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Kinetics of transport systems dependent on periplasmic binding proteins

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Rate equations are derived for a transport model involving a water-soluble binding protein outside the plasma membrane. On addition of the substrate, the conformation of the binding protein changes; the complex then combines with the membrane carrier, transferring the substrate to the carrier site. The free binding protein leaves and the carrier shifts inward, releasing the substrate inside the cell. Exit follows the reverse path. The predicted behaviour is as follows. (i) Uptake does not necessarily conform to Michaelis-Menten kinetics. (ii) In both the energized and de-energized states, the maximum rate of exit is far lower than that of entry; the asymmetry is determined by the conformational change in the binding protein, which is independent of the energy state of the system. (iii) Exchange transport is inhibited by external substrate and is extremely slow; consequently counter-transport is not expected. (iv) The half-saturation constant in uptake can differ from the dissociation constant of the binding protein. (v) The maximum rate of uptake depends on the intrinsic substrate affinity of the membrane carrier relative to that of the binding protein. (vi) The maximum rate of uptake and the substrate half-saturation constant depend on the concentration of the binding protein.

Introduction

Transport systems in Gram-negative bacteria commonly depend on two different components: a carrier-protein complex associated with the plasma membrane, and a separate water-soluble binding protein in the periplasmic space outside the plasma membrane [1–8]. Why transport across a lipid bilayer should involve a carrier in the aqueous phase has not been satisfactorily explained. It seems paradoxical, as Boos [9] has remarked, that though the membrane carrier is capable of transporting the substrate across the main permeability barrier, into the cytoplasm, a protein outside this barrier appears to be the substrate recognition site for the system. Whatever the role of the binding proteins, they must confer a decisive advantage in survival since the cell goes to the expense of producing many copies of the protein for each copy of the membrane carrier.

While further structural studies, on the effects of site-directed mutagenesis for example, should contribute to an understanding of these systems, progress may also be made by examining the kinetics of transport. Until now no kinetic analysis has been carried out, a surprising omission considering the unusual behaviour. Transport models in accord with the structural evidence have been suggested but it has not been shown whether they explain even Michaelis-Menten kinetics. Perhaps because the behaviour has not been explained, the periplasmic systems are often ignored in general accounts of transport, such as 'The Biochemistry of Membrane Transport' by I.C. West [10] and 'Transport and Diffusion across Cell Membranes' by W.D. Stein [11].

Here, I present a kinetic analysis of a reaction scheme involving a soluble binding protein. The scheme is based on a mechanism proposed by Hengge and Boos [1]. The analysis reveals properties unlike those of simpler transport models. In the paper following this one, the predictions of the analysis are compared with the observed behavior, with a view of accepting or rejecting the model.

Behaviour of the system

The binding proteins are essential for substrate entry. Exit, in either active or passive transport, is extremely slow and does not involve the binding protein;

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any loss of substrate that occurs is probably through some other route [12–14]. Exchange transport is not seen, nor is counter-transport, which distinguishes carriers from simple channels. In these respects the behaviour is unprecedented. Every reaction, including transport, is reversible. The equilibrium constant, the ratio of the forward and reverse reaction rates, is a function of the free energies of the reactants and products, and is unchanged in the presence of a catalyst. A catalytic agent, therefore, increases the forward and reverse rates equally. And however much the products of a reaction are favoured, an equilibrium is dynamic, and in the final equilibrium or steady state the forward and reverse rates (in the case of a carrier, unidirectional influx and efflux) are equal.

Other findings also have to be explained. (a) Despite the complexity of the mechanism, uptake follows the Michaelis-Menten rate law over a wide range of substrate concentrations. (b) The binding protein, at a concentration of about 1 mM in the periplasm, is at least 20 times as abundant as the membrane carrier. (c) Though the binding protein undoubtedly interacts with the membrane component, attempts to isolate the reversible complex have not been successful. (d) The measured Michaelis constant for transport can be either larger or smaller than the substrate dissociation constant for the binding protein. (e) The Michaelis constant and the maximum transport rate depend on the content of the binding protein in the periplasm.

The systems dependent on periplasmic proteins are notable for high-affinity and high-uptake ratios. K_m values are commonly micromolar or less, and the final ratio of internal to external substrate concentrations can be as high as 10^5. ATP is thought to supply the energy for active transport [5,6,15–18]. Spheroplasts or proteoliposomes, without the periplasmic proteins, lack transport activity, but 30–80% of the activity can be restored when the protein is added back [18–20].

The binding proteins, which have molecular weights of 25 000 to 50 000, form a tight, bimolecular complex with the transported substrate but have no enzymatic activity. The protein molecule is divided into two functionally and genetically separable binding domains, one involved in holding the substrate, the other in interacting with the membrane-bound components of the system [21,22]. On addition of the substrate, there is a conformational change [23] in which two similar lobes of the protein joined by a flexible hinge region swing together, enclosing the substrate and largely cutting it off from contact with the solvent [24–26]. The conformational change is probably essential for function, in view of its universal occurrence [2], and of the finding that a mutant protein capable of forming a complex with the substrate (histidine) but unable to undergo the normal change in conformation, fails to mediate transport [21,27,28]. The binding protein interacts directly with the membrane component of the system [29], and the interaction is said to be enhanced by the membrane potential [30]. The membrane component is itself a complex of three distinct proteins.

Transport models

There is no doubt that the membrane component of the system contains a substrate binding site [4]. Double mutants have been studied, in which the first mutation has destroyed transport activity by eliminating the binding protein, and the second has restored transport activity by altering the membrane component. The substrate site in the latter is accessible in the absence of the binding protein, though the affinity for the substrate appears to be low. The double mutants behave like an ordinary carrier [31–33]. In the wild type the site must be inaccessible, since transport is not observed in spheroplasts or membrane vesicles [2].

In a model suggested by Ames and co-workers [29,34], translocation across the membrane involves a pore that opens when the membrane component combines with the periplasmic protein. A model of this kind is ruled out, however, by the finding of mutants of the membrane-bound components with changed specificity, or with transport activity in the absence of the binding protein [2]. In models suggested by Shuman [32], Hengge and Boos [1], and Quiocho [8], the membrane component resembles the familiar carrier model, with a transport site alternately exposed on the outer and inner sides of the cytoplasmic membrane; though with the difference that the outward-facing carrier site is not readily available to substrate in the periplasmic space. The outer site is inaccessible, in the model of Hengge and Boos, because the substrate site is blocked in the outward-facing carrier, and only becomes unblocked in the complex with the binding protein; and in the model of Shuman, because the carrier faces predominantly inward. An objection to the latter device is that the inward-facing carrier is unavailable to the binding protein as well as the substrate, and that when it faces outward it becomes available to both.

