Methods of Determining Rate Constants in Single-Substrate–Single-Product Enzyme Reactions

USE OF INDUCED TRANSPORT: LIMITATIONS OF PRODUCT INHIBITION

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1. The calculation of the rate constants from steady-state kinetics of a single-substrate–single-product enzyme reaction in which there is an isomerization of the enzyme is described. 2. It is shown that even with the use of isotopically labelled substrates a set of solutions for the constants is obtained rather than a unique solution. However, limits are derived within which they must lie. 3. The most appropriate observations to determine the rate constants are measurements of $V_{\max}$ and K_m for both substrate and product, and measurement of the degree of countertransport in an induced-transport test. 4. Experimental procedures for induced-transport tests and the quantitative interpretation of the results obtained are discussed. 5. Product inhibition is shown to be an ambiguous and imprecise means of determining the rate constants. Further, the absence of a [substrate] × [product] term in the denominator of the steady-state rate equation does not necessarily mean that the isomerization of the enzyme is rapid, since the term also disappears when the isomerization is very slow. 6. Similar considerations apply to carrier mechanisms.

In the present paper, methods of determining the rate constants from steady-state kinetics in a single-substrate–single-product enzyme reaction of the type shown below (Mechanism 1) are described:

S and P are substrate and product, X is the enzyme–substrate complex, and E and F are isomeric forms of the enzyme.

If the step $\text{F} \rightleftharpoons \text{E}$ (Mechanism 1) does not exist (or is very rapid, which is kinetically equivalent) the rate constants may be readily determined from the maximum velocities and Michaelis constants for the substrate and product. However, there appear to have been no systematic investigations into how the rate constants may be determined when the step is rate-limiting. Cleland (1963) noted that the steady-state velocity equation for Mechanism 1 contains an $[\text{S}][\text{P}]/K_{\text{SP}}$ term in the denominator (see eqn. 1 below) whereas this term is absent if the step $\text{F} \rightleftharpoons \text{E}$

does not exist. The presence or absence of such a term has therefore been regarded as evidence for or against an enzyme isomerization. Several workers (Ray & Roscelli, 1964; Taraszka & Alberty, 1964; Darvey, 1972) have described methods to determine from the observed kinetics whether such a term is present in the steady-state velocity equation. Only Ray & Roscelli (1964), however, consider how the rate constants may be derived from the measurement of the parameters in the steady-state velocity equation, and they limit their calculations to the condition that the isomerization of the enzyme should be much more rapid than the other steps in the reaction.

The calculations in the present paper lead to the surprising conclusion that the $[\text{S}][\text{P}]/K_{\text{SP}}$ term in the denominator of the steady-state velocity equation disappears not only when the isomerization of the enzyme is very fast, which is kinetically equivalent to the existence of only one form of the enzyme, but also when it is very slow. Further, if the parameter K_{SP} should be finite, two different sets of solutions from the rate constants will, in general, satisfy the parameters of the steady-state velocity equation. Other considerations also suggest that determination of the rate constant based on K_{SP} must be inherently imprecise. In view of these limitations an alternative method of determining the rate constants is presented, depending on the measurement of induced transport of isotopically labelled substrates.

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Notation

F and E represent different conformational forms of the enzyme. X is the enzyme-substrate complex, (E_T) is the total quantity of enzyme. k_{+1} , k_{-1} etc. (Mechanism 1) are rate constants. v is the steady-state velocity. V_S is the maximum velocity per mol of enzyme with S as substrate. V_P is the maximum velocity per mol of enzyme with P as substrate. K_S is the Michaelis constant for S, K_P is the Michaelis constant for P, and K is the equilibrium constant ($[S]/[P]$). The coefficients α and K_{SP} are defined below. $[S]$ is the concentration of substrate, $[P]$ is the concentration of product and $[T]$ is the total concentration of substrate and product.

