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Expression of Substrate Specificity in Facilitated Transport Systems

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Summary. In facilitated transport systems the carrier reorientation step is shown to be largely independent of the forces of interaction between the substrate and the carrier site, whereas in coupled systems (obligatory exchange or cotransport) reorientation proceeds at the expense of the binding force developed in the transition state. In consequence, the expression of substrate specificity is expected to differ in the two systems. In the facilitated transport of analogs no larger than the normal substrate, the affinity but not the maximum rate of transport can vary widely; with larger analogs, both the affinity and rate can vary if steric constraints are more severe in the translocation step than in binding. In coupled transport, by contrast, the translocation step can be highly sensitive to the structure of the substrate, and binding much less sensitive. The theory agrees with published observations on facilitated systems for choline and glucose in erythrocytes, as well as on Na^+-coupled systems for the same substrates in other cells. The following mechanism, which could account for the behavior, is proposed. In facilitated systems, the transport site fits the substrate closely and retains its shape as the carrier undergoes reorientation. In coupled systems, the site is initially looser, but during carrier reorientation it contracts around the substrate. In both systems, the carrier encloses the substrate during the translocation step, though for a different reason: in coupled but not in facilitated systems the binding force enormously increases in the enclosed state, through a chelation effect. In both systems, steric interference with enclosure retards the translocation of bulky substrate analogs.

Key Words facilitated transport · active transport · substrate specificity · substrate binding forces · transition state · carrier conformational change

Introduction

In facilitated transport systems, substrate specificity is expressed in a different way than in exchange-only systems. The patterns may be summarized as follows: in a facilitated system such as the glucose carrier of red cells, specificity for monosaccharides is shown in the affinity alone (Stein, 1967); in an exchange-only system such as the anion-exchanger of red cells, specificity for substrates as unalike as singly and doubly charged anions is shown in the maximum rate of translocation alone (Krupka, 1989*b*). Facilitated systems permit substrates to flow from regions of high to regions of low concentration; exchange-only systems permit substrates on either side of the membrane to change places, but forbid net flow.

The question to be explored here is whether the underlying mechanism of transfer across the membrane decides how the preference of each type of system is expressed. In exchange-only transport, the carrier-substrate complex is mobile but the free carrier is not; the mobility of the complex has been shown to depend on the utilization of substrate binding forces, which convert the carrier from the immobile to a mobile form (Krupka, 1989*a*). In facilitated transport, by contrast, both the free carrier and the complex are mobile, so that substrate binding forces can play no such role. Thus, the translocation step depends on the utilization of substrate binding forces in one system but not in the other, and this, it will be shown, can account for the expression of substrate specificity in each.

Transition-State Theory

The treatment of the problem of substrate specificity given here is based on the ordinary carrier model in Fig. 1. The conclusions should not depend on the choice of model, however, inasmuch as the relationship between coupling and the binding force in the transition state, on which the analysis is based, has been shown to hold for both a bilateral transport model, with substrate sites simultaneously exposed on opposite sides of the membrane, and the carrier model (Krupka, 1989*a*).

The carrier model may be expanded to include the transition states in movement of the substrate site across the membrane, as in Fig. 2. From transition-state theory, the rate constant for reorientation of the substrate site in the free carrier, f_1 in Fig. 1, may be written (Krupka, 1989*a*)

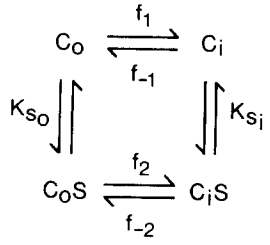

Fig. 1. The carrier model. The carrier equilibrates between inward-facing and outward-facing conformations, C_i and C_o respectively; substrate in the external compartment forms a complex with C_o, substrate in the internal compartment with C_i

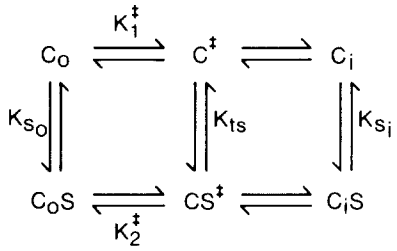

Fig. 2. The carrier model, showing the transition states in reorientation of the free carrier, C^*, and of the carrier-substrate complex, C^*S

$$f_1 = kK_1^{\ddagger} \tag{1}$$

where $K_1^{\ddagger}$ is the quasi-equilibrium constant for formation of the transition state from the reactant, and k is the constant for break-down of the transition state. Similarly, the rate constant for reorientation of the carrier-substrate complex, f_2 in Fig. 1, may be written