The model of Hengge and Boos [1] involves the following steps: (1) the protein-substrate complex adds to an outward-facing form of the membrane carrier; (2) the substrate passes to the now-accessible site in the carrier, and the binding protein conformation relaxes to that of the free form; (3) as the binding protein dissociates, the outward-facing carrier is converted to the inward-facing form, translocating the substrate inward; (4) the return of the carrier site to the outer surface of the membrane is coupled to the hydrolysis of ATP. Transport is made irreversible by the conformational change in the binding protein induced by the substrate (since the substrate complex but not the free binding protein interacts with the membrane compo-

nent), and by the coupling of ATP hydrolysis to return of the carrier. The model of Quiocho [8] is similar. No pathway is shown for reversal of the reaction.

Proposed reaction scheme (Fig. 1)

The mechanism to be treated here is based on the model of Hengge and Boos, but is reversible, as thermodynamics requires, and is less restrictive, in that the point of coupling to ATP hydrolysis is not specified. In principle the release of energy in active transport can occur anywhere along the cycle of carrier reactions [35]. The effects of coupling at any given step can be deduced later, from the rate equations for the general scheme.

To account for a conformational change induced by the substrate, the binding protein must be assumed to be an equilibrium mixture of two conformations, called P′ and P. P′, which is abundant, has low affinity for the substrate, and P, which is scarce, has high affinity. Addition of the substrate necessarily shifts the equilibrium to favour the high affinity form. By hypothesis, the predominant conformation of the free binding protein, P′, does not interact with the carrier, but the other conformation does, whether the substrate is bound or not.

Substrate uptake depends on addition of the protein-substrate complex to the carrier, the complex being largely in the correct conformation – PS, not P′S. In the complex, the substrate S migrates to the carrier site; the carrier than takes the substrate across the membrane and into the cell. Exit follows the reverse pathway. Substrate inside the cell adds directly to the inward-facing form of the carrier, which swings outward. The free binding protein P then adds to the outward-facing carrier-substrate complex, C_oS, and the substrate jumps to the binding protein. Finally, the protein-substrate complex dissociates from the carrier, and the substrate is released.

The external substrate site is closed off in the free carrier but open in the complex with the binding protein. In other respects the membrane component, though a complex of three distinct proteins [2], behaves like the ordinary carrier model. The substrate site alternates between the outer and inner surfaces of the membrane (in C_o and C_i, respectively). A region of the carrier linked to the substrate site, which is only exposed in the outward-facing carrier, bonds specifically

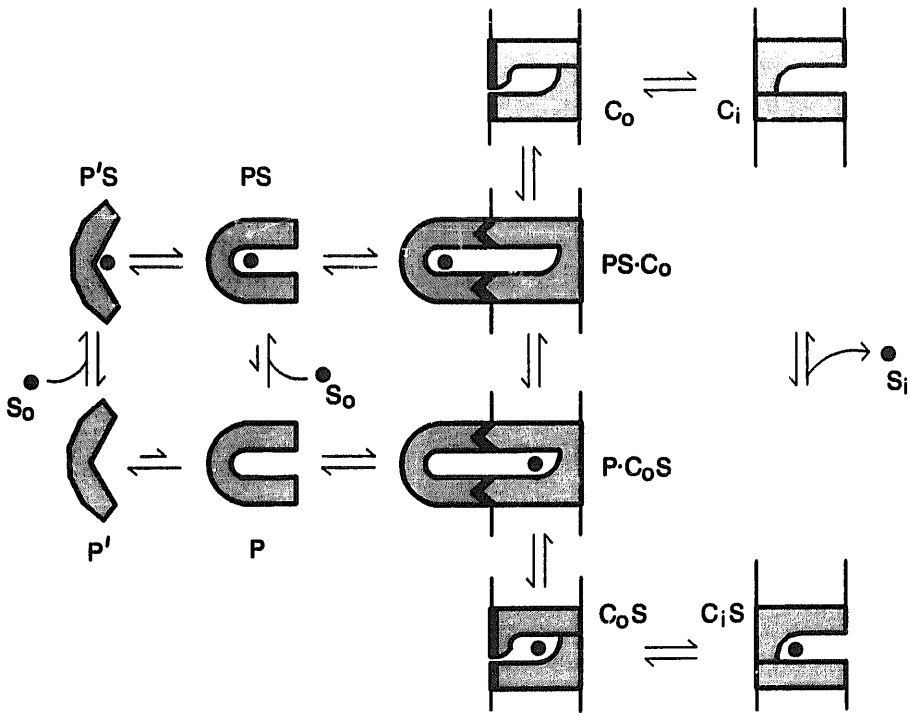

Fig. 1. A transport mechanism in which a binding protein, located in the periplasmic space between the outer membrane and the cytoplasmic membrane, transfers the external substrate S_o to the outward-facing carrier. The external substrate cannot add to the carrier directly. The transporter, imbedded in the cytoplasmic membrane, is like the ordinary carrier model, alternating between outward-facing and inward-facing forms, C_o and C_i. When the binding protein, P or PS, adds to the outward-facing carrier, the carrier site becomes unblocked, allowing the substrate to pass between binding sites in the protein and the carrier. The binding protein is an equilibrium mixture of two conformational forms, P′ and P, and only the second combines with the carrier; free of substrate, the protein is mainly in conformation P′, but as the substrate complex a good proportion is in conformation PS. The binding protein cannot combine with the inward-facing carrier, whereas the internal substrate, S_i, can.