Steady-state rate equation

The steady-state velocity for Mechanism 1 is (Britton, 1966):

$$v = \left[\frac{V_S (1 + \alpha K [P]) [S] (E_T)}{K_S \left(1 + \frac{[S]}{K_S} + \frac{[P]}{K_P} + \frac{[S][P]}{K_{SP}} \right)} \right] - \left[\frac{V_P (1 + \alpha [S]) [P] (E_T)}{K_P \left(1 + \frac{[S]}{K_S} + \frac{[P]}{K_P} + \frac{[S][P]}{K_{SP}} \right)} \right] \quad (1)$$

Also:

$$K V_S / K_S = V_P / K_P \quad (2)$$

The parts in the large square brackets (eqn. 1) refer to the fluxes of S to P and P to S respectively. As previously defined (Britton, 1966) the flux of S to P is the rate of conversion of individual S molecules into P molecules and the flux of P to S is the rate of conversion of individual P molecules into S molecules. Clearly the velocity of the chemical reaction (v) will

$$k_{+1} = \frac{(n+1)^2 \{ V_S / [1 - n\alpha K_S / (n+1)] + V_P / [1 - \alpha K_S V_P / (n+1) V_S] \}}{n\alpha K_S^2 \{ 1 / [1 - n\alpha K_S / (n+1)] + (n+1) / n\alpha K_S \}} \quad (12)$$

and

$$k_{-2} = \frac{(n+1)^2 V_S \{ V_S / [1 - n\alpha K_S / (n+1)] + V_P / [1 - \alpha K_S V_P / (n+1) V_S] \}}{n\alpha K_S K_P \{ V_P / [1 - \alpha K_S V_P / (n+1) V_S] + (n+1) V_S / \alpha K_S \}} \quad (13)$$

be the difference between these two fluxes. Each of the fluxes contains a term in α . When the difference is taken (in eqn. 1) these α terms cancel, and they do not appear in the final velocity equation. The steady-state velocity equation, however, is presented in the form given in eqn. (1), since the parameter α leads to a solution for the rate constants.

To measure the individual fluxes and therefore to determine α requires the use of isotopically labelled substrates, as discussed below.

From Britton (1966) the parameters in eqn. (1) are related to the rate constants as follows:

$$V_S = k_{+2} k_{+3} / (k_{+2} + k_{+3}) \quad (3)$$

$$V_P = k_{-1} k_{-3} / (k_{-1} + k_{-3}) \quad (4)$$

$$K_S = (k_{+3} + k_{-3}) (k_{-1} + k_{+2}) / k_{+1} (k_{+2} + k_{+3}) \quad (5)$$

$$K_P = (k_{+3} + k_{-3}) (k_{-1} + k_{+2}) / k_{-2} (k_{-1} + k_{-3}) \quad (6)$$

$$\alpha = k_{+1} k_{+2} / k_{-3} (k_{-1} + k_{+2}) = k_{-1} k_{-2} / K k_{+3} (k_{-1} + k_{+2}) \quad (7)$$

$$K_{SP} = (k_{+3} + k_{-3}) (k_{-1} + k_{+2}) / k_{+1} k_{-2} \quad (8)$$

Determination of the rate constants from the parameters V_S , V_P , K_S , K_P and α

Let $k_{-3} = nk_{+3}$ when n is an arbitrary constant, then from eqns. (3), (5) and (7):

$$k_{-3} = nk_{+3} = (n+1) V_S / \alpha K_S \quad (9)$$

Substituting the values for k_{+3} and k_{-3} in eqn. (9) into eqns. (3) and (4) yields solutions:

$$k_{+2} = V_S / [1 - n\alpha K_S / (n+1)] \quad (10)$$

$$k_{-1} = V_P / [1 - \alpha K_S V_P / (n+1) V_S] \quad (11)$$

Substituting the values for k_{+3} , k_{-3} , k_{+2} and k_{-1} from eqns. (9)–(11) into eqns. (5) and (6) yields the solutions:

The solutions (eqns. 9–13) for the six rate constants involve the arbitrary constant n , since only five out of the six equations (eqns. 3–7) were employed in their derivation. The sixth equation (eqn. 8), however, is not an independent equation as shown below. Consequently a unique solution for the rate constants cannot be obtained by steady-state kinetics. Nevertheless, limits may be set to their values, as discussed below. The sum $[(1/k_{+3}) + (1/k_{-3})]$ is independent of n and equal to $V_S / \alpha K_S$.

