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Role of Substrate Binding Forces in Exchange-Only Transport Systems: I. Transition-State Theory

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Summary. An analysis of transition-state models for exchange-only transport shows that substrate binding forces, carrier conformational changes, and coupled substrate flow are interrelated. For a system to catalyze exchange but not net transport, addition of the substrate must convert the carrier from an immobile to a mobile form. The reduction in the energy barrier to movement is necessarily paid for out of the intrinsic binding energy between the substrate and the transport site, and is dependent on the formation of two different types of complex: a loose complex initially and a tight complex in the transition state in carrier movement. Hence the site should at first be incompletely organized for optimal binding but, following a conformational change, complementary to the substrate structure in the transition state. The conformational change, which may involve the whole protein, would be induced by cooperative interactions between the substrate and several groups within the site, involving a chelate effect. The tightness of coupling, i.e., the ratio of exchange to net transport, is directly proportional to the increased binding energy in the transition state, a relationship which allows the virtual substrate dissociation constant in the transition state to be calculated from experimental rate and half-saturation constants. Because the transition state is present in minute amount, strong bonding here does not enhance the substrate's affinity, and specificity may, therefore, be expressed in maximum exchange rates alone. However, where substrates largely convert the carrier to a transport intermediate whose mobility is the same with all substrates, specificity is also expressed in affinity. Hence the expression of substrate specificity provides evidence on the translocation mechanism.

Key Words exchange · transport · transition state · binding forces · substrate specificity · coupled transport

Introduction

Two fundamental problems are encountered in studies of the transport of small molecules or ions across biological membranes. One is the problem of translocation itself—how a protein molecule imbedded in the membrane can with great specificity conduct its substrate from one side to the other. The second is the problem of coupling—how the flow of one substrate across the membrane can entrain the flow of another. The mechanism of neither simple transport nor coupled transport is well understood, despite their importance in cell physiology.

Both active transport, sustained by an ion gradient or proton motive force, and exchange-only transport depend on some kind of coupling. Exchange-only transport, because of its simplicity, is a particularly favorable subject for theoretical and experimental study. The kinetic analysis of exchange, which is given below, shows that a direct relationship exists between the tightness of coupling and the strength of the binding forces developed in the substrate complex. The conclusions are the same whether we treat the carrier model or a bilateral transport model. These findings have definite implications for the structure and function of the transport site.

Theory

The Carrier Model

For the carrier model (Fig. 1) to bring about exchange-only transport, the carrier must be free to move across the membrane with the substrate bound, but not otherwise. The restriction is necessary, for if the carrier returned empty there would be a net transfer of substrate from one compartment to the other. The increased carrier mobility on substrate binding is a crucial property of the system.

It should be understood that in this mechanism the substrate site, and not necessarily the whole carrier, shifts from one side of the membrane to the other. In many biological transport systems the carrier is a fixed, membrane-spanning protein, which is probably an equilibrium mixture of two different conformations. In one form, the substrate site is exposed to the solution outside the cell, and in the other it is exposed inside. The carrier may be said to

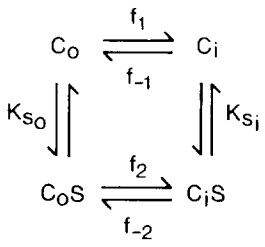

Fig. 1. The carrier model, in which the carrier exists in inward-facing and outward-facing forms, C_i and C_o, respectively. Substrate in the internal compartment forms a complex with C_i, and substrate in the external compartment with C_o

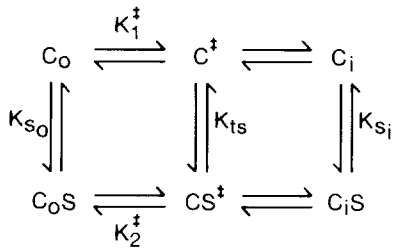

Fig. 2. The transition-state carrier model. The transition states in the interconversion of the outward-facing and inward-facing carrier forms are shown, both for the free carrier, $C^{\ddagger}$, and the substrate complex, $CS^{\ddagger}$