$$f_2 = kK_2^{\ddagger}. \tag{2}$$

The key to the analysis is the relationship that exists between the transition-state constants, $K_1^{\ddagger}$ and $K_2^{\ddagger}$, and the dissociation constants for substrate binding in the initial state ($C_o + S_o \rightleftharpoons C_oS$) and in the transition state ($C^* + S \rightleftharpoons C^*S$)

$$K_1^{\ddagger}K_{S_o} = K_2^{\ddagger}K_{\mathrm{ts}} \tag{3}$$

where K_{ts} is a virtual dissociation constant for the substrate in the transition state (Fig. 2). The ratio of rate constants for reorientation of the carrier-substrate complex and the free carrier f_2/f_1 is, from Eqs. (1), (2), and (3)

$$f_2/f_1 = K_2^{\ddagger}/K_1^{\ddagger} = K_{S_o}/K_{\mathrm{ts}}. \tag{4}$$

Exchange *versus* Net Transport

The maximum rate of exit of substrate from cells suspended in a medium containing no substrate (referred to as "zero *trans* exit") was previously (Devés & Krupka, 1979*b*) shown to be

$$\bar{V}_{S_i} = C_t/(1/f_1 + 1/f_{-2}) \tag{5}$$

where C_t is the total carrier concentration in all forms. The maximum rate of exchange, measured with one substrate pool (inside the cell, for example) labeled with radioactivity, and the other (outside the cell) unlabeled, is given by

$$\bar{\bar{V}}_S = C_t/(1/f_2 + 1/f_{-2}). \tag{6}$$

From Eqs. (5) and (6) the ratio of the maximum rates of exchange and net flow is

$$\frac{\bar{\bar{V}}_S}{\bar{V}_{S_i}} = \frac{1/f_1 + 1/f_{-2}}{1/f_2 + 1/f_{-2}}. \tag{7}$$

In an exchange-only system, this ratio can be very large, above 10^4 in the case of the anion exchanger of red cells (Fröhlich, 1984; Fröhlich & King, 1987). Hence, from Eq. (7), f_1 must be much smaller than f_2 or f_{-2}; for it is the low mobility of the free carrier, governed by f_1, that limits the rate of net transport (Eq. (5)), and the high mobility of the substrate complex, governed by f_2 and f_{-2}, that determines the rapid exchange rate (Eq. (6)). In a facilitated transport system, where net transport is rapid, the mobility of both the free carrier and the complex, governed by f_1 and f_{-2}, must be high (*see* Eq. (5)). The system would derive little advantage from an increased mobility of the carrier-substrate complex, which would only marginally increase the net transport rate.

Expected Patterns of Substrate Specificity

The expression of substrate specificity is found to depend on the role of substrate binding forces in determining carrier mobility. In an exchange-only system, where, as seen above, $f_2 \gg f_1$, the tightness of coupling, which is the ratio of exchange to net transport, $\bar{\bar{V}}_S/\bar{V}_{S_i}$, is directly related to the increased binding force in the transition state: from Eqs. (4) and (7), assuming that $f_2 \gg f_1$ and $f_2 \approx f_{-2}$

$$\overline{\overline{V}}_S/\overline{V}_{S_i} \approx K_{S_o}/2K_{ts} \gg 1. \quad (8)$$

In a facilitated transport system, where $\overline{\overline{V}}_S$ and $\overline{V}_{S_i}$, though not necessarily equal, are not too different, the simplifying assumption may be made that $f_2 \approx f_1$. The relationship corresponding to Eq. (8), again from Eqs. (4) and (7), is then found to be

$$\overline{\overline{V}}_S/\overline{V}_{S_i} \approx K_{S_o}/K_{ts} \approx 1. \quad (9)$$

Equation (8) tells us that in exchange-only mechanisms there is a tremendous increase in the substrate binding force as the complex passes through the transition state in reorientation of the carrier: a good substrate should be comparatively weakly bound in the initial complex and tightly bound in the transition state. Hence, the substrate site in the free carrier might be expected to be unconfining, and specificity in the binding step low. The anion exchanger of red cells, for example, readily forms a complex with a variety of anions, some much larger than the normal substrate, suggesting that the site exists in a dilated configuration. On the other hand, tight binding in the transition state in carrier reorientation depends on a close fit between the now contracted site and the substrate, and consequently the translocation step is highly specific.

In facilitated transport, the predictions are different. Equation (9) tells us that the substrate is bound equally well in the initial state and the transition state, implying that the carrier site should as well match the substrate in one as in the other. Hence, any substrate analog capable of binding to the carrier and able to avoid repulsive interactions in the transition state should be translocated about as fast as the normal substrate. Discrimination, at least with analogs no larger than the normal substrate, should therefore be exercised in the binding step but not the translocation step, and specificity should be expressed in the affinity but not the maximum rate of transport. The affinity of an analog would be low if it interacted weakly with the constellation of bonding points within the carrier site.