Permissible values for n and α

The parameters V_s , V_p , K_s , K_p , K_{SP} and α have positive values and the insertion of these values into eqns. (9)–(13) must yield positive values for the rate constants. Inspection of eqns. (9)–(13) shows that this requirement demands that the denominators of eqns. (10) and (11) should always be positive. From eqn. (11) it follows that:

$$n > \alpha K_s V_p / V_s - 1 \quad (14)$$

and from eqn. (10) if $\alpha K_s > 1$:

$$n < 1/(\alpha K_s - 1) \quad (15)$$

Eqn. (14) sets a lower limit to n and eqn. (15) an upper limit. The larger the parameter α , for given values of K_s , V_s and V_p the smaller will be the range of possible values for n . The upper limit for n (eqn. 15) must always exceed the lower limit (eqn. 14) and therefore:

$$\alpha < (V_s + V_p) / K_s V_p \quad (16)$$

At the limiting value of α given by eqn. (16):

$$n = V_p / V_s \quad (17)$$

Inspection of eqns. (9)–(13) shows that when α approaches the limiting value given by eqn. (16), k_{+2} and k_{-1} approach infinity whereas the other rate constants remain finite. V_s and V_p (eqns. 3 and 4) are then entirely determined by k_{+3} and k_{-3} respectively. The isomerization of the enzyme is therefore the rate-limiting step in the reaction under these conditions.

Parameter K_{SP}

If the solutions for the rate constants (eqns. 9–13) are substituted into the equation for K_{SP} (eqn. 8) the following expression is obtained:

$$K_{SP} = V_s K_p / \alpha (V_s + V_p - \alpha K_s V_p) \quad (18)$$

K_{SP} is therefore not an independent parameter but is related to the other parameters in the steady-state rate equation. Consequently eqn. (8) is not an independent equation involving the rate constants, and as stated above there are only five independent equations from which the rate constants may be obtained.

When the isomerization of the enzyme in Mechanism 1 is very rapid and k_{+3} and k_{-3} approach infinity, α (eqn. 7) approaches zero. Correspondingly, from eqn. (18) K_{SP} approaches infinity and the $[S][P]/K_{SP}$ term in the denominator of the steady-state rate equation (eqn. 1) disappears. This conclusion has been reached by others (Cleland, 1963; Ray & Roscelli, 1964; Taraszka & Alberty, 1964). However, K_{SP} will also approach infinity and the $[S][P]/K_{SP}$ term will disappear from the denominator of eqn. (1) when α approaches the equality:

$$\alpha = (V_s + V_p) / K_s V_p \quad (19)$$

From eqn. (16) and the discussion related to this equation, α can only approach the equality in eqn. (19) as a limiting case. This corresponds to the condition that k_{+3} and k_{-3} are finite and that k_{-1} and k_{+2} approach infinity. The $[S][P]/K_{SP}$ term therefore also disappears from the denominator of the steady-state rate equation (eqn. 1) when the isomerization is the major rate-limiting step in the reaction.

The conclusion that the absence of an $[S][P]/K_{SP}$ term in the denominator does not necessarily mean that any isomerization of the enzyme is rapid may seem surprising. A numerical example has therefore been calculated and is illustrated in Fig. 1. For simplicity a completely symmetrical mechanism has been taken and it is supposed that the equilibrium constant for the overall reaction is 1. The arbitrary constant m is the ratio of the rate constant for isomerization of the free enzyme, to the rate constant for dissociation of the enzyme–substrate complex into product. Not only does K_{SP} approach infinity both when $m \rightarrow 0$ and $m \rightarrow \infty$, but the parabolic shape of the curve means that in general two values of m may be found to satisfy a given value of K_{SP} . The shallow minimum of the curve also demonstrates that in this range, even without the ambiguity, K_{SP} is not a good measure of the value of m . Beyond this range, the measurement of K_{SP} itself will tend to become difficult and imprecise.