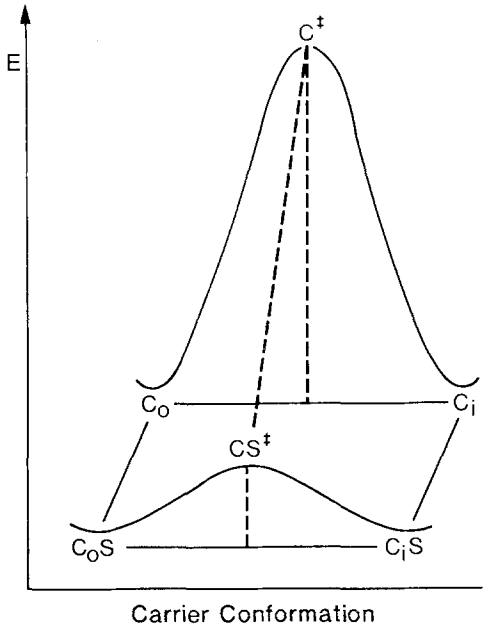

Fig. 3. Energy profile for carrier isomerization in exchange transport. The four stable carrier states, C_o, C_i, C_oS, and C_iS, are represented as being at the same energy level and, therefore, on the same plane in the diagram. (The relative levels of the complex and the free carrier would of course depend on the concentration of the substrate in relation to its dissociation constant.) The transition state for the free carrier, $C^{\ddagger}$, is at a higher energy level (vertical axis) than that for the substrate complex, $CS^{\ddagger}$. The rate of reorientation is, therefore, faster with the substrate complex than with the free carrier, and to exactly the same degree the substrate complex is more stable in the transition state ($C^{\ddagger} + S \rightleftharpoons CS^{\ddagger}$) than in the initial state ($C_o + S_o \rightleftharpoons CS_o$). The diagram makes it clear that the tightness of coupling of inward and outward substrate flows is directly related to the tightness of substrate binding in the transition state

alternate between inward-facing and outward-facing conformations, C_i and C_o, respectively, and in the conversion of one to the other a bound substrate molecule is shut off from the solution on one side of the membrane and exposed to the solution on the other side.

In terms of the coupling mechanism, it is clear that if the substrate complex undergoes reorientation readily and the free carrier not at all, then the energy barrier between the inward-facing and outward-facing conformations must fall on addition of the substrate. Some source of energy must account for this lowering of the barrier, and in the absence of chemical transformations the only possible source would be the forces of binding in the complex. The magnitude of these binding forces, and the implications for the transport mechanism, are brought out by a kinetic analysis of the carrier model.

Transition-State Model

In the simplest case, the carrier model is expanded to include the transition states, $C^{\ddagger}$ and $CS^{\ddagger}$, which are formed in the interconversion of the outward-facing and inward-facing carriers (Fig. 2). In the potential energy diagram corresponding to this scheme (Fig. 3), the transition state for the free carrier, $C^{\ddagger}$, is represented as being at a much higher energy level than the transition state for the carrier-substrate complex, $CS^{\ddagger}$. This accounts for the low reactivity of the free carrier, and at the same time shows that the substrate should be bound more strongly in the transition state than in either the outward-facing or inward-facing forms of the complex.

The binding constant in the transition state, K_{ts}, can be calculated from maximum flux and half-saturation constants for substrate transport. Expressions for the required experimental parameters are readily found when the individual rate constants for carrier reorientation, f_1, f_2, etc., which appear in these expressions, are written in terms of the transition state in each reaction.