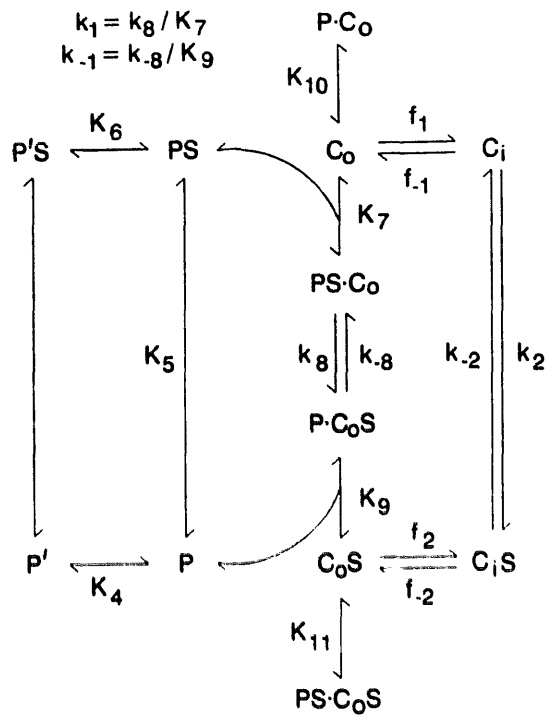

Fig. 2. Transport scheme for the binding-protein model, corresponding to the diagram in Fig. 1. Steps considered to be in rapid equilibrium in the kinetic treatment of the model are indicated by single arrows, and steps that may be rate-limiting by double arrows. The binding protein can form two types of complex with the carrier; one productive, PS with C_o and P with C_oS, and one non-productive, P with C_o and PS with C_oS. The non-productive complex is at a dead-end in the reaction sequence and is therefore inhibitory. The substrate dissociation constant of the binding protein is K_S ($K_S = K_S(1 + K_4)/(1 + K_6)$), and the intrinsic substrate dissociation constant of the outer carrier site is K_{S_o} ($K_{S_o} = [C_o][S_o]/[C_oS] = k_{-1}K_S/k_1$). k_1 and k_{-1} are rate constants governing addition of the substrate to, and its dissociation from, the outer carrier site, both steps mediated by the binding protein.

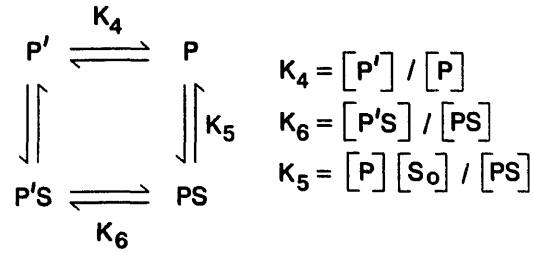

Fig. 3. The equilibrium between the external substrate and the two conformations of the binding protein. If the substrate induces a sharp conformational change (with the free protein almost exclusively in conformation P' and the substrate complex almost exclusively in conformation P), then $K_4 \gg 1$ and $K_6 \ll 1$, and the dissociation constant for the binding protein is $K_S \approx K_4K_5$.

and reversibly with the binding protein; consequently the latter, located outside the membrane, does not add to the inward-facing carrier. Substrate inside the cell adds directly to the inner carrier site, without the intervention of a binding protein.

In Fig. 1, the complex between the binding protein and the carrier is always the right combination for reaction (PS with C_o and P with C_oS), but the possibility that the wrong combinations can also be formed should be allowed for: P with C_o and PS with C_oS, as in Fig. 2. This non-productive complex is assumed to be at a dead-end in the reaction sequence and is therefore inhibitory.

Kinetics of the model

Method of derivation

In order to make the derivation manageable, the transport scheme is treated as a partial equilibrium mechanism in which reaction intermediates connected by rapid equilibrium steps are grouped together in the steady-state treatment of the over-all reaction. The method is that described by Cha [36]. The binding protein and the substrate are taken to be in equilibrium (Fig. 3), a reasonable assumption considering that dissociation of the protein-substrate complex is at least as fast as the maximum transport rate [37]. Also, addition of the binding protein to the carrier is treated as an equilibrium step; the weak interaction of the two proteins indicates that the dissociation step would be rapid, a point discussed in the following paper. In Fig. 2, equilibrium steps are indicated by single arrows and slower steps by double arrows.

Given these conditions, together with the condition that the binding protein is in great excess over the membrane component [1,2,38], the partial reactions in Figs. 3 and 4 can be treated separately. The scheme in Fig. 3 represents the equilibrium between the substrate and the binding protein, and that in Fig. 4 the interaction between the binding protein and the membrane carrier. In the latter the transport machinery is reduced to a carrier model in which the outward-facing carrier, C_o' or $C_o'S$, combines with the binding protein in conformation P. In entry the protein-substrate complex, PS, adds to the carrier to form the carrier-substrate complex, $C_o'S$, with release of the free binding protein, P. The addition of substrate is represented as

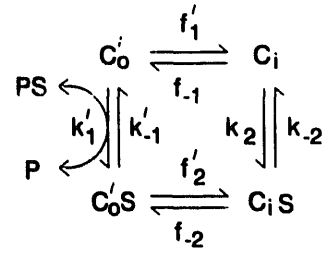

Fig. 4. Abbreviated transport scheme for a partial equilibrium mechanism. C_o, PS·C_o, and P·C_o in Fig. 2 are assumed to be in rapid equilibrium, and are represented by C_o'; C_oS, P·C_oS, and PS·C_oS, also in rapid equilibrium, are represented by $C_o'S$. The binding protein is in rapid equilibrium with the substrate (Fig. 3).

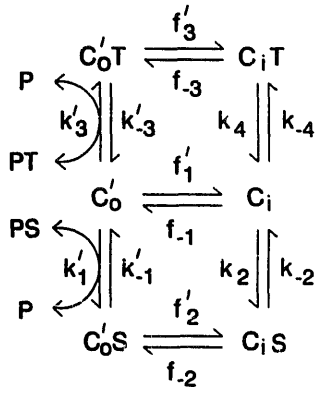

Fig. 5. Transport scheme for the labelled and unlabelled forms of the substrate, S and T, respectively, corresponding to the scheme for one substrate in Fig. 4.

a single step rather than three steps with two intermediates as in the full scheme in Fig. 2. In exit the free binding protein P adds to the carrier-substrate complex, releasing the free carrier and the protein-substrate complex, PS. The more inclusive scheme in Fig. 5 shows the labelled substrate S exchanging with an unlabelled substrate, T.

The strategy for deriving rate equations is as follows. In the first stage, an expression is derived for the partial reaction scheme in Fig. 4 (or Fig. 5), without assumptions as to which steps are rate-limiting. In this derivation, the 'on' term on the outside is the product of the rate constant k'_1 and the concentration term [PS], and the 'off' term is the product of the rate constant k'_{-1} and the concentration term [P]. That is, PS (or PT), the substrate-binding protein complex, is treated as the external substrate. In the second stage of the derivation, equilibrium expressions are introduced for the external substrate and the various forms of the binding protein.