Fig. 1. Relationship between K_{SP} (eqn. 1) and the rate constants for isomerization of the enzyme

$K_s = K_p = 1 \text{ mM}$. The mechanism (Mechanism 1) is assumed to be symmetrical. $k_{+1} = k_{-2}$, and $k_{+3} = k_{-3} = mk_{-1} = mk_{+2}$, where m is an arbitrary constant. K_{SP} tends to infinity asymptotically both when m is small, corresponding to a slow isomerization, and when m is large, corresponding to a fast isomerization. ---- illustrates how, in general, two values of m can be found to satisfy any given value of K_{SP} . Further, since the minimum is shallow, estimates of m from K_{SP} will be imprecise in this region. The equilibrium constant for the overall reaction is 1.

Determination of rate constants from V_s , V_p , K_s , K_p and K_{sp}

If isotopically labelled substrates are not available the parameter α cannot be directly measured. α can be determined indirectly, however, from eqn. (18) by inserting the other parameters into this equation. Eqn. (18) gives a quadratic expression for α so that two values of α will be obtained. When these values are inserted into eqns. (9)–(13) two sets of solutions for the rate constant will be found. This ambiguity has already been discussed in relation to Fig. 1.

Isotopic methods for determining rate constants

It is apparent from the above that measurement of V_s , V_p , K_s , K_p and K_{sp} alone must give rise to an ambiguity in the calculated values of the rate constants. This ambiguity can be removed by measuring α , although as mentioned in relation to eqn. (1), this will involve the use of isotopically labelled substrates. Since measurement of α renders the parameter K_{sp} largely redundant (see eqn. 18), any method of determining α should preferably not require prior measurement of K_{sp} .

Direct measurement of fluxes. If the individual fluxes (see eqn. 1) are measured, and if the parameters V_s , V_p , K_s , K_p and K_{sp} are known, then α can be determined by equating the expressions for the fluxes (eqn. 1) with the measured values. Fluxes may be measured under any steady-state conditions; the methods of measurement are briefly discussed in Britton (1966). In many cases it will be most convenient to make the measurements at chemical equilibrium, when the fluxes are equal. The term flux is then synonymous with equilibrium reaction rate. This method of determination of α , however, requires the prior measurement of the other parameters in the steady-state velocity equation (eqn. 1) including K_{sp} . It will clearly be subject to many errors.

Induced-transport test. The induced-transport test (Britton, 1965a, 1966; Britton & Clarke, 1968) provides a direct measure of the parameter α and no knowledge of the other parameters in the steady-state rate equation is required. In this test (Fig. 2) isotopically labelled substrate is incubated with the enzyme until equilibrium is reached. Unlabelled substrate (S) is then added and the isotopic label (total amount of label, not specific radioactivity) in the substrate is observed during the ensuing chemical reaction. Since the unlabelled and labelled molecules can be regarded as separate species, the equilibrium of the labelled species should not be disturbed by the addition of unlabelled material. The amount of label in the substrate should therefore remain constant. If it does not, there must be a transfer of energy from the unlabelled to the labelled molecules.

Such a transfer of energy may be brought about by a slow isomerization of the enzyme. Thus, when the unlabelled substrate is added and there is a net chemical reaction, the ratio $[F]/[E]$ will be increased above its equilibrium value. As a consequence labelled molecules of product will have a greater chance of finding F than labelled molecules of substrate of finding E. A transient flow of labelled material is therefore induced in the opposite direction to the net chemical flow, and correspondingly the amount of the radioisotope in the substrate transiently rises. This phenomenon has been termed countertransport. The condition for its occurrence in terms of flux measurements, assuming a net flow from S to P and assuming the activity coefficients of S and P to be equal, is (Britton & Clarke, 1968):

$$\frac{\text{Flux of S to P}}{\text{Flux of P to S}} < \frac{[S]}{[P]K} \quad (20)$$

From eqns. (1) and (2) for Mechanism 1:

$$\frac{\text{Flux of S to P}}{\text{Flux of P to S}} = \frac{[S](1 + \alpha K[P])}{[P]K(1 + \alpha[S])} \quad (21)$$

