(1 + \frac{K'}{[B]}\right) \quad (28)$$

By the rules given earlier, for mechanism 25:

$$V = k_5[E_t], \quad V/K_b = k_3k_5[E_t]/(k_4 + k_5)$$

(A is saturating for this calculation), and thus $K_b = (k_4 + k_5)/k_3$. Comparison of P^*_{max} with E_t then allows calculation of the ratio of k_7 to $V/[E_t]$:

$$\frac{k_7}{V/[E_t]} = \left(\frac{[E_t]}{[P^*_{max}]}\right) - 1 \quad (29)$$

From the ratio of K' and K_b one obtains limits on the ratio of k_2 to $V/[E_t]$:

$$\left(\frac{K'}{K_b}\right)\left(\frac{[E_t]}{[P^*_{max}]}\right) \geq \left(\frac{k_2}{V/[E_t]}\right) \geq \left(\frac{K'}{K_b}\right) \quad (30)$$

In an ordered mechanism (where $k_7 = 0$), or in a random one where $k_7 \ll k_5$ (as has been shown for yeast hexokinase by Danenberg and Cleland (1975)):

$$[E_t] = [P^*_{max}]$$

and

$$\frac{k_2}{V/[E_t]} = \frac{K'}{K_b} \tag{31}$$

Note that these equations, which do not describe a steady-state situation, but rather the fate of EA* during a single turnover, are easily obtained by partition methods, as here (and as was done by Rose et al. (1974)) but would be difficult to derive by integration of the differential equations describing the system (see the Appendix of Rose et al. (1974)).

Isotope Exchange. Since the work of Boyer (1959), measurement of the rates of isotope exchange between reactants has been a very useful tool for studies of enzymic mechanisms. Rate equations for isotope exchange are easily derived by a schematic method (Cleland, 1967), but they may also be derived by using partition theory to calculate net rate constants for the movement of label as follows. In any isotope exchange experiment, labeled reactant will add to an unlabeled enzyme form to give a labeled enzyme form, and then labeled product is released after a number of steps. To compute net rate constants for label transfer, one starts with the step for release of labeled product, and works backwards, calculating net rate constants for each step as described earlier until one reaches the step where labeled reactant adds. The net rate constant for this step, multiplied by the concentration of the enzyme form to which the labeled reactant adds, is the initial rate of isotope transfer from reactant to product. For example, in the following scheme:

$$E_1 \underset{k_2}{\overset{k_1[A]}{\rightleftharpoons}} E_2 \underset{k_4P}{\overset{k_3}{\rightleftharpoons}} E_3 \xrightarrow{k_5} E_1 \tag{32}$$

which describes the hydrolysis of A to P and Q in the presence of labeled P*, but absence of Q, the net rate constant for release of A* is k_2, while that for converting E_3 to E_2 (addition of P*) is: $(k_4[P])k_2/(k_2 + k_3)$. The rate of exchange from P* to A is then:

$$v^*_{P\rightarrow A} = \frac{[E_3]k_2k_4[P]}{(k_2 + k_3)} \tag{33}$$

Since the system is not at equilibrium, but rather in a steady state, the concentration of E_3 can be obtained by calculating net rate constants for the chemical reaction in the forward direction:

$$k_5' = k_5$$

$$k_3' = \frac{k_3k_5}{(k_5 + k_4[P])}$$

$$k_1' = \frac{k_1[A]k_3k_5}{k_2(k_5 + k_4[P]) + k_3k_5} \tag{34}$$

and then determining the distribution of the enzyme among the various forms on the basis of the resistance to flow in each step, as was done in equation 3:

$$\frac{[E_3]}{[E_t]} = \frac{1/k_5'}{1/k_1' + 1/k_3' + 1/k_5'} = \frac{k_1k_3[A]}{k_1k_4[A][P] + k_1(k_3 + k_5)[A] + (k_2 + k_3)k_5 + k_2k_4[P]} \tag{35}$$

$[E_2]/[E_t]$ is $1/k_3'$ over the same denominator, and $[E_1]/[E_t]$ is $1/k_1'$ over the same denominator. Substitution of eq 35 into eq 33 gives the rate of isotope exchange from P* to A:

$$v^*_{P\rightarrow A} = k_1k_2k_3k_4[A][P][E_t]/(k_2 + k_3)\Delta \tag{36}$$

where Δ is the denominator of the last part of eq 35.

Since the reactants in mechanism 32 are not at equilibrium, the rate of A* → P transfer will not equal the rate of P* → A exchange (nor will it equal the rate of chemical reaction). To evaluate A* → P transfer, we calculate net rate constants for the steps involving label as follows:

$$k_3'^* = k_3$$

$$k_1'^* = k_1[A]k_3/(k_2 + k_3) \tag{37}$$

Note that these net rate constants do not equal those for the chemical reaction given by eq 34. The initial rate of isotope transfer from A* to P is then:

$$v^*_{A\rightarrow P} = k_1k_3[A][E_1]/(k_2 + k_3) \tag{38}$$

and since from eq 34 the equation analogous to 35 is:

$$\frac{[E_1]}{[E_t]} = \frac{(k_2 + k_3)k_5 + k_2k_4P}{\Delta} \tag{39}$$

where Δ is again the denominator of the last part of eq 35

$$v^*_{A\rightarrow P} = \left(k_1k_3k_5[A] + \frac{k_1k_2k_3k_4[A][P]}{k_2 + k_3}\right)\frac{[E_t]}{\Delta} \tag{40}$$

The second term here is the same as the rate of P* → A exchange (eq 36), while the first term is the net chemical rate in the forward direction (= $k_5[E_3]$).