From a comparison of the two reaction schemes, Figs. 1 and 2, it is seen that the rate of conversion of C_o to C_i is given by

$$f_1[C_o] = k[C^{\ddagger}] \qquad (1)$$

where k is a universal constant for decomposition of the transition state. The transition state is considered to be in equilibrium with C_o (for a brief account of the absolute rate theory *see*, for example, Chang, 1981):

$$[C^{\ddagger}] = K_1^{\ddagger}[C_o]. \tag{2}$$

It follows that $f_1[C_o] = kK_1^{\ddagger}[C_o]$, and therefore,

$$f_1 = kK_1^{\ddagger}. \tag{3}$$

Similarly,

$$f_2 = kK_2^{\ddagger}. \tag{4}$$

The increase in the binding force in the transition state is calculated from the ratio of the maximum exchange rate and the rate of leakage (slippage) in the system, i.e., coupled relative to uncoupled flow. In the carrier mechanism in Fig. 1, the maximum rate of exchange is given by

$$\overline{\overline{V}}_S = C_t/(1/f_2 + 1/f_{-2}) \tag{5}$$

where C_t is the total carrier concentration in all forms (Devés & Krupka, 1979). The maximum rate of carrier-mediated leakage (the rate for example with substrate inside the cell but not outside) is

$$\overline{V}_{Si} = C_t/(1/f_1 + 1/f_{-2}). \tag{6}$$

In an exchange-only system, $\overline{\overline{V}}_S \gg \overline{V}_{Si}$, and therefore, from Eqs. (5) and (6), $(1/f_1 + 1/f_{-2}) \gg (1/f_2 + 1/f_{-2})$; hence $f_2 \gg f_1$. As f_2 and f_{-2} will be of comparable magnitude (otherwise exchange, Eq. (5), would be slow), $f_{-2} \gg f_1$, and Eq. (6) reduces to

$$\overline{V}_{Si} = f_1 C_t. \tag{7}$$

The experimental value of the substrate half-saturation constant in exchange transport is, for the scheme in Fig. 1 (Déves & Krupka, 1979)

$$\begin{aligned} \tilde{K}_{So}^{S} &= K_{So}(f_1 + f_{-2})/(f_2 + f_{-2}) \\ &\simeq K_{So}/(1 + f_2/f_{-2}) \end{aligned} \tag{8}$$

(since $f_{-2} \gg f_1$). $\tilde{K}_{So}^{S}$ is defined as the half-saturating concentration of external substrate, with substrate inside the cell at a saturating concentration. From Eqs. (5), (7), and (8)

$$\frac{\overline{V}_{Si}}{\overline{\overline{V}}_S} \tilde{K}_{So}^{S} = \frac{f_1}{f_2} K_{So}. \tag{9}$$

The relationship between K_{So} and K_{ts} is found from the principle of detailed balance, for the constants

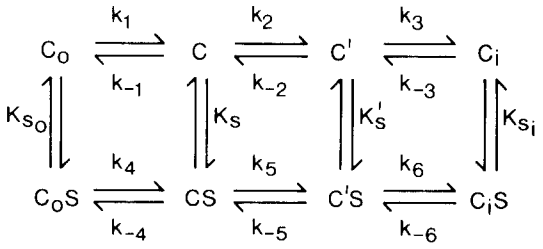

Fig. 4. An expanded carrier model involving intermediates in translocation (C and C' in the conversion of C_o to C_i, and CS and $C'S$ in the conversion of C_oS to C_iS)

in the circle of carrier forms in Fig. 2, C_o, $C^{\ddagger}$, $CS^{\ddagger}$, and C_oS, are interrelated

$$K_1^{\ddagger} K_{So} = K_2^{\ddagger} K_{ts}. \tag{10}$$

Substitution into Eq. (9) of K_{So} (from Eq. (10)) and of expressions for f_1 and f_2 (Eqs. (3) and (4)) yields

$$K_{ts} \simeq \tilde{K}_{So}^{S} \overline{V}_{Si}/\overline{\overline{V}}_S. \tag{11}$$

The increase in the exchange rate over the rate of net transport is seen to be directly dependent on the increase in the binding force in the transition state. That is, the ratio of binding strengths in the transition state and the initial complex, $\tilde{K}_{So}^{S}/K_{ts}$, is equal to the ratio of exchange to net transport, $\overline{\overline{V}}_S/\overline{V}_{Si}$ (Eq. (11)).