Comparison of eqns. (20) and (21) shows that with Mechanism 1 there should be no inequality in eqn. (20) when α is zero and consequently no induced transport. This corresponds to an infinitely rapid isomerization of the enzyme. As α becomes larger, the inequality in eqn. (20) will become larger and the amount of countertransport will increase progressively. Quantitatively the radioactivity may be shown to follow the equation:

$$\frac{[S^*]}{[S_0^*]} = 1 + \frac{[S] - [S_\infty]}{[S_\infty]} \left[1 - \left(\frac{[S] - [S_\infty]}{[S_0] - [S_\infty]} \right)^{\alpha[S_\infty]} \right] \quad (22)$$

where $[S^*]$ represents the concentration of labelled S at any instant in time and $[S_0^*]$ represents the concentration of labelled S at zero time. $[S_0]$ and $[S_\infty]$ represent the total concentration of substrate at zero time and at the end of the reaction respectively. $[S_\infty]$ will be related to the total concentrations of substrate and product T by the relationship:

$$[S_\infty] = K[T]/(1 + K) \quad (23)$$

Eqn. (22), which is a special case of eqn. (26) below, is derived in the Appendix.

The maximum in the countertransport curve will be given by:

$$[S^*]/[S_0^*] = 1 + \alpha([S_0] - [S_\infty])/(1 + \alpha[S_\infty])^{(1 + 1/\alpha[S_\infty])} \quad (24)$$

or if

$$\alpha[S_\infty] \ll 1$$

$$[S^*]/[S_0^*] = 1 + \alpha([S_0] - [S_\infty])/e \quad (25)$$

where $e = 2.718 \dots$

α may therefore be derived directly from the maximum of the countertransport curve.

From eqns. (24) and (25), countertransport for a given value of α will be at a maximum when $[S_\infty]$ is at a minimum. The reaction should therefore be studied in the direction in which the equilibrium lies towards the formation of product. The concentration of labelled material in the initial equilibration step should also be low, although very low concentrations should perhaps be avoided because of the possibility of adsorption effects and because an appreciable fraction of the label may then be bound to the enzyme.

From a theoretical and practical point of view the induced-transport test has a number of advantages. It is not necessary to know the amount of enzyme added and it is unimportant if the enzyme becomes partially inactivated during the experiment. The qualitative conclusions are also not affected if the rate constants should change during the reaction or by isotope effects. The only mechanism other than a slow isomerization that would lead to countertransport would appear to be an interaction between active centres (Britton, 1966, 1967). Thus the occurrence of countertransport must be very good evidence for an isomerization of the enzyme.

Modified induced-transport tests. In some cases it may be inconvenient to allow an initial period for the radioactivity to become distributed between substrate and product. This could be because of side reactions or because the enzyme becomes rapidly inactivated and it is difficult to obtain complete equilibration. The general equation for induced transport is:

$$\frac{[S^*]}{[S_\infty^*]} = \frac{[S]}{[S_\infty]} + \left(\frac{[S_0^*]}{[S_\infty^*]} - \frac{[S_0]}{[S_\infty]} \right) \left(\frac{[S] - [S_\infty]}{[S_0] - [S_\infty]} \right)^{(1 + \alpha[S_\infty])} \quad (26)$$

From eqn. (26), if $\alpha = 0$, $[S^*]$ should be linearly related to $[S]$. $[S^*]$ should therefore be plotted against

Fig. 2. Induced-transport tests

In each case $[S^*]$ is the concentration of labelled S (absolute concentration of label, not specific radioactivity), ---- represents the theoretical behaviour of $[S^*]$ when the isomerization of the enzyme (Mechanism 1) is very rapid, and represents the theoretical behaviour of $[S^*]$ when the isomerization is rate-limiting. In (a) isotopically-labelled substrate (S^*) and product are initially at equilibrium. At zero time unlabelled substrate is added. — represents the total substrate concentration and shows the chemical reaction that follows the addition of the unlabelled substrate. If the isomerization is rapid there is no change in the amount of label in the substrate. In (b), a mixture of unlabelled substrate and labelled product is added to the enzyme. The unlabelled substrate is present in excess, so that there is a net conversion of substrate into product. The concentration of labelled substrate ($[S^*]$) is plotted against the total concentration of substrate. $[S_0]$ and $[S_0^*]$ are the initial concentrations and $[S_\infty]$ and $[S_\infty^*]$ the final concentrations of S and S^* respectively. A straight line indicates that any isomerization is not rate-limiting. If the points lie above this line a rate-limiting isomerization is present. In (c), the experimental procedure is similar to that in (b) but the label is initially present in the substrate only. If the points lie above the straight line a rate-limiting isomerization is present.