An expression for the flux (v) of substrate S, derived for the scheme in Fig. 5, has the form:

$$v = \left(\frac{\bar{V}'_{PS}}{\bar{K}'_{PS}}([PS]-[S_i]/\alpha') + \frac{\tilde{V}^{T'}_{PS}}{\bar{K}'_{PS}\tilde{K}^{PS'}_{T_i}}([PS][T_i]-[PT][S_i])\right) \times \left(1+\frac{[PS]}{\bar{K}'_{PS}}+\frac{[S_i]}{\bar{K}'_{S_i}}+\frac{[PT]}{\bar{K}'_{PT}}+\frac{[T_i]}{\bar{K}'_{T_i}}+\frac{[PS][S_i]}{\bar{K}'_{PS}\tilde{K}^{PS'}_{S_i}}+\frac{[PT][T_i]}{\bar{K}'_{PT}\tilde{K}^{PT'}_{T_i}} + \frac{[PS][T_i]}{\bar{K}'_{PS}\tilde{K}^{PS'}_{T_i}}+\frac{[PT][S_i]}{\bar{K}'_{PT}\tilde{K}^{PT'}_{S_i}}\right)^{-1} \quad (1)$$

With the exception of $\bar{V}'_{PS}$, the parameters of Eqn. 1 – K', V' and α' – are functions of [P]: each is a combination of rate constants in the carrier scheme, where the 'off' rate on the outside is governed by the term k'_{-1}[P]. At this stage of the derivation the parameters are marked with a prime (') to distinguish them from constants appearing in the final rate equations. Eqn. 1 applies to both passive and active transport systems: α' is the ratio of $[S_i]$ to [PS] at equilibrium or in the final steady-state in an active system. The relationship of α' to the ratio of the final internal and external substrate concentrations, $[S_i]/[S_o]$, is given below (Eq. 22). The complete expressions for the parameters will be drawn, as needed, from an earlier treatment of the carrier model [39].

Since S and T in Eqn. 1 represent the same substrate (one labelled, the other not), $\bar{K}'_{PS} = \bar{K}'_{PT}$, $\tilde{K}^{S'}_{PS} = \tilde{K}^{S'}_{PT} = \tilde{K}^{T'}_{PS} = \tilde{K}^{T'}_{PT}$, and $\bar{K}'_{S_i} = \bar{K}'_{T_i}$. Gathering terms, Eqn. 1 becomes

$$v = \frac{\frac{\bar{V}'_{PS}}{\bar{K}'_{PS}}([PS]-[S_i]/\alpha') + \frac{\tilde{V}^{S'}_{PS}}{\bar{K}'_{PS}\tilde{K}^{PS'}_{S_i}}([PS][T_i]-[PT][S_i])}{\left\{1+\frac{([PS]+[PT])}{\bar{K}'_{PS}}+\frac{([S_i]+[T_i])}{\bar{K}'_{S_i}}+\frac{([PS]+[PT])([S_i]+[T_i])}{\bar{K}'_{PS}\tilde{K}^{PS'}_{S_i}}\right\}} \quad (2)$$

The following relationships among experimental parameters are also required:

$$\bar{V}'_{PS}/\bar{K}'_{PS} = \alpha'\bar{V}'_{S_i}/\bar{K}'_{S_i} \quad (3)$$

$$\bar{K}'_{PS}\tilde{K}^{PS'}_{S_i} = \bar{K}'_{S_i}\tilde{K}^{S'}_{PS} \quad (4)$$

The binding protein-substrate complex

If the substrate induces a sharp conformational change in the binding protein (with the free protein almost exclusively in one conformation and the substrate complex in another), then $K_4 \gg 1$ and $K_6 \ll 1$. The substrate dissociation constant for the binding protein, K_S, takes into account both conformational forms, P and P':

$$K_S = \frac{([P]+[P'])[S_o]}{([PS]+[P'S])} = \frac{[P][S_o](1+K_4)}{[PS](1+K_6)} = \frac{K_5(1+K_4)}{(1+K_6)} \approx K_4K_5 \quad (5)$$

Expressions for [P] and [PS] are found by substitution from Fig. 3 and Eqn. 5 into an expression for the total binding protein:

$$[P_t] = [P]+[P']+[PS]+[P'S] \quad (6)$$

$$[PS] = [P_t]/\{1+K_S/[S_o]\} \quad (7)$$

$$[P] = [P_t]/\{K_4(1+[S_o]/K_S)\} \quad (8)$$

The sum in Eqn. 6 omits complexes of the binding protein with the carrier: as the number of copies of the binding protein exceeds that of the membrane component by a factor of at least 20, the amount tied up by the carrier is a negligible fraction of the total.

Rate constants for the carrier

The constants for addition of the substrate to the carrier in the full scheme in Fig. 2 are related to those in the abbreviated scheme in Fig. 4 in the following way. C_o' in Fig. 4 represents C_o, $PS \cdot C_o$, and $P \cdot C_o$ in the full scheme in Fig. 2, and $C_o'S$ represents C_oS, $P \cdot C_oS$, and $PS \cdot C_oS$:

$$[C_o'] = [C_o] + [PS \cdot C_o] + [P \cdot C_o]$$

$$= [C_o](1 + [PS]/K_7 + [P]/K_{10}) \quad (9)$$

from which

$$k_1'[C_o'][PS] = k_8[PS \cdot C_o] = k_8[C_o][PS]/K_7$$

$$= (k_8/K_7)[C_o'][PS]/(1 + [PS]/K_7 + [P]/K_{10}) \quad (10)$$

where $k_1 = k_8/K_7$. Hence,

$$k_1' = k_1/(1 + [PS]/k_7 + [P]/K_{10}) \quad (11)$$

similar expressions may be written for k_{-1}', f_1', and f_2':

$$k_{-1}' = (k_{-8}/K_9)/(1 + [P]/K_9 + [PS]/K_{11})$$

$$= k_{-1}/(1 + [P]/K_9 + [PS]/K_{11}) \quad (12)$$

$$f_1' = f_1/(1 + [PS]/K_7 + [P]/K_{10}) \quad (13)$$

$$f_2' = f_2/(1 + [P]/K_9 + [PS]/K_{11}) \quad (14)$$

If the concentration of the binding protein is low enough, the terms in [P] and [PS] drop out and the constants become: $k_1' = k_1$, $k_{-1}' = k_{-1}$, $f_1' = f_1$, $f_2' = f_2$. In any case, [P] (Eqn. 8) should always be low if $K_4 \gg 1$ (low in the absence of external substrate and lower still in its presence), so that Eqns. 11–14 become, approximately,

$$k_1' = k_1/(1 + [PS]/K_7) \quad (15)$$

$$f_1' = f_1/(1 + [PS]/K_7) \quad (16)$$

$$k_{-1}' = k_{-1}/(1 + [PS]/K_{11}) \quad (17)$$

$$f_2' = f_2/(1 + [PS]/K_{11}) \quad (18)$$

From the principle of detailed balance the constant α is

$$\left(\frac{[S_i]}{[S_o]}\right)_{final} = \alpha = \frac{f_{-1}f_2k_8k_{-2}K_9}{f_1f_{-2}k_{-8}k_2K_7K_5} = \frac{f_{-1}f_2K_{S_i}}{f_1f_{-2}K_{S_o}} = \frac{f_{-1}f_2k_{-2}k_1}{f_1f_{-2}k_2k_{-1}K_5} \quad (19)$$