Mechanisms Involving an Intermediate in Carrier Reorientation

Substrate binding forces could be utilized to stabilize both the transition state and an intermediate in transport. Such a mechanism, shown in Fig. 4, is of interest because it can be distinguished experimentally, either by detection of the intermediate, or from the expression of substrate specificity. To see the implications of the mechanism, it is sufficient to treat the simplest case, in which the intermediate, C and CS, is in equilibrium with the initial carrier forms: $[C] = K_1[C_o]$ and $[CS] = K_4[C_oS]$, where $K_1 = k_1/k_{-1}$ and $K_4 = k_4/k_{-4}$. The derivation is similar to that for the transition-state model except that the concentration of the intermediate cannot be assumed to be negligible. The half-saturation constant for external substrate, K_{mo}, must then take into account both external forms of the carrier, C_o and C:

$$K_{mo} = \frac{([C_o] + [C])}{([C_oS] + [CS])}[S_o] = K_{So}\frac{(1 + K_1)}{(1 + K_4)} \tag{12}$$

and the experimental value of the half-saturating concentration, $\tilde{K}_{So}^{S}$ (Eq. (8)), is given by

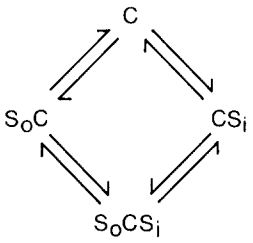

Fig. 5. The bilateral transport model, in which substrate sites are simultaneously exposed on both sides of the membrane. Substrate in both compartments can add to the carrier C at the same time, forming a ternary complex, S_oCS_i

$$\tilde{K}^S_{So} = K_{mo}/(1 + f_2/f_{-2}) = \frac{K_{So}(1 + K_1)}{(1 + f_2/f_{-2})(1 + K_4)}. \quad (13)$$

The rate constant for carrier reorientation, f_1, is found by means of a comparison of the scheme in Fig. 4 with the simple carrier scheme in Fig. 1

$$f_2[C_oS^t] = k_5[CS] = k_5[C_oS^t]/(1 + 1/K_4) \quad (14)$$

where C_oS^t is the total of both forms of carrier-substrate complex in equilibrium with the external substrate

$$[C_oS^t] = [C_oS] + [CS] = [CS](1 + 1/K_4). \quad (15)$$

From Eq. (14),

$$f_2 = k_5/(1 + 1/K_4). \quad (16)$$

Similarly,

$$f_1 = k_2/(1 + 1/K_1). \quad (17)$$

From Fig. 4, the dissociation constant for the mobile carrier form is given by $K_S = K_{So}K_1/K_4$, and by combining this expression with Eqs. (5), (7), (13), (16) and (17) it is found that

$$\frac{\overline{V}_{Si}}{\overline{\overline{V}}_S}\tilde{K}^S_{So} = K_S(k_2/k_5). \quad (18)$$

If the intermediates C and CS react at the same rate ($k_2 = k_5$), the calculated substrate dissociation constant for the intermediate (Eq. (18)) is identical to that for the transition state (Eq. (11)). The reason is that, where $k_2 = k_5$, the energy barrier to reaction of the intermediates C and CS is identical. The difference in energy levels for these two intermediates, which is also a measure of the binding energy, is then identical to the difference in energy levels for the transition states. If $k_2 \neq k_5$, the binding energies will be different in the intermediate and transition state.

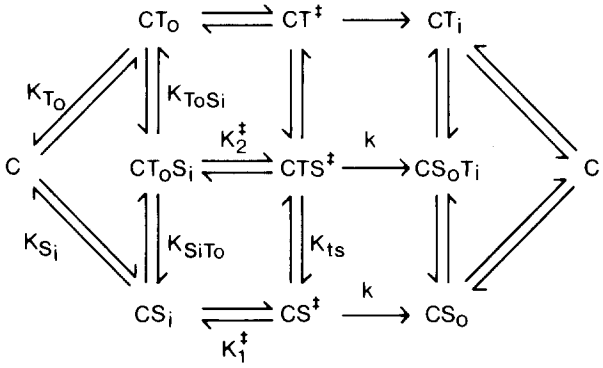

Fig. 6. The bilateral transport model, showing the transition state in translocation. Two substrates are present, S_i and T_o, which form a binary complex with the carrier, CS_i and CT_o, and also a ternary complex, CT_oS_i; the corresponding transition states are $CS^‡$, $CT^‡$, and $CTS^‡$