[S]. If there is a rate-limiting isomerization of the enzyme and α is finite, $[S^*]$ will lie above the straight line (see the Appendix). This is illustrated in Fig. 2 where the two extreme cases of $[S^*] = 0$ and $[P^*] = 0$ are considered. As in the case where there is initially equilibrium, these modified induced-transport tests are not influenced by any inactivation of the enzyme that may occur during the reaction, and the qualitative interpretations of the tests do not require that the rate constants should remain constant. They will be sensitive, however, to isotope effects. If the rate constants for isotopically labelled substrates should be different from those for the unlabelled substrates, the labelled material will equilibrate at a different rate from the unlabelled material.

Discussion

The systematic calculation of the rate constants described in the present paper demonstrates that calculations based on the K_{SP} term will be ambiguous, and also imprecise. Further, the absence of an $[S][P]/K_{SP}$ term in the denominator of the steady-state rate equation does not necessarily indicate that the isomerization of the enzyme is very fast or that the step does not exist, since the term also disappears when the isomerization is the slowest step in the reaction. To determine the rate constants without ambiguity, the parameters that should be primarily determined are V_s , K_s , V_p , K_p and α . The first four can be derived from conventional initial-velocity measurements: only three need be determined if the equilibrium constant is known, since the fourth can be calculated from the Haldane relationship. The measurement of the fifth parameter, α , requires the use of isotopically labelled substrates. The most appropriate experimental procedure would appear to be the induced-transport test, since the degree of

countertransport is solely dependent on α and independent of the other parameters. If only a qualitative estimate of α is required a second substrate for the enzyme might be used rather than an isotopically labelled substrate (Britton & Clarke, 1968).

The kinetics of carrier-mediated passive transport, with a univalent carrier, can also be represented by the steps in Mechanism 1 (Britton, 1965b, 1966; Regen & Morgan, 1964). It therefore follows that the present conclusions also apply to such membrane-transport systems.

In two-substrate-two-product reactions of the type $A+B \rightleftharpoons P+Q$, product inhibition is commonly studied to obtain information about the order of addition and dissociation of products and substrates. In such investigations it is usual to omit one of the products while the effect of the other product is studied. An equation similar to eqn. (18) applies, but since one product has been omitted V_p is zero. Under these conditions the K_{SP} term becomes infinite only when α is zero, and there is no ambiguity in the interpretation of product inhibition.

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APPENDIX

Calculation of induced transport

Following Britton & Clarke (1968), from eqn. (21) of the main paper (Britton, 1973) and the relation: Net flux of S to P = (flux of S to P) – (flux of P to S):

$$\text{Flux of S to P} = \frac{[S](1 + \alpha K[P])}{[S] - K[P]} (\text{net flux of S to P}) \quad (1)$$

and

$$\text{Flux of P to S} = \frac{K[P](1 + \alpha[S])}{[S] - K[P]} (\text{net flux of S to P}) \quad (2)$$

also:

Rate of transfer of label from P to S assuming no isotope effects

$$\begin{aligned} &= (\text{flux of P to S}) (\text{specific radioactivity of P}) \\ &= (\text{flux of P to S}) [P^*]/[P] \end{aligned} \quad (3)$$

and similarly:

Rate of transfer of label from S to P

$$= (\text{flux of S to P}) [S^*]/[S] \quad (4)$$

Consequently:

$$\begin{aligned} \text{Net rate of transfer of label from P to S} &= V \frac{d[S^*]}{dt} \\ &= (\text{flux of P to S}) [P^*]/[P] - (\text{flux of S to P}) [S^*]/[S] \end{aligned} \quad (5)$$

where V is the volume of the solution.