where $K_{S_i} = k_{-2}/k_2$, and where

$$K_{S_o} = [C_o][S_o]/[C_oS] = K_5k_{-1}/k_1$$

$$= K_5K_7k_{-8}/(K_9k_8) = K_5k_{-1}/(k_1K_4) \quad (20)$$

Also, because $[PS][C_o]/([P][C_oS]) = k_{-1}/k_1$, and $[PS]/[P] = [S_o]/K_5$,

$$[S_o]/K_{S_o} = k_1[PS]/k_{-1}[P] \quad (21)$$

α', which appears in the intermediate expressions, Eqns. 1 and 2, is found by substituting [PS] into Eqn. 19:

$$\left(\frac{[S_i]}{[PS]}\right)_{final} = \alpha' = \frac{f_{-1}f_2'k_1'k_{-2}}{f_1'f_{-2}k_{-1}'[P]k_2} = \alpha\{K_S + [S_o]_{final}\}/[P_t] \quad (22)$$

Note that binding protein-mediated addition of the substrate to the carrier is governed by the rate constant $k_1 = k_8/K_7$, and binding protein-mediated loss of substrate from the carrier-substrate complex by the constant $k_{-1} = k_{-8}/K_9$ (see Fig. 2). Note, too, that K_{S_o} is the apparent substrate dissociation constant for the outer carrier site (Eqn. 20), and K_S that for the binding protein (Eqn. 5).

To find rate equations for individual experiments (exit, entry, etc.), the constants for the full scheme, as given in Eqns. 15–18, are substituted for k_1', f_1', k_{-1}', and f_2' in the general expressions for the parameters of Eqns. 1 and 2. Next, substitution is made for [P] and [PS] (Eqns. 7 and 8), as well as [PT], in terms of the concentrations of the binding protein and external substrate. Finally, terms in external and internal substrate concentration, $[S_o]$, $[S_i]$ etc., are gathered together, allowing the experimental parameters to be defined in terms of rate constants, equilibrium constants, and the total concentrations of the binding protein and carrier ($[P_t]$ and $[C_t]$). As a check on the derivation, a theorem is applied requiring the ratio of first-order rate constants for zero-trans entry and exit to be equal to α, the ratio of internal to external substrate concentrations, $[S_i]/[S_o]$, in the final steady-state.

Naming the experimental constants

The convention followed in naming experimentally measured constants imitates that in enzyme kinetics [39]: V is a maximum flux, K a half-saturation constant, and small v the measured rate of transport of substrate S under a particular set of conditions. Attached to these symbols are subscripts and superscripts – subscripts indicating the substrate followed and its location either outside the cell, 'o', or inside, 'i', and superscripts indicating the type of experiment in which the constant is measured. A zero-trans experiment is indicated by a bar, '–', the minus sign denoting the complete absence of substrate in the trans (i.e., opposite) compartment. An equilibrium exchange experiment is indicated by a double bar, ' = ', the equal sign denoting an equal concentration of substrate in the

trans compartment (or in active transport, denoting the concentration achieved in the final steady-state). An infinite-trans experiment is indicated by a squiggle or tilde, '~', which denotes a saturating concentration of substrate in the trans compartment. (The squiggle may be viewed as the letter s on its side – s for 'saturating'. Thus, '~T' indicates a saturating concentration of substrate T in the trans compartment). The ratio of internal to external substrate concentrations in the final steady-state or equilibrium is α.

The kinetics of individual experiments

Zero-trans entry (Uptake into cells containing no substrate). The rate of appearance of substrate S inside the cell under zero-trans conditions is found from Eqn. 2 by setting $[S_i] = [T_i] = [T_o] = [PT] = 0$.

$$v = \bar{V}'_{ps}/(1 + \bar{K}'_{ps}/[PS]) \tag{23}$$

$$\bar{V}'_{ps} = \frac{k_{-2} f_{-1} f'_2 [C_t]}{k_{-2}(f_{-1} + f'_2) + f_{-1}(f'_2 + f_{-2})}$$

$$\bar{K}'_{ps} = \frac{(f'_1 + f_{-1})\{k'_{-1}[P](f_{-2} + k_{-2}) + k_{-2} f'_2\}/k'_1}{k_{-2}(f_{-1} + f'_2) + f_{-1}(f'_2 + f_{-2})}$$

$[C_t]$ is the total concentration of the carrier in the membrane. After substitution for [PS] and [P]/[PS] (Eqns. 7 and 21), the rate equation becomes a function of $[S_o]$:

$$v = f_{-1} k_{-2} [C_t]/(B + D/[S_o]) \tag{24}$$

$$B = f_{-1} + k_{-2} + (k_{-2} + f_{-2}) f_{-1}/f_2$$

$$+ (f_1 + f_{-1}) k_{-2}/(k_1[P_t]) + f_{-1} k_{-2}/(k_1 K_7)$$

$$+ (k_{-2} + f_{-2}) f_{-1}[P_t]/\{f_2 K_{11}(1 + K_S/[S_o])\}$$

$$+ f_{-1} k_{-1}[P_t](k_{-2} + f_{-2})/\{k_1 f_2 K_7 K_4 (1 + [S_o]/K_S)\}$$

$$D = \frac{K_S(f_1 + f_{-1})}{k_1[P_t]} \left\{ k_{-2} + \frac{k_{-1}[P_t](f_{-2} + k_{-2})}{f_2 K_4} \right\}$$