A Bilateral Model

Transport mechanisms are conveniently divided into two types, those in which a substrate site alternately appears on opposite sides of the membrane, as in the carrier model in Fig. 1, and those in which substrate sites are simultaneously exposed on both sides, as in the bilateral model in Fig. 5. We can expect substrate binding forces to play a similar role in the two mechanisms, but whether the relationship to coupling is the same should be determined.

According to the bilateral model (Fig. 6), the substrate can add on both sides of the membrane at once, forming a ternary complex, CT_oS_i. Exchange transport involves the simultaneous translocation of the two bound substrate molecules in the ternary complex in opposite directions, and net transport the translocation of a single bound substrate molecule in the binary complex, CS_i or CT_o. For coupling to be tight, the binary complex should be immobile. The relationship between binding forces in the transition state and the rate of translocation of the ternary complex may be determined as follows.

The maximum rate of net exit from cells containing a saturating concentration of substrate, with all the carrier in the form of CS_i, is

$$\overline{V}_{Si} = k[CS^‡] = kK_1^‡[CS_i] = kK_1^‡[C_t]. \quad (19)$$

The maximum rate of exchange, with all the carrier in the form of CT_oS_i, is

$$\overline{\overline{V}}_{Si} = k[CTS^‡] = kK_2^‡[CT_oS_i] = kK_2^‡[C_t]. \quad (20)$$

It follows that the ratio of exchange to net transport is

$$\frac{\overline{\overline{V}}_{Si}}{\overline{V}_{Si}} = \frac{kK_2^‡}{kK_1^‡} = \frac{K_2^‡}{K_1^‡}. \quad (21)$$

The reaction scheme in Fig. 6 shows that $K_2^{\ddagger}$ and $K_1^{\ddagger}$ are related to K_{ts} and K_{SiTo}:

$$K_1^{\ddagger} K_{SiTo} = K_2^{\ddagger} K_{ts} \tag{22}$$

and substitution of this relationship into Eq. (21) yields

$$\frac{\overline{\overline{V}}_{Si}}{\overline{V}_{Si}} = \frac{K_{SiTo}}{K_{ts}}. \tag{23}$$

In terms of the general nomenclature used above, $K_{SiTo} = \tilde{K}^S_{To}$, both constants representing the half-saturating concentration of external substrate (T_o), with substrate inside at a saturating concentration. Further, if S and T stand for labeled and unlabeled forms of the same substrate S, then $\tilde{K}^S_{To} = \tilde{K}^S_{So}$. Hence, from Eq. (23),

$$K_{ts} = \tilde{K}^S_{So} \overline{V}_{Si} / \overline{\overline{V}}_{Si}. \tag{24}$$

Equation (24) for a bilateral transport model is seen to be the same as Eq. (11) for the carrier model.

Net Transport by Pathways Other Than Slippage

The rate of slippage, $\overline{V}_{Si}$, may be overestimated if net transport occurs by other routes such as simple diffusion through the membrane or transport by another carrier. A specific inhibitor of the exchange-only system could be used to detect leaks of this kind. Another possible leak, which has been called tunneling (Fröhlich, 1988), involves passage of the substrate through the closed carrier channel, in the absence of carrier reorientation. Specific inhibitors would fail to discriminate between this mechanism of translocation and true slippage.

The point to notice is that if $\overline{V}_{Si}$ is overestimated because of such leaks, the affinity of the substrate in the transition state will be underestimated. Hence, in a system catalyzing exchange-only transport, the actual increase in binding forces in the transition state should be at least as great as calculated from Eqs. (11) and (24).