According to this mechanism for facilitated transport, the carrier discriminates against analogs smaller than the normal substrate in the binding step. It could discriminate against larger analogs in the translocation step as well. Steric interference in translocation could be severe if at some stage the carrier site encloses the substrate; in this case, substituents in an analog that do not weaken binding at a surface site could get in the way of the contraction of the substrate site required for carrier reorientation.

In facilitated transport systems, it follows, substrate specificity should be expressed in quite a different way than in exchange-only systems. With analogs no larger than the normal substrate, specificity should be seen in affinity but not in the maximum transport rate. With analogs larger than the normal substrate, the maximum transport rate should become sensitive to the structure of the substrate; the affinity too could be sensitive, depending on the structure of the substituent and the contours and environs of the carrier site, for bulky substituents might either interfere with binding at the surface site, reducing affinity, or bond in adjacent regions of the carrier, increasing affinity.

The Effect of Accelerated Exchange

In facilitated transport systems, the maximum rate of exchange, $\overline{\overline{V}}_S$, is commonly found to be somewhat higher than the maximum rate of net transport measured in a zero *trans* experiment, $\overline{V}_{S_i}$, and the implications of this fact for the expression of substrate specificity have to be considered. The ratio of the rates, $\overline{\overline{V}}_S/\overline{V}_{S_i}$, in several erythrocyte systems may be typical: choline, 5.0 (Martin, 1968); glucose, 2.3 (Miller, 1971); uridine, 3.4 (Jarvis et al., 1983); leucine, 2.0 (Hoare, 1972). The ratio depends on the relative mobilities of the carrier-substrate complex and the free carrier, governed by f_2 and f_1 in Fig. 1 (Hoare, 1972). Provided $f_2 = f_{-2}$, Eq. (7) yields

$$f_2/f_1 = (2\overline{\overline{V}}_S/\overline{V}_{S_i}) - 1 \quad (10)$$

from which the f_2/f_1 ratios are estimated to be: choline, 9; glucose, 3.6; uridine, 6; leucine, 3. In every case the mobility of the carrier is increased by addition of the substrate. In the analysis of facilitated transport given above, the mobilities of the free carrier and the substrate complex were assumed to be equal. Nevertheless, taking accelerated exchange into account and allowing for a difference in mobility does not fundamentally alter the predictions. A substrate analog could well have a smaller effect on the mobility of the carrier than the preferred substrate has, but still the complex with the analog would be as mobile as the free carrier. Consequently, its rate of net transport, which is always limited by the mobility of the free carrier (Eq. (5)), would be little changed—halved at most.

Experimental Observations on Facilitated Transport Systems

The theory may be checked against the behavior of two facilitated transport systems whose substrate specificity has been amply documented, those for

Table 1. The choline transport system of human erythrocytes: half-saturation constants and maximum rates of translocation (f_2 in Fig. 1)

Substrate analog	Half-Saturation constant (μM)	Relative translocation rate (f_2)	Reference
$Me_3\overset{+}{N}C_2H_4OH$	6.3	1.0	a
$Me_4\overset{+}{N}$	205	~1.0	a
$Me_3\overset{+}{N}$—Et	110	~1.0	b
$Me_3\overset{+}{N}$—Pr	55	~1.0	b
$Me_2\overset{+}{N}HC_2H_4OH$	20	~1.0	a,d
Imidazole	<100	~1.0	d
$Me_3\overset{+}{N}$—Bu	133	<0.1	b
$Me_3\overset{+}{N}$—$C_2H_4CONH_2$	430	<0.17	b
$Et_3\overset{+}{N}C_2H_4OH$	700	<0.06	a
$Et_2\overset{+}{N}(Pr)$—C_2H_4OH	590	0.0	a
$Me_2\overset{+}{N}(i\text{-}Pr)$—$C_2H_4OH$	300	<0.06	a
$Me_2\overset{+}{N}(Bu)$—C_2H_4OH	30	0.0	a
$Me_2\overset{+}{N}(Pentyl)$—$C_2H_4OH$	7.3	0.0	a
$Me_2\overset{+}{N}(Decyl)$—$C_2H_4OH$	0.3	0.0	a
$Me_2\overset{+}{N}(Dodecyl)$—$C_2H_4OH$	0.1	0.0	a

[a] Devés & Krupka, 1979*b*.
[b] Martin, 1969.
[c] Krupka & Devés, 1980.
[d] Devés & Krupka, 1987.

choline and glucose in human erythrocytes. Agreement between the observed and predicted behavior in these cases would corroborate the role assigned to substate binding forces in the transition state in carrier reorientation. Results on other transport systems extensive enough to test the theory do not appear to have been reported.