Since the experimental parameters $\bar{K}_{S_o} = D/B$ and $\bar{V}_{S_o} = f_{-1} k_{-2} [C_t]/B$ contain terms in $[S_o]$, the behaviour is not of the Michaelis-Menten type. These terms could drop out under various circumstances: (i) $K_4 \gg 1$, and the dead-end complex, $PS \cdot C_o S$, is not formed ($[P_t] \ll K_{11}$); (ii) the concentration of the binding protein is low ($[P_t] \ll K_7$, $[P_t] \ll K_{11}$); (iii) $K_4 \gg 1$ and the step governed by k_1 is rate-limiting: $k_1[P_t] \ll f_2(1 + f_1/f_{-1})/(1 + f_{-2}/k_{-2})$; (iv) $f_2 \gg (k_{-2} + f_{-2})$. The maximum rate and half-saturation constant for entry are then

$$\bar{V}_{S_o} = \frac{f_{-1} k_{-2} [C_t]}{f_{-1} + k_{-2} + (k_{-2} + f_{-2}) f_{-1}/f_2 + \dfrac{(f_1 + f_{-1}) k_{-2}}{k_1[P_t]} + \dfrac{f_{-1} k_{-2}}{k_1 K_7}} \tag{25}$$

$$\bar{K}_{S_o} = \frac{K_S(f_1 + f_{-1})\{k_{-2} + k_{-1}[P_t](f_{-2} + k_{-2})/(f_2 K_4)\}/k_1[P_t]}{f_{-1} + k_{-2} + (k_{-2} + f_{-2}) f_{-1}/f_2 + \dfrac{(f_1 + f_{-1}) k_{-2}}{k_1[P_t]} + \dfrac{f_{-1} k_{-2}}{k_1 K_7}}$$

$$= \frac{K_{S_o}(f_1 + f_{-1})\{k_{-2} K_4/(k_{-1}[P_t]) + (f_{-2} + k_{-2})/f_2\}}{f_{-1} + k_{-2} + (k_{-2} + f_{-2}) f_{-1}/f_2 + \dfrac{(f_1 + f_{-1}) k_{-2}}{k_1[P_t]} + \dfrac{f_{-1} k_{-2}}{k_1 K_7}} \tag{26}$$

Zero-trans exit (Exit into a suspending medium free of substrate). With $[S_o] = [T_o] = [PS] = [PT] = [T_i] = 0$, the rate of exit is found from Eqn. 2 to be

$$v = \bar{V}'_{S_i}/(1 + \bar{K}'_{S_i}/[S_i]) \tag{27}$$

$$\bar{V}'_{S_i} = \frac{k'_{-1}[P] f'_1 f_{-2}[C_t]}{k'_{-1}[P](f'_1 + f_{-2}) + f'_1(f'_2 + f_{-2})}$$

$$\bar{K}'_{S_i} = \frac{(f'_1 + f_{-1})\{k'_{-1}[P](f_{-2} + k_{-2}) + k_{-2} f'_2\}/k_2}{k'_{-1}[P](f'_1 + f_{-2}) + f'_1(f'_2 + f_{-2})}$$

After substitution for f'_1, k'_1, f'_2, k'_{-1}, [P], [PS], and [P]/[PS], and with $k_{-2}/k_2 = K_{S_i}$ and $[S_o] = 0$, Eqn. 27 becomes

$$v = \bar{V}_{S_i}/(1 + \bar{K}_{S_i}/[S_i])$$

$$\bar{V}_{S_i} = \frac{f_{-2}[C_t]}{1 + (f_{-2}/f_1) + (f_2 + f_{-2}) K_4/(k_{-1}[P_t])} \tag{29}$$

$$\bar{K}_{S_i} = \frac{(1 + f_{-1}/f_1)\{K_{S_i} + (f_{-2}/k_2) + f_2 K_{S_i} K_4/(k_{-1}[P_t])\}}{1 + (f_{-2}/f_1) + (f_2 + f_{-2}) K_4/(k_{-1}[P_t])} \tag{30}$$

Infinite-trans entry (Unidirectional uptake into cells containing a saturating concentration of substrate). From Eqn. 2, uptake of labelled substrate, S_o, into cells containing unlabelled substrate T_i at a saturating concentration, is found by setting $[S_i] = [PT] = 0$ and $[T_i]/K'_{S_i} \gg 1$:

$$v = \tilde{V}'^{S'}_{PS}/(1 + \tilde{K}'^{S'}_{PS}/[PS]) \tag{31}$$

$$\tilde{V}'^{S'}_{PS} = \frac{k_{-2} f_{-2} f'_2 k'_{-1}[P][C_t]}{(f'_2 + f_{-2})\{k'_{-1}[P](k_{-2} + f_{-2}) + f'_2 k_{-2}\}}$$

$$\tilde{K}'^{S'}_{PS} = \frac{k'_{-1}[P](f'_1 + f_{-2}) + f'_1(f'_2 + f_{-2})}{k'_1(f'_2 + f_{-2})}$$

Substitution for the various rate constants, as before, yields a rate equation that may be written as

$$v = \tilde{V}^{>}_{S_o} / \{1 + \tilde{K}^{>}_{S_o} / [S_o]\} \tag{32}$$

$$\tilde{V}^{>}_{S_o} = \left(k_{-2} f_{-1} [C_i] / \left\{k_{-2} + f_{-2} + \frac{k_{-2} f_2 K_4 (1 + [S_o]/K_S)}{k_{-1}[P_i]}\right\}\right) \times \left\{1 + \frac{f_1 (1 + f_{-2}/f_2)}{k_1 [P_i]} + \left(1 + \frac{f_1}{k_1 K_{11}}\right)(f_{-1}/f_2) + \frac{f_{-2}[P_i]/K_{11}}{f_2 (1 + K_S/[S_o])}\right\}^{-1} \tag{33}$$

$$\tilde{K}^{>}_{S_o} = \left[K_S \left\{\frac{f_1 (1 + f_{-2}/f_2)}{k_1 [P_i]} + \frac{k_{-1}(f_1 + f_{-2})}{f_2 k_1 K_4} + \frac{f_{-2} k_{-1} [P_i]/K_7}{f_2 k_1 K_4 (1 + K_S/[S_o])}\right\}\right] \left[1 + \frac{f_1 (1 + f_{-2}/f_2)}{k_1 [P_i]} + \left(1 + \frac{f_1}{k_1 K_{11}}\right)(f_{-2}/f_2) + \frac{f_{-2}[P_i]/K_{11}}{f_2 (1 + K_S/[S_o])}\right]^{-1} \tag{34}$$

$\tilde{V}^{>}_{S_o}$ and $\tilde{K}^{>}_{S_o}$ are seen to contain terms in $[S_o]$, indicating that the kinetics are not of the Michaelis-Menten type.

The maximum flux in infinite-trans entry ($\tilde{V}^{>}_{S_o}$) is identical to that in equilibrium exchange ($\bar{V}_S$) or in infinite-trans exit ($\tilde{V}^{>}_{S_i}$), since the conditions are the same – saturating concentrations of substrate on both sides of the membrane, one labelled and the other not. With $K_4 \gg 1$ and $K_{11} \gg [P_i]$ (one of the conditions giving Michaelis-Menten kinetics in entry), the rate expression is:

$$\tilde{V}^{>}_{S_o} = \bar{V}_S = \tilde{V}^{>}_{S_i} = \frac{k_{-1}[P_i][C_i]/(1 + f_2/f_{-2})}{K_4 (1 + [S_o]/K_S)\{1 + f_1/k_1 [P_i]\}} \tag{35}$$

An essential difference should be noted in the expressions for the maximum rate of entry ($\bar{V}_{S_o}$, Eqn. 24) and exchange ($\bar{V}_S$, Eqn. 35). Both equations contain terms in $[S_o]$ that give rise to non-Michaelis-Menten behaviour. But the same conditions that reduce the entry equation to a simple form by eliminating terms in $[S_o]$, allow the term $(1 + [S_o]/K_S)$ to act with full force in the exchange equation. The result is substrate inhibition, which at high concentrations drops the rate to zero.