Substrate Specificity

The transition-state reaction schemes for the carrier and bilateral models (Figs. 2 and 6) predict that substrate specificity should be expressed in the maximum rate of translocation but not in the affinity. The mechanism may be explained as follows. As the exchange rate depends on increased binding forces in the transition state relative to the substrate complex, strong initial binding is a disadvantage, and good substrates are likely to be no more strongly bound in the initial complex than poor substrates. Good substrates are distinguished by being tightly bound in the transition state, where poor substrates are weakly bound. The tightness of binding at this stage determines the transport rate but has no effect on the substrate's half-saturation constant, since the transition state exists at a vanishingly low concentration.

In one special case, where a high steady-state concentration of a carrier intermediate is formed in transport, substrate affinity may also be involved in specificity. Provided the intermediate reacts at the same rate with all substrates, the transport rate depends on the steady-state concentration of the intermediate, which in the scheme for the carrier model (Fig. 4) is a function of K_4 ($K_4 = k_4/k_{-4}$). Here the substrate half-saturation constant is inversely proportional to $1 + K_4$ (*see* Eq. (13), where $K_1 \ll 1$ in an exchange-only system), while the maximum exchange rate, governed by f_2 (Eq. (5)), is inversely proportional to $1 + 1/K_4$ (Eq. (16)). If for various substrates K_4 has a range of values all greater than unity, the affinity can vary widely, with little change in the maximum rate; K_4 for a good substrate must then be very large, meaning that in its presence the carrier is converted entirely to the intermediate form. If the values of K_4 are all less than unity, the maximum rate varies but not the affinity; here even the best substrates only partially convert the carrier to the intermediate. If K_4 is about the same with all substrates and only k_5 varies, the result is similar: the maximum rate varies (Eqs. (5) and (16)) with little or no change in the affinity (Eq. (13)).

The results of the analysis may be summed up as follows. Where only rates are sensitive to substrate structure, we infer either that intermediates are formed in insignificant amounts or that the mobility of an intermediate, not its concentration, determines the transport rate. Where only affinities are sensitive to substrate structure, we infer that all substrates convert the carrier largely to an intermediate form, whose concentration determines the rate. Where both rates and affinities are sensitive to substrate structure, we infer that good substrates produce much and poor substrates little of the intermediate.

The Energy Barrier

Exchange-only transport depends, as we have noted, on the inability of the carrier to undergo reorientation unless the substrate is bound at the transport site (both sites in the case of the bilateral model, Fig. 5). Why one form of the carrier is mobile and the other not, and how mobility is related to substrate binding energy, are problems of wide sig-

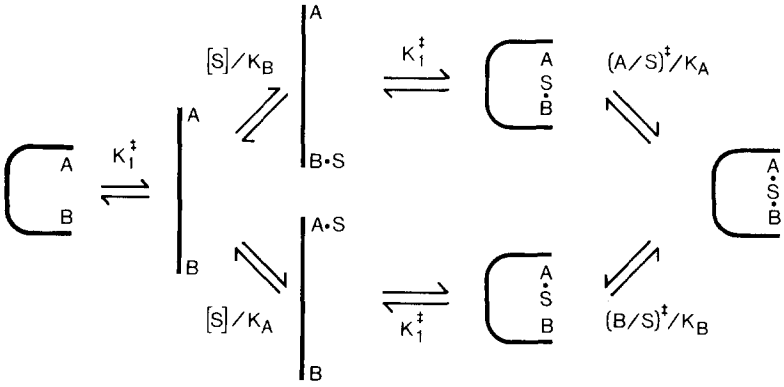

Fig. 7. Kinetic scheme for the induction of a conformation change in a polypeptide chain by a substrate, *S*. The chain contains two binding sites, *A* and *B*, which are distant in the unfolded conformation but close together in the folded conformation (represented by a straight bar and a U-shaped bar, respectively). In the substrate complex the folded conformation is stabilized by a chelate effect, which is dependent on heightened interaction between the substrate and the two binding sites

nificance. On such coupling depends all active transport involving cotransport of driving and driven substrates, as in systems that expend the transmembrane proton-motive force to build up a substrate concentration gradient.