Exchange in Ping Pong Mechanisms. When isotope exchanges are measured for ping pong mechanisms, the system is generally at equilibrium, since only the reactants from one-half of the reaction are usually present. For example, in mechanism 41:

$$E_1 \underset{k_2}{\overset{k_1[A]}{\rightleftharpoons}} E_2 \underset{k_4[P]}{\overset{k_3}{\rightleftharpoons}} E_3 \rightleftharpoons E_1 \tag{41}$$

the exchange between A and P can be measured in the absence of the reactants involved in converting E_3 back into E_1 through the second part of the ping pong scheme. To calculate the rate of A* → P exchange, one determines net rate constants for label transfer:

$$k_3'^* = k_3$$

$$k_1'^* = k_1[A]k_3/(k_2 + k_3) \tag{42}$$

and then calculates the rate as:

$$v^*_{A\rightarrow P} = k_1'^*[E_1] = k_1k_3[A][E_1]/(k_2 + k_3) \tag{43}$$

Since the system is at chemical equilibrium, the method of eq 3 and 35 cannot be applied, but rather one uses the rule:

$$\frac{[E_i]}{[E_t]} = \frac{1}{1 + K_{eq\,i} + (K_{eq\,i})(K_{eq\,i+1}) + (K_{eq\,i})(K_{eq\,i+1})(K_{eq\,i+2}) + \ldots} \tag{44}$$

where there is one term in the denominator for each enzyme form present, and $K_{eq\,i}$ is K_{eq} for the step for converting E_i into E_{i+1}. In the present case we have:

$$\frac{[E_1]}{[E_t]} = \frac{1}{1 + (k_1[A]/k_2) + (k_1[A]k_3/k_2k_4[P])} = \frac{k_2/k_1[A]}{k_2/k_1[A] + 1 + k_3/k_4[P]} \tag{45}$$

and substituting the second form given into eq 43 gives:

$$v^*_{A\rightarrow P} = \frac{[k_2k_3/(k_2 + k_3)][E_t]}{1 + k_2/k_1[A] + k_3/k_4[P]} \quad (46)$$

The rate of P* → A exchange must equal the rate of A* → P exchange, since the system is at equilibrium.

Exchange at Equilibrium in Sequential Mechanisms. A similar approach can be applied to sequential mechanisms where all reactants are present and the system is at chemical equilibrium. Equation 44 still applies, except that with the same number of terms in the denominator as there are enzyme forms, the rate constants for one step (the conversion of the last enzyme form back into E_i) will not appear in this denominator.

Discussion

As described above, the calculation of net rate constants by partition methods allows one to write down directly a large number of useful kinetic expressions and rate equations. Thus V is E_t times the reciprocal of sums of reciprocals of the net rate constants when all substrates are saturating, and V/K is normally E_t times the net rate constant for combination of enzyme and substrate, divided by substrate concentration, when substrate level is very low. The net rate constant for combination of labeled reactant with enzyme, multiplied by the concentration of that enzyme form, gives the rate of isotope exchange, either in the steady state or at equilibrium. The technique works equally well for situations in the steady state, at equilibrium, or in a single turnover, as with the isotope partition example. No doubt further applications will become apparent with time. In this laboratory this technique has become the method of choice for routine derivations where it is desired to determine quickly the result of expanding a mechanism by adding extra steps with extra rate constants associated with them, and an immense amount of time has been saved thereby. Hopefully this paper will serve to make the method equally available to others with an interest in enzyme kinetics.

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A Study of Protein–Sodium Dodecyl Sulfate Complexes by Transient Electric Birefringence†

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ABSTRACT: The method of transient electric birefringence has been applied to study the conformation of protein–sodium dodecyl sulfate complexes. A model of a deformable prolate ellipsoid has been proposed for the protein–dodecyl sulfate complex. This model is compared to the models proposed by J. A. Reynolds and C. Tanford ((1970), *J. Biol. Chem. 245,* 5161) and K. Shirahama, K. Tsujii, and T. Takagi ((1974), *J. Biochem. 75,* 309). Differences between these latter two models are resolved by the model presented here. In addition, it has been demonstrated that protein molecular weights may be obtained from the slow relaxation time for transient electric birefringence of protein–dodecyl sulfate complexes.

The generally accepted basis for molecular weight determination of proteins by sodium dodecyl sulfate gel electrophoresis has been proposed by Reynolds and Tanford (1970), who studied the hydrodynamic behavior of several protein–sodium dodecyl sulfate complexes by means of intrinsic viscosity. Their data suggested that the complex is a rod-like particle, the length of which varies uniquely with the molecular weight of the protein moiety.

The simplest model suggested by their data is a prolate ellipsoid, with constant semi-minor axis, and a semi-major axis which is proportional to the molecular weight of the protein moiety. The average value of the semi-minor axis is 18.1 Å with a range of 3.4 Å for 12 protein–dodecyl sulfate complexes at the binding level of 1.4 g of dodecyl sulfate/g of protein. The semi-major axis varies linearly with molecular weight over the range studied: cytochrome *c* (11,700) 44.8 Å to bovine serum albumin (69,000) 230.5 Å.

Recently, Shirahama et al. (1974) have proposed an alternative model of protein–dodecyl sulfate complexes, which they call the "necklace model". In this model, the polymer chain is flexible and micelle-like clusters of dodecyl sulfate are scattered along the chain. The authors state that the necklace model is at variance with the ellipsoid model in the following points: (1) the former is flexible and free-

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