Choline Transport

According to the theory, substrate analogs fall into two groups with respect to specificity: those no larger than the substrate, and those larger. Five analogs in the first group have been examined: tetramethylammonium ion, N-ethyltrimethylammonium ion, N-propyltrimethylammonium ion, N,N-dimethylaminoethanol, and imidazole. In agreement with the predictions of the theory, the maximum rates of transport are similar to that of choline, while the affinities are lower by factors of 3 to 30 (Table 1). The rapid translocation of imidazole seems surprising until it is noticed that choline can round into a cyclic structure somewhat larger than imidazole.

These maximum rates were determined from *trans* effects of the unlabeled analogs on the exit of labeled choline and are, therefore, measures of ex-

change transport (Devés & Krupka, 1979*a*). As with choline, exchange transport is in every case faster than net transport, which means that the carrier complex is more mobile than the free carrier (in the case of choline by a factor of about 9, as noted above). As the various analogs are all ammonium ions, it seems likely that electrostatic interaction between the cationic charge and an anionic site in the carrier is responsible for increasing the mobility of the complex. The exceptionally strong ionic bond formed between choline and the transport site (Krupka & Devés, 1980) may have a bearing on such effects.

The affinity of the analogs does vary, as expected (Table 1). Tetramethylammonium ion, the smallest, has least affinity, 30 times less than choline. In the case of N-ethyltrimethylammonium ion, larger by one methylene group, the factor is 15, and it is 9 in the case of N-propyl-trimethylammonium ion, with the hydroxyl group of choline replaced by methyl. Dimethylaminoethanol, a cation at neutral pH where the experiments were run, has an affinity closer to that of choline. The affinity of imidazolium ion has not been precisely determined.

Among analogs larger than choline, some, only slightly more bulky, have diminished affinity and a low rate of translocation. For example, the triethyl analog, with the N-methyl groups of choline replaced by ethyl, has less than 1/100 the affinity of choline, and the isopropyl analog, with one N-methyl replaced by isopropyl, about 1/50. Substitution at the hydroxyl group of choline also reduces affinity, as in carbamylcholine and N-butyltrimethylammonium ion (Table 1). Such observations have suggested that the boundaries of the substrate site, at the surface of the membrane, are sharply defined, matching one side of the choline molecule (Devés & Krupka, 1979*a*).

The translocation step is even more sensitive than the binding step to bukly substituents in choline analogs. Thus, the N-butyl, N-pentyl, N-decyl and N-dodecyl analogs are not transported at all. The alkyl substituents in these analogs may, it seems, protrude from the substrate site and bond in adjacent nonpolar regions of the carrier, increasing the affinity but interferring with carrier reorientation.

Glucose Transport

The glucose carrier is extremely sensitive to the conformation of simple monosaccharides, but it is in the affinity and not in the rate of translocation that this sensitivity is manifest (Table 2). For example, the half-saturation constants for D-glucose, an aldohexose, and D-arabinose, an aldopentose, differ by a factor of over 2,000, but the maximum rates of net transport are nearly indistinguishable. Again, the affinity of fructose, a ketohexose, is even lower than that of D-arabinose, but the maximum transport rates are similar (Stein, 1967). Two molecules considerably smaller than glucose are also transported, though the rates have not been reported: erythritol (Bowyer & Widdas, 1955; Wieth, 1971) and pentaerythritol (Bowyer & Widdas, 1956).

Table 2. The glucose transport system of human erythrocytes: Half-saturation constants and relative maximum rates of transport

Substrate	Half saturation constant (mM)	Relative maximum rate of transport
Glucose[a]	2.5	1.0
2-Deoxyglucose[a]	2.0	1.05
Mannose[a]	5.0	0.8
Galactose[a]	14	0.8
Xylose[a]	12	0.8
Ribose[b]	~140	~1.0
L-Arabinose[b]	~230	~1.0
Arabinose[b]	5,500	~1.0
L-Sorbose[b]	3,100	~1.0
Fructose[b]	9,300	~1.0

[a] Krupka, 1971.
[b] Stein, 1967: Stein has collected various estimates of K_m and V_{max}, which are not in close agreement, but which indicate that V_{max} is nearly constant.