The anion exchanger of red cells, discussed in the following paper, is an example of a transport system in which both substrates are simple ions. Here, two different mechanisms can be envisaged to explain the increased mobility of the complex. One involves purely electrostatic effects and the other, conformational changes. The first depends on the free energy of an electrostatic charge, opposite to the charge in the substrate, at the transport site. The hypothesis is as follows. At the surface of the membrane, the charged group in the free carrier is surrounded by water, but as it moves through the membrane in the course of carrier reorientation it becomes immersed in a nonpolar medium, such as the interior of the lipid bilayer. Transfer of an ion from an aqueous to a nonpolar environment is an unfavorable process, which in this mechanism stands as a barrier to reorientation of the carrier. When the substrate, which is an ion of opposite charge, is bound at the transport site, the resulting ion-pair has a net charge of zero, and if the ion-pair were more readily immersed in the nonpolar medium than the single ion, the effect of substrate binding would be to lower the barrier to movement of the carrier, as required in exchange-only transport. The increased substrate binding energy in the mobile forms, as predicted by Eq. (11), derives in this case from the effect of the dielectric constant of the medium, polar *vs.* nonpolar, on the strength of electrostatic interactions.

This mechanism, however, cannot be correct, because the energy of an ion-pair in a nonpolar medium has been shown to be as great as that of a single ion (Parsegian, 1969). The proposed transition state would, therefore, form no more readily with the ion-pair (in the substrate complex) than with a single ion at the transport site. Aside from this objection, such a mechanism would not be general, in that it would not explain coupled transport of a neutral substrate, as in Na^+-glucose cotransport.

Some other mechanism must, therefore, be sought. As translocation involves a substantial carrier conformational change, in which an inward-facing carrier is converted to an outward-facing form, the role of substrate binding forces could well be to stabilize an intermediate conformation. In such a mechanism, the driving force in producing the transition state is the increasing strength of interaction between the substrate and the transport site as the carrier conformation changes. At first, the substrate would necessarily fit the site inexactly or incompletely, but as the protein conformation changed the fit would become more exact and more encompassing, and the interaction energy greater. The additional binding forces between the substrate and the transport site then compensate for the unfavorable energy change in the conversion of the immobile to the mobile carrier form.

The Connection Between Binding Forces and Protein Conformational Changes

The stabilization of an altered carrier conformation by the forces of substrate binding is akin to the stabilization of the folded state of a polypeptide chain by cooperative intramolecular interactions of groups within the chain. A simple treatment of the problem of protein folding has been outlined by Creighton (1984), and a similar treatment for the effect of substrate binding forces on the conformation of a polypeptide chain is given in Fig. 7. Two binding sites in the chain, *A* and *B*, are assumed to interact with the substrate. In the extended chain, where the sites are distant, each bonds singly with the substrate, and the complex is, therefore, loose. In the folded chain, where the two sites are in a position to interact simultaneously with the sub-

strate, a chelate effect makes binding stronger. In practice more than two groups may be involved. The interactions are cooperative, because the first tend to fix the positions of successive interacting groups, raising their effective concentrations. The same phenomenon accounts for rate accelerations in intramolecular catalysis, where the entropy of activation is made more favorable by restrictions on the freedom of movement, translational and rotational, of interacting atoms (Page & Jencks, 1971).

The relation of substrate binding forces to the conformational change, from extended to folded chain, may be found by artificially dividing the reaction into two steps: first, the extended chain with the substrate bound at one site folds, attaining its final conformation, though without additional binding interactions; next the second site bonds with the substrate (Fig. 7). If in the folded substrate complex the concentrations of the groups A and B relative to the substrate S are written $(A/S)^{\ddagger}$ and $(B/S)^{\ddagger}$, respectively, then the equilibrium constant for formation of the folded from the unfolded substrate complex, $K_2^{\ddagger}$, is given by

$$K_2^{\ddagger} = \frac{\text{Sum of folded forms of the complex}}{\text{Sum of unfolded forms of the complex}} = \frac{K_1^{\ddagger}\{1 + [1 + K_A/(A/S)^{\ddagger}](B/S)^{\ddagger}/K_B}{1 + K_A/K_B}. \quad (25)$$