Infinite-cis net exit (Exit of substrate at a high concentration into a solution of substrate at low concentration). With the internal substrate concentration saturating ($[S_i] \gg \bar{K}'_{S_i}$ and $[S_i] \gg \alpha'[PS]$), the rate is given by

$$v = \bar{V}'_{S_i} / \{1 + [PS]/\tilde{K}^{>'}_{PS}\} \tag{36}$$

$\bar{V}'_{S_i}$ and $\tilde{K}^{>'}_{PS}$ are defined above (Eqns. 27 and 31).

Substitution into Eqn. 36 yields

$$v = \bar{V}_{S_i} / \{1 + [S_o]/\tilde{K}^{>}_{S_o}\} \tag{37}$$

$\bar{V}_{S_i}$ and $\tilde{K}^{>}_{S_o}$ are given in Eqns. 29 and 34.

Consistency of entry and exit equations

In the mechanism in Fig. 2 the rate becomes first-order at a low substrate concentration, and the first-order rate constant is a V/K_m ratio (see Eqns. 24 and 28 with $[S_i]$ and $[S_o]$ approaching zero). Further, the ratio of the first-order constants for zero-trans entry and exit is equal to α, the ratio of internal to external substrate concentrations in the final steady-state or equilibrium [40]:

$$\bar{V}_{S_o} / \bar{K}_{S_o} = \alpha \bar{V}_{S_i} / \bar{K}_{S_i} \tag{38}$$

Substitution from Eqns. 25, 26, 29, and 30 confirms the relationship, which serves a check on the derivation.

Binding forces at the carrier site

The efficiency of any transport mechanism in which the substrate is handed on from a binding protein to a membrane carrier depends on their relative substrate affinity; the lower the affinity of the carrier, the more the substrate tends to remain on the binding protein, which retards transport. The principle may be demonstrated in the present case, where the substrate dissociation constants for the carrier and the binding protein, K_{S_o} and K_S, are necessarily related: $K_{S_o} = K_S \cdot k_{-1}/(k_1 K_4)$. It is because of this that the half-saturation constant for uptake, $\bar{K}_{S_o}$, may be written in terms of either (Eqn. 26). The connection between rate and affinity is implicit in the dissociation constants themselves: for the binding protein ($P + S_o \rightleftharpoons PS$) the constant is K_S, and for the carrier ($C_o + S_o \rightleftharpoons C_oS$) the constant is $K_{S_o} = K_S(k_{-1}/k_1)$. Their ratio is k_1/k_{-1}; $k_1 = k_8/K_7$, the rate constant for transfer of the substrate to the free outward-facing carrier (mediated by the loaded binding protein, PS), and $k_{-1} = k_{-8}/K_9$, the constant for loss of the substrate from the outward-facing carrier-substrate complex (mediated by the free binding protein, P):

$$\frac{k_1}{k_{-1}} = \frac{(k_8/K_7)}{(k_{-8}/K_9)} = \frac{K_S}{K_{S_o}} = \frac{K_S}{K_{S_o}} = \frac{K_S(1 + K_9)}{K_{S_o}(1 + K_4)} \approx \frac{K_S}{K_4 K_{S_o}} \tag{39}$$

As K_{S_o} increases, the rate of transfer of the substrate to the carrier falls.

Low carrier affinity ($K_{S_o} \gg K_S$) has two effects. First, Michaelis-Menten kinetics are not expected: from Eqn. 39, $k_1 K_4 \ll k_{-1}$ if $K_{S_o} \gg K_S$, and as a result the term in $[S_o]$ in B (Eqn. 24) cannot be neglected.

Second, uptake is slow, because with $k_1K_4 \ll k_{-1}$ and $K_4 \gg 1$, $k_1 \ll k_{-1}$. If k_1 is now rate-limiting, the maximum transport rate, from Eqn. 24, is:

$$\bar{V}_{S_o} \approx k_1[P_i][C_i]/\{1 + f_1/f_{-1} + [P_i]/K_7\} \quad (40)$$

If k_1 is still a fast step, the maximum rate is

$$\bar{V}_{S_o} \approx f_2 k_{-2}[C_i]K_7(K_S/K_{S_o})/\{(k_{-2} + f_{-2})[P_i]\} \quad (41)$$

Either way the rate is low: according to Eqn. 40 because k_1 is small, and according to Eqn. 41 because $K_S/K_{S_o} \ll 1$.

The conformational change in the binding protein, too, affects the k_1/k_{-1} ratio. If there is no conformational change ($K_4 = K_6 = 0$), or equivalently, if both conformations add to the carrier, then $k_1/k_{-1} = K_S/K_{S_o}$ (Eqn. 39). But if the conformational change is sharp ($K_4 \gg 1 \gg K_6$) and only the conformation induced by the substrate adds to the carrier, then $k_1/k_{-1} \ll K_S/K_{S_o}$. For k_1 to be as high as k_{-1} the carrier must have, in the first case, the same substrate affinity as the binding protein, and in the second case, much higher affinity. In general, the lower the affinity of the carrier relative to the binding protein, and the sharper the change in conformation, the slower the rate of substrate transfer to the carrier site.