The situation is different with analogs larger than glucose. In general, sugar derivatives only slightly larger are transported, though at a low rate, while those larger still are not transported at all. The first group includes α-methyl-D-glucoside, which is probably very weakly bound (LeFevre, 1961) and has a maximum translocation rate 1/70 of that of glucose (Bowyer, 1957); and 3-O-methyl-D-glucose, with an affinity comparable to that of glucose and a transport rate half as high (Baker & Widdas, 1988). Various smaller analogs are transported, though the rates have not been measured precisely: 6-O-methylgalactose, 6-deoxy-6-iodogalactose (Barnett et al., 1975), 4-deoxy-4,4-azo-D-glucose, and 6-deoxy-6,6-azo-D-glucose (Midgley et al., 1985). Other, larger, analogs are not transported, for example the 6-O-propyl, 6-O-pentyl, and 6-O-benzyl derivatives of galactose (Barnett et al., 1975), as well as 4-azido-4-deoxy-D-galactose, 4-(2′-diazo-3′,3′,3′-trifluoropropionyl)-D-glucose (Midgley et al., 1985), and 4,6-O-ethylidene-D-glucose (Baker & Widdas, 1973; Baker, Basketter & Widdas, 1978). In general, sugars with two or more hydroxyl groups cross-linked by iso-

propylidene substituents are not transported (Novak & LeFevre, 1974), and the same is true of still larger molecules such as maltose (Lacko & Burger, 1962) and bis-mannosyl compounds (Midgley et al., 1985).

All these sugar derivatives, transported or not, are bound at the carrier site. The smaller ones tend to have a lower affinity than glucose, while some of the larger ones have a higher affinity. For example phenyl-β-D-glucose (Barnett et al., 1975), 4,6-O-benzilidene-D-glucose (Novak & LeFevre, 1974) and bis-mannosyl compounds bearing a phenyl substituent (Midgley, Parker & Holman, 1985) are bound about 10 times more strongly than glucose. Such observations on the glucose carrier parallel those on the choline carrier, and both agree with expectation.

Experimental Observations on Facilitated and Coupled Systems Compared

The hypothesis may be put to a further test by comparing the facilitated systems for choline and glucose with cotransport systems for the same substrates in which transport is coupled to the movement of sodium ions. As shown in the Appendix, the tightness of coupling in a cotransport system bears the same relation to transition-state binding forces as in an exchange-only system, provided there is an ordered addition of substrates to the carrier, with the driving substrate, Na^+, adding first, and driven substrate, glucose or choline, adding second. This order appears to be followed in both cotransport systems to be considered: the glucose system of rat intestine (Hopfer, 1987) and the choline system of synaptosomes (Wheeler, 1979). By comparison with the facilitated systems, the experimental data on substrate specificity are limited in scope, but the contrasting behavior strikingly supports the analysis.

By hypothesis, the maximum rates of transport of analogs no larger than the normal substrate can vary widely in coupled but not in facilitated systems. Indeed, in the Na^+-dependent cotransport system for glucose in rat intestine, the maximum rates for D-galactose, 6-deoxy-D-galactose, and 6-deoxy-6-fluoro-D-galactose have relative values of 1.0, 0.14 and 0.52, respectively (Barnett, Jarvis & Munday, 1968). More remarkable, sugars lacking the C-2 hydroxyl group, though weakly bound at the carrier site, are not transported; on the basis of such evidence, Barnett, Ralph and Munday (1970*a* and *b*) proposed that the C-2 hydroxyl group enters into a *trans*-esterification reaction during translocation, forming a covalent bond at the substrate site.

Table 3. Relative values for the half-saturation constant for choline analogs in the active transport system in rat brain synaptosomes and in the facilitated system in human erythrocytes

Analog			Relative half-saturation constant[a]	
			Synaptosome[b–d]	Erythrocyte[e]
R_1	R_2	R_3		
Methyl	Methyl	Ethyl	2.2	2.0
Methyl	Ethyl	Ethyl	1.0–2.3	5.6
Ethyl	Ethyl	Ethyl	2.4	109
Methyl	Methyl	Propyl	3.0	5.2
Methyl	Methyl	Isopropyl	7.9	48

[a] Half-saturation constant for an analog divided by the half-saturation constant for choline.
[b] Simon et al., 1975.
[c] Barker & Mittag, 1975.
[d] Batzold et al., 1980.
[e] Devés & Krupka, 1979*a*.

Specificity in binding may be compared in two types of choline transport system—the facilitated system in erythrocytes described above and the high-affinity system in rat-brain synaptosomes (Simon, Mittag & Kuhar, 1975; Barker & Mittag, 1975; Batzold et al., 1980). The relative values of the affinity constants for several analogs, listed in Table 3, evidence the milder constraints upon binding in coupled transport. The triethyl analog, for example, is bound 100 times more weakly than choline by the facilitated system but almost as well as choline by the coupled system.