The term $(B/S)^{\ddagger}/K_B$, representing the strength of the second interaction with the substrate, accounts for the stabilization of the folded conformation. $(B/S)^{\ddagger}$, the effective concentration of B relative to S in the chelate, can be large; for example, in intramolecular reactions, where a covalent bond is formed, the factor can easily be 10^8 M, though for ionic or hydrophobic bonding where the interacting groups are not rigidly positioned it may be only 10^2 to 10^3 M (Creighton, 1984). For hydrogen-bonding, which is moderately sensitive to orientation, the factor could be intermediate. In substrate chelation, $(B/S)^{\ddagger}$ should be somewhat lower than these estimates, because the substrate is not covalently anchored and, therefore, retains some freedom of movement in the complex. With each additional link, the substrate is further restrained, however, making successive interactions increasingly tight.

If the unfolded chain in Fig. 7 is taken to represent the transport site in its initial state, and the folded chain the site in the transition state, then Eq. (25) relates the substrate binding forces to the rate of carrier reorientation. The equilibrium constant for activation of the substrate complex is $K_2^{\ddagger}$, and that for activation of the free carrier $K_1^{\ddagger}$ (Figs. 2 and 7). Their ratio, which determines the relative rates of exchange and net transport (Eqs. (3) and (4)) is found by rewriting Eq. (25):

$$\frac{\overline{\overline{V}}_S}{\overline{V}_{Si}} = \frac{K_2^{\ddagger}}{K_1^{\ddagger}} = \frac{1 + K_A/(A/S)^{\ddagger}](B/S)^{\ddagger}/K_B}{1 + K_A/K_B}. \quad (26)$$

Concluding Remarks

The binding forces that must develop between the substrate and the carrier site during translocation appear to be independent of the transport mechanism. Thus, the predicted affinity in the transition state is identical for the carrier model (Fig. 1), where the carrier-substrate complex is mobile and the free carrier immobile, and for a bilateral transport model (Fig. 5), where a ternary carrier-substrate complex, CS_2, is mobile, and the binary complex, CS, is immobile (Eqs. (11) and (24)). It seems likely that these forces overcome the resistance to carrier movement by stabilizing an altered, and mobile, carrier conformation.

A related point, which is implicit in the transition-state theory, is that no exchange system could entirely prohibit net transport. The increase in mobility of the substrate complex compared to the free carrier, which determines the exchange rate and the tightness of coupling, is proportionate to the increased force of substrate binding. The force would have to be infinitely strong to convert the carrier to a mobile form, were the free carrier completely immobile. The term "exchange-only" transport, though vivid, may be misleading. This point could be overlooked in the ordinary carrier scheme in Fig. 1, where f_1 and f_{-1}, the rate constants for movement of the free carrier, may be assumed to be zero, with no apparent violation of the thermodynamics of the system.

Finally, it is of interest to compare the roles of substrate binding forces in coupled transport and enzyme catalysis. In enzymes, the substrate site is designed to form a loose complex with the substrate in its ground state and a very tight complex with the altered substrate structure in the transition state (Wolfenden, 1972; Lienhard, 1973). In exchange transport too the carrier forms a loose complex initially and a tight complex in the transition state, but the protein, not the substrate, is transformed, and the substrate rather than the protein is the catalyst. The carrier reaction, an isomerization between inward-facing and outward-facing forms, has been studied in the case of the ATP-ADP exchanger of mitochondria (Klingenberg et al., 1976).

The difference in the role of substrate binding forces in carriers and enzymes has practical consequences for the design of inhibitors. In a coupled

transport system, a powerful inhibitor should be complementary in structure to the transport site in its initial conformation. It is then unlikely to fit the transition state conformation of the carrier, and as a result should be strongly bound but not transported. In an enzyme system, a powerful inhibitor should differ from the substrate in resembling the transition state in the substrate reaction, since this structure fits the active site exactly.

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