Other models

(i) A mechanism in which the binding protein undergoes no conformational change

Assuming that the binding protein is entirely in conformation P, $K_4 = K_6 = 0$, and $K_S = K_S$. To simplify the derivation, assume that the dead-end complex is not formed: $[P_i] \ll K_{10}$ or K_{11}. Expressions for [PS], [P], k_1', k_{-1}', f_1', and f_2' are found from Eqns. 7, 8, 11, 12, 13, and 14, and the derivation is carried through as before. The rate equation for zero-trans entry is not of the simple Michaelis-Menten form. The maximum rates of entry and exit are:

$$\bar{V}_{S_o} = \frac{f_{-1}k_{-2}[C_i]}{f_{-1} + k_{-2} + f_{-1}\left(\frac{f_{-2} + k_{-2}}{f_2}\right) + \frac{(f_1 + f_{-1})k_{-2}}{k_1[P_i]} + \frac{f_{-1}k_{-2}}{k_1K_7}} \quad (42)$$

$$\bar{V}_{S_i} = \frac{f_1 f_2 k_{-1}[P_i][C_i]}{f_1(f_2 + f_{-2}) + k_{-1}[P_i](f_1 + f_{-2}) + f_1 f_{-2}[P_i]/K_9} \quad (43)$$

(ii) A mechanism in which the free binding protein but not the substrate complex interacts with the carrier

Assume that the free binding protein is mainly in conformation P, the form that interacts with the carrier, and the substrate complex in conformation P′, which does not; then $K_4 \ll 1$ and $K_6 \gg 1$. Assume also that the non-productive complex is not formed. Again the entry equation is not Michaelis-Menten in form. The maximum rates of entry and exit are:

$$\bar{V}_{S_o} = \{f_{-1}k_{-2}[C_i]\}\{f_{-1} + k_{-2} + (f_{-2} + k_{-2})f_{-1}/f_2 + (f_1 + f_{-1})k_{-2}K_6/(k_1[P_i])\}^{-1} \quad (44)$$

$$\bar{V}_{S_i} = \frac{f_1 f_{-2}[C_i]}{f_1 + f_{-2} + (f_2 + f_{-2})f_1/(k_{-1}[P_i]) + f_1 f_{-2}/(k_{-1}K_9)} \quad (45)$$

Summary of the behaviour

Though translocation across the membrane depends on an ordinary carrier mechanism, the involvement of the binding protein increases the complexity of the kinetic equations and alters the behaviour. The main characteristics of the system are as follows.

(1) The rate equation for zero-trans entry is not of the Michaelis-Menten form but may reduce to this form under any of the following conditions: (i) $K_4 \gg 1$ and the dead-end complex, $PS \cdot C_oS$, is not formed ($K_{11} \gg [P_i]$); (ii) the concentration of the binding protein is low ($[P_t] \ll K_7$, $[P_i] \ll K_{11}$); (iii) $K_4 \gg 1$ and the step governed by k_1 is rate-limiting; (iv) $f_2 \gg (k_{-2} + f_{-2})$.

(2) The substrate half-saturation constant for entry, $\bar{K}_{S_o}$, need not be equal to the dissociation constant for the binding protein, K_S (Eqn. 26). If condition (ii) for Michaelis-Menten kinetics applies (see above), $\bar{K}_{S_o}$ is equal to K_S. If condition (iii) or (iv) applies, $\bar{K}_{S_o}$ is less than K_S:

$$\bar{K}_{S_o} = K_S/\{1 + [P_i]/\{K_7(1 + f_1/f_{-1})\}\} \quad (46)$$

If condition (i) applies, $\bar{K}_{S_o}$ may be smaller or larger than K_S. As $\bar{K}_{S_o}$ is proportional to K_{S_o} (Eqn. 26), $\bar{K}_{S_o}$ should be greater than K_S if $K_{S_o} \gg K_S$. But here the rate equation for zero-trans entry ceases to be of the Michaelis-Menten form, because with $k_{-1} \gg k_1K_4$ (Eqn. 20), a term in $[S_o]$ in Eqn. 24, in the expression for B, is no longer negligible. For uptake to follow Michaelis-Menten kinetics, therefore, $\bar{K}_{S_o}$ should not be too much larger than K_S.

(3) A low substrate affinity of the carrier site ($K_{S_o} \gg K_S$) retards transport.

(4) The conformational change in the binding protein ($K_4 \gg 1$ and $K_6 \ll 1$) causes the maximum rate of entry, $\bar{V}_{S_o}$, to be far higher than the maximum rate of exit, $\bar{V}_{S_i}$:

$$\frac{\bar{V}_{S_o}}{\bar{V}_{S_i}} = \frac{K_4(1 + f_2/f_{-2})k_1/k_{-1}}{\{1 + f_1/f_{-1} + [P_i]/K_7\}} \quad (47)$$

The ratio of rates is seen to be proportional to K_4, which is independent of α (Eqn. 19) and of the mecha-

nism of coupling to ATP. As a result, asymmetry persists in the de-energized state. The binding protein facilitates substrate entry but makes exit difficult, even under conditions of passive transport.

The symmetry is reversed if the free binding protein but not the complex interacts with the carrier, i.e., if $K_6 \gg 1 \gg K_4$. Here exit is much slower than entry (Eqns. 44 and 45):

$$\frac{\bar{V}_{S_0}}{\bar{V}_{S_i}} = \frac{(f_2 + f_{-2})k_1/k_{-1} + k_1[P_t](1 + f_{-2}/f_1) + f_{-2}k_1[P_t]/(k_{-1}K_9)}{f_{-2}K_6(1 + f_1/f_{-1})} \tag{48}$$

In a model in which the binding protein exists in a single conformation, where $K_4 = K_6 = 0$, there is no asymmetry other than that introduced by steps determining the uptake ratio, α (Eqns. 19, 42, and 43). The comparison of the three models makes it clear that the binding protein independently controls symmetry.

(5) Two factors combine to make exchange transport extremely slow: (a) an inverse dependence on K_4 (exactly as in exit), and (b) an unusual type of substrate inhibition (Eqn. 35). The inhibition constant is K_S, the dissociation constant for the binding protein. As the Michaelis constant for entry, $\bar{K}_{S_0}$, is in many cases close to K_S, exchange may be largely blocked at the substrate concentrations required for maximal rates of uptake.

(6) Counter-transport, which depends on exchange, is not expected.

(7) The maximum rate and the half-saturation constant in entry, $\bar{V}_{S_0}$ and $\bar{K}_{S_0}$, are functions of the concentration of the binding protein in the periplasm, $[P_t]$. $\bar{V}_{S_0}$ should rise to a limit as $[P_t]$ increases, while $\bar{K}_{S_0}$ may either rise or fall: $\bar{V}_{S_0} = a/(1 + K_p/[P_t])$ and $\bar{K}_{S_0} = b(1 + c/[P_t])/(1 + K_p/[P_t])$, where a, b, c, and K_p are constants.

(8) Formation of the complex of the binding protein with the membrane component depends on rate constants for transport as well as on the dissociation constant for the complex, K_7. From the dependence of the maximum rate of entry on the concentration of the binding protein (Eqn. 25), the half-saturating concentration, K_p, is given by

$$K_p = \frac{K_7(1 + f_1/f_{-1})}{1 + k_1K_7\{(1/f_{-1}) + (1/k_{-2}) + (1 + f_{-2}/k_{-2})/f_2\}} \tag{49}$$

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