Implications for the Mechanism of Facilitated Transport

Judging by the experimental observations cited above, the transport site senses only one side of the substrate molecule in the binding step, allowing protruding substituents on the opposite side to bond in adjacent regions of the carrier; in the translocation step, the site appears to sense the entire surface and volume of the substrate molecule, rejecting any analog bulkier than the normal substrate. The substrate is probably first adsorbed to a site on the surface of the carrier molecule, and then in the course of translocation is enclosed by another section of the carrier. Any molecule small enough to enter the crypt formed in this way may be readily translocated, the main limitation being the fraction of carrier sites bearing the substrate: a molecule

lacking affinity for the carrier is unlikely to occupy the site and ride across the membrane. Analogs larger than the normal substrate, on the other hand, are not readily transported, since they obstruct the closing up of the site, on which carrier reorientation depends. If the analog is not too much larger than the substrate, the interference is partial and transport proceeds at a low rate. If the analog is very much larger, blockage is complete, though the affinity may be increased by attachments outside the substrate site.

The Basis of Specificity in Facilitated and Coupled Transport Systems

To achieve specificity, coupled and facilitated transport systems appear to employ different strategies, which are related to the role played by substrate binding forces in carrier reorientation. The coupling of the translocation of two substrate molecules, as occurs in an exchange-only system or a cotransport system, entails the conversion of an immobile to a mobile carrier form. The energy driving the transformation comes from the forces of substrate binding; the substrate is loosely bound in an initial complex, but as the interaction with the carrier site becomes closer, an inclusion or chelate complex is formed, and a new mobile conformation is stabilized. The induction of this conformational change, which is required for translocation, can be extremely sensitive to the structure of the substrate.

The situation is different in facilitated transport systems, where the carrier is in a mobile state in the absence of the substrate. Specificity must be achieved by other means: instead of the coupled system's discrimination in favor of a preferred substrate (in the translocation step), there is discrimination against other substrates through either weak binding in the initial complex or steric interference with carrier reorientation. It may be in order to discriminate against analogs larger than the true substrate that the carrier site encloses the substrate. In coupled systems, also, the carrier probably forms an enclosed complex, but for a different reason—in order to strengthen binding through chelation (Krupka, 1989*a* and *b*); here, too, enclosure gives rise to the possibility of steric interference by bulky analogs in the translocation step.

The experimental observations suggest, further, that the substrate site is initially less confining in coupled than in facilitated systems. The anion exchanger of red cells indiscriminately binds a variety of substrate analogs, in contrast to the facilitated systems for choline and glucose in these cells. Again, the coupled system for choline uptake in synaptosomes was seen to be far less sensitive to the size of the quaternary ammonium group than is the facilitated system in red cells. This laxness in the carrier site in coupled systems appears to be functional: as coupling depends on the increment in binding energy in the transition state in carrier reorientation, it is an advantage for the initial complex to be loose fitting and the binding force weak. The required contraction of the site around the substrate could then involve a massive adjustment in the carrier structure, producing a new, and mobile, conformation. In a facilitated transport system a loose-fitting site would be detrimental, since it would only diminish the specificity of the carrier.

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Appendix

Transition-State Theory for Cotransport Systems

In the cotransport scheme in Fig. A1, either the driven or the driving substrate, S or A respectively, could add to the carrier first. The implications of each pathway may be considered separately.

In the scheme in Fig. A2, where the order of addition is A followed by S, A may be assumed to induce a conformational change in which the binding site for S appears. For a gradient of A to drive the movement of S against its own concentration gradient, the free carrier (C_o and C_i) and the ternary complex (C_oAS and C_iAS) must be mobile, and the binary complex (C_oA and C_iA) immobile; i.e., $f_1, f_{-1}, f_3, f_{-3} \gg f_2, f_{-2}$. The full derivation of the rate equations need not be gone over here, since the kinetics of cotransport have been treated in the past (*see* Turner, 1981; Stein, 1986). What is required now are expressions for the constants measured in the zero *trans* entry of substrate S ($[S_i] = [A_i] = 0$) under conditions where the concentration of the driving substrate A_o is saturating ($[A_o] \gg K_{Ao}$): namely, the maximum rate of entry, $\bar{V}^{Ao}_{So}$, and the substrate half-saturation constant, $\bar{K}^{Ao}_{So}$. In addition, an expression is needed for the rate of the slow, uncoupled leakage of the driving substrate into the cell, $\bar{V}_{Ao}$, a leak that dissipates the concentration gradient of A. These constants are derived for the case of rapid equilibrium between the free carrier and the substrate complex; i.e., carrier movement, governed by f_1, f_{-1}, f_3 and f_{-3}, is assumed to be much slower than dissociation of the substrate from the transport site

$$\bar{V}^{Ao}_{So} = C_t \Big/ \left(\frac{1}{f_3} + \frac{1}{f_{-1}}\right) \tag{A1}$$