Substituting eqns. (1) and (2) into eqn. (5):

$$V \frac{d[S^*]}{dt} = \frac{K[P^*](1 + \alpha[S]) - [S^*](1 + \alpha K[P])}{[S] - K[P]} \text{ (net flux of S to P)} \quad (6)$$

or since:

$$\text{Net flux of S to P} = -V \frac{d[S]}{dt} \quad (7)$$

$$\frac{d[S^*]}{d[S]} = \frac{[S^*](1 + \alpha K[P]) - K[P^*](1 + \alpha[S])}{[S] - K[P]} \quad (8)$$

From the main paper (Britton, 1973):

$$[S] + [P] = [T] \quad (9)$$

Also let:

$$[S^*] + [P^*] = [T^*] \quad (10)$$

where $[T^*]$ = total amount of label per unit volume.
Substituting eqns. (9) and (10) into eqn. (6):

$$\frac{d[S^*]}{d[S]} = \frac{[S^*]\{1 + \alpha K[T]/(1 + K)\} - \alpha K[T^*][S]/(1 + K) - K[T^*]/(1 + K)}{[S] - K[T]/(1 + K)} \quad (11)$$

Since

$$[S_\infty] = K[T]/(1 + K) \quad (12)$$

and

$$[S_\infty^*] = K[T^*]/(1 + K) \quad (13)$$

Where $[S_\infty]$ and $[S_\infty^*]$ represent the values at the end of the reaction, eqn. (11) may be rearranged to the form:

$$\frac{d([S^*]/[S_\infty^*])}{d([S] - [S_\infty])} = \frac{([S^*]/[S_\infty^*])(1 + \alpha[S_\infty]) - (1 + \alpha[S_\infty])}{([S] - [S_\infty])} - \alpha \quad (14)$$

Integration of eqn. (14) yields eqn. (26) of the main paper (Britton, 1973). Eqn. (22) is a special case of this equation with $[S_0^*]/[S_\infty^*] = 1$. As discussed in the text of the main paper if $\alpha = 0$, $[S^*]/[S_\infty^*]$ should follow a straight line when plotted against $[S]$. If α is finite, then for any given value of $[S]$, $[S^*]/[S_\infty^*]$ will be larger than the value given by this straight line. From eqn. (26) of the main paper the difference is given by:

$$\frac{[S^*]}{[S_\infty^*]} - \left(\frac{[S^*]}{[S_\infty^*]} \right)_{\alpha=0} = \frac{([S_0^*]/[S_\infty^*] - [S_0]/[S_\infty])([S] - [S_\infty])}{[S_0] - [S_\infty]} \left[\left(\frac{[S] - [S_\infty]}{[S_0] - [S_\infty]} \right)^{\alpha[S_\infty]} - 1 \right] \quad (15)$$

By differentiation, the maximum difference occurs when:

$$[S] - [S_\infty] = ([S_0] - [S_\infty])/(1 + \alpha[S_\infty])^{1/\alpha[S_\infty]} \quad (16)$$

and from eqn. (26) of the main paper the maximum difference is given by:

$$\frac{[S^*]}{[S_\infty^*]} - \left(\frac{[S^*]}{[S_\infty^*]} \right)_{\alpha=0} = \frac{([S_0] - [S_\infty])[S_0^*]/[S_\infty^*]\alpha}{(1 + \alpha[S_\infty])^{(1+1/\alpha[S_\infty])}} \quad (17)$$

Eqn. (24) of the main paper is a special case of this equation when $[S_0^*]/[S_\infty^*] = 1$. Under these conditions [see eqn. (22) of main paper] $([S^*]/[S_\infty^*])_{\alpha=0} = 1$.

As discussed in the main paper, Mechanism 1 and eqns. (1) and (2) also apply to carrier-mediated

passive transport with a univalent carrier. In this case $[S]$ represents the concentration on one side of the membrane and $[P]$ the concentration on the other. The equilibrium constant will be 1. To calculate induced transport it is necessary to take into account that S will be distributed in the solute volume V_s present on one side of the membrane and P will be distributed in the solute volume V_p present on the other side. This approach, assuming V_s and V_p are constant leads to an equation identical with eqn.

(14) of the Appendix. Thus under these conditions eqns. (15)–(17) of the Appendix apply to induced transport across a membrane brought about by a passive univalent carrier.

References

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