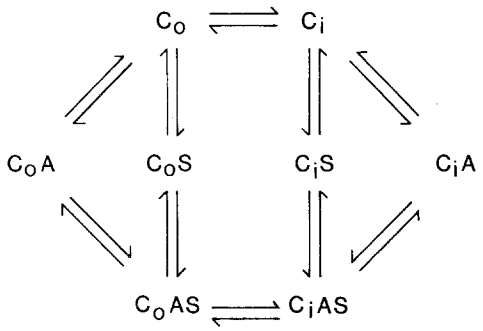

Fig. A1. A carrier model for cotransport. S and A represent the driven and driving substrates, respectively. For tight coupling of the flow of the two substrates, only the free carrier (C_o and C_i) and the ternary complex with both substrates (C_oAS and C_iAS) can be mobile

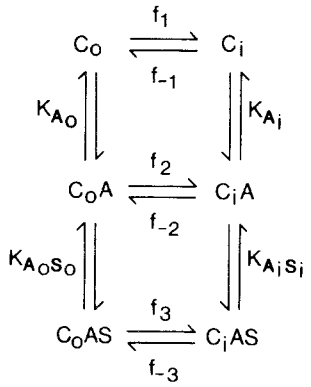

Fig. A2. A simplified carrier scheme for cotransport, in which the driving substrate A adds to the carrier before the driven substrate S can add. For tight coupling, f_2 and f_{-2} must be small relative to f_1, f_{-1}, f_3 and f_{-3}

$$\check{K}_{S_o}^{A_o} = K_{A_oS_o}(1 + f_1/f_{-1})/(1 + f_3/f_{-1}) \tag{A2}$$

$$\overline{V}_{A_o} = C_t \Big/ \left(\frac{1}{f_2} + \frac{1}{f_{-1}}\right) \simeq f_2 C_t \tag{A3}$$

where C_t is the total carrier concentration. $\overline{V}_{A_o}$ is the maximum rate of zero *trans* entry of A in the absence of the driven substrate S.

The substrate binding force generated in the transition state may be found from the expanded scheme in Fig. A3, which shows the transition states for reorientation of the substrate site in the free carrier C^*, in the complex with the driving substrate C^*A, and in the complex with both substrates C^*AS. By proceeding as in the treatment of facilitated transport (Eqs. (1) and (2), above), the following relationships are found:

$$f_1 = kK_1^{\ddagger} \tag{A4}$$

$$f_2 = kK_2^{\ddagger} \tag{A5}$$

$$f_3 = kK_3^{\ddagger} \tag{A6}$$

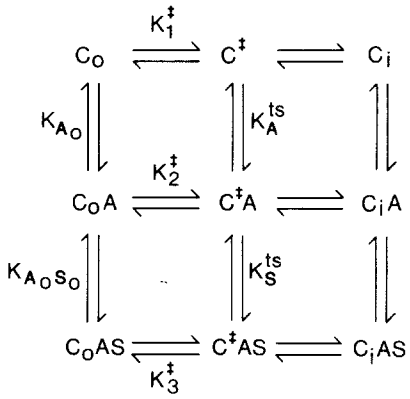

Fig. A3. A carrier scheme for cotransport expanded to show the transition states in carrier reorientation (C^*, C^*A, and C^*AS)

where k is a rate constant for breakdown of the transition state and K_1^*, K_2^*, and K_3^* the quasi-equilibrium constants for formation of the transition state from the reactant. The equilibrium constants linking C_oA, C^*A, C_oAS, and C^*AS are interrelated

$$K_{A_oS_o}K_2^* = K_S^{ts}K_3^* \tag{A7}$$

and substitution of Eqs. (A4) and (A6) into Eq. (A7) yields

$$K_S^{ts} = K_{A_oS_o}f_2/f_3. \tag{A8}$$

From Eqs. (A1)–(A3)

$$\check{K}_{S_o}^{A_o}\overline{V}_{A_o}/\check{V}_{S_o}^{A_o} = K_{A_oS_o}(1 + f_1/f_{-1})f_2/f_3 \tag{A9}$$

and combining Eqs. (A8) and (A9) yields

$$K_S^{ts} = \frac{\check{K}_{S_o}^{A_o}\overline{V}_{A_o}}{\check{V}_{S_o}^{A_o}(1 + f_1/f_{-1})} \leq \frac{\overline{V}_{A_o}\check{K}_{S_o}^{A_o}}{\check{V}_{S_o}^{A_o}}. \tag{A10}$$

According to Eq. (A10), the tightness of coupling of the gradients of the two substrates S and A, defined as the ratio of coupled and uncoupled transport, $\check{V}_{S_o}^{A_o}/\overline{V}_{A_o}$, is equivalent to the increase in the binding force, $\check{K}_{S_o}^{A_o}/K_S^{ts}$, in moving from the initial complex, S_oA_oC, to the transition-state in translocation, C^*AS.

The relationship between coupling and binding forces in the transition state does not depend on the transport model, inasmuch as a very similar relationship is found for the bilateral transport scheme in Fig. A4. As substrate sites are exposed on both sides of the membrane at the same time in this model, only one form of the free carrier need be considered. In deriving an equation for coupling, rapid equilibrium between the substrates and the enzyme is assumed again. The maximum rate of zero *trans* entry of substrate S (with $[A_o]$ and $[S_o]$ saturating, and $[A_i]$ and $[S_i]$ equal to zero) is given by

$$\check{V}_{S_o}^{A_o} = k[C^*AS] = kK_2^*[S_oA_oC] = kK_2^*C_t \tag{A11}$$

where C_t is the total carrier concentration and k is the constant for breakdown of the transition state. Similarly, the maximum

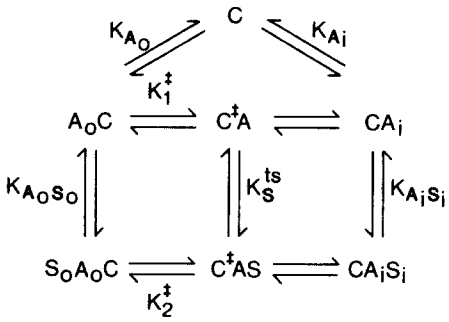

Fig. A4. A bilateral carrier mechanism for cotransport, in which substrate sites are exposed on both sides of the membrane simultaneously. The substrates add to the carrier in an ordered sequence, A first and then S

rate of uncoupled entry of the driving substrate A (a "leak") in the absence of the driving substrate S is given by

$$\bar{V}_{A_o} = k[C^*A] = kK_1^*[A_oC] = kK_1^*C_t. \tag{A12}$$

The equilibrium constants linking A_oC, C^*S, S_oA_oC, and C^*AS are related to one another

$$K_{A_oS_o}K_1^* = K_S^{ts}K_2^* \tag{A13}$$

and substitution of Eqs. (A11) and (A12) into (A13) yields

$$K_S^{ts} = K_{A_oS_o}K_1^*/K_2^* = \tilde{K}_{S_o}^{A_o}\bar{V}_{A_o}/\tilde{V}_{S_o}^{A_o}. \tag{A14}$$

Equation (A10), for the unilateral model in Fig. A3, differs from Eq. (A14) only in allowing for the partition of the free carrier between its inward-facing and outward-facing forms. Hence, the virtual dissociation constant for the driven substrate S in the transition state is not greater than $\tilde{K}_{S_o}^{A_o}\bar{V}_{A_o}/\tilde{V}_{S_o}^{A_o}$. Moreover, where the driving substrate A can cross the membrane in some other way, involving another carrier or channel, or by unmediated diffusion through the membrane, the measured value of $\tilde{V}_{S_o}^{A_o}$ could be higher than the true value. Therefore K_S^{ts} calculated from Eq. (A14) should be an upper limit.

In both these transport models, with the driving substrate adding first, the relationship of the tightness of coupling to the development of substrate binding forces in the transition state is the same as in exchange-only transport, and the implications for the expression of substrate specificity would be the same. That is, specificity should find expression mainly in the maximum transport rate rather than in the affinity of various substrate analogs. However, if the two substrates add in the opposite order, the driven substrate S first, followed by the driving substrate A, the predictions are different. The relationship (Eq. (A14)) between coupling and the binding force now applies to the driving substrate, A, rather than the driven substrate S. In this mechanism the binary complex with the driven substrate, C_oS is immobile; hence binding forces, for the driven substrate, are not utilized to increase the mobility of the carrier. Instead, addition of S to the carrier exposes the binding site for A, the binding forces being used to induce the required shift in conformation from a carrier form lacking the binding site for A to the form having this site. A similar induced fit mechanism would operate in allosteric enzymes. Under these circumstances a substrate analog smaller than the normal substrate should have an undiminished maximum transport rate.