J. Membrane Biol. 167, 35–41 (1999)

The Journal of
Membrane
Biology

Limits on the Tightness of Coupling in Active Transport

R.M. Krupka
Agriculture Canada, 1391 Sandford St., London, Ontario, Canada N5V 4T3

Received: 3 June 1998/Revised: 22 September 1998

Abstract. Control of the coupled reaction sequence in active transport depends on systematic changes in the properties of the carrier protein as the reaction proceeds. These changes would have to be brought about by specific interactions with the substrate, the binding forces being used to stabilize either (i) a carrier state with altered properties or (ii) the transition state in a carrier transformation. In the first case the tightness of coupling (the ratio of the coupled rate to slippage) will at first rise with the increment in binding energy in the altered state but will approach an upper limit when overly strong binding forces retard substrate dissociation in a subsequent step in the coupled reaction sequence. Primary and secondary active transport are subject to this limitation because the coupling mechanism necessarily involves intermediates in which the substrate is strongly bound. Exchange-only transport is not necessarily subject to the same limitation because the mechanism can involve only a substrate-catalyzed change in carrier state. The available data, although scant, agree with these conclusions.

Key words: Slippage — Binding energy — Coupling efficiency — Energy transfer — Active transport — Exchange-only transport

Introduction

In active transport, the coupling of the driving reaction to the driven process, in which a substrate is pumped uphill, cannot be perfectly tight. There will be slippage; but how much, is still unclear. Under the pressure of natural selection, coupling should have become as efficient as possible and slippage reduced to a minimum. Indeed, some enzymes have evolved to the point where no improvement in catalysis would be of benefit because the slowest step in the reaction is diffusion of the substrate in solution up to the active center, over which the enzyme has no control (Knowles & Albery, 1977). What limits coupling efficiency, and how slippage is related to the coupling mechanism, are questions that will be explored here.

Two processes will be coupled if they are combined in a single overall reaction sequence. For the coupling protein to guide the reaction along a path involving both processes, while avoiding byways involving only one, its specificity and transport properties have to be systematically altered in the course of the reaction. These changes can be brought about through the use of substrate binding energy; it can be shown that the greater the increase in the strength of substrate binding at branch points between coupled and uncoupled paths, the greater the preference can be for the coupled path. Hence, the ratio of coupled to uncoupled rates, which may be called the tightness of coupling, is a function of binding energy. From this it seems to follow that the only limit on tight coupling would be the binding energy that can be generated in the substrate complex. But, although the tightness of coupling does rise in a simple manner at lower binding energies, sufficiently strong binding will be shown to limit the tightness of coupling by reducing the rate of substrate dissociation in a subsequent step in the coupled reaction sequence (for example, by retarding the unloading of the substrate after it has been transferred through the membrane).

The Basis of Coupling

In the ordered mechanism for the cotransport of substrates *S* and *T* in Fig. 1, transport is coupled insofar as reaction is around the perimeter of the scheme, the path by which both substrates react; transport is uncoupled

Correspondence to: R.M. Krupka

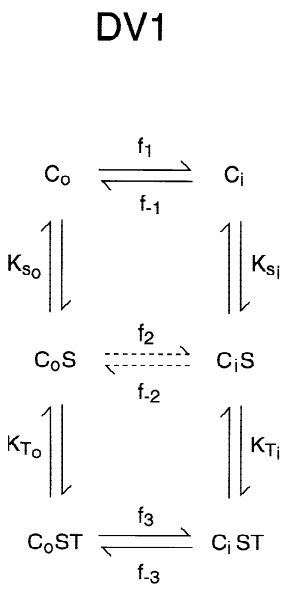

Fig. 1. A scheme for cotransport of substrates *S* and *T*. The substrates add in fixed order, *S* first, *T* second. Subscript *o* indicates the outward-facing carrier form, subscript *i* the inward-facing form. For coupling, the binary complex is required to be much less mobile than the free carrier or the ternary complex (that is, C_o and C_i, as well as C_oST and C_iST, are readily interconvertible, whereas C_oS and C_iS are not). For the substrates to add in fixed order the free carrier is required to have a site for only *S*, the binary complex sites for both *S* and *T*.

insofar as there is a short-circuit midway via the binary complexes C_oS and C_iS, with net reaction of only one of the substrates. In every coupled reaction, as in this one, there are rules as to which paths are permissable. The rules come into play at control points in the reaction sequence; here the properties of the carrier are required to change abruptly in order to shunt the reaction along the coupled path. The nature of the changes may be illustrated by noting the rules for the scheme in Fig. 1: (1) The free outward-facing carrier, C_o, is mobile (meaning that C_o and C_i, the outward and inward-facing carrier forms, are interconvertible); further, C_o specifically binds external *S* (S_o), but lacks sites for internal *S* (S_i) and for *T* on either side (T_o or T_i). (2) The substrate complex C_oS is immobile (it cannot be converted to C_iS); further, it has sites for both external substrates — for S_o (which is already bound) and for T_o. (3) The ternary complex C_oST is mobile (it can be converted to C_iST). Analogous rules govern the properties of the internal carrier forms. The first transformation of the carrier, which occurs on addition of *S*, can be explained as a shift in equilibrium between two carrier states — a mobile one-site state and an immobile two-site state. The second transformation, on addition of *T*, depends on catalysis of the conversion of one state to another, outward-facing to inward-facing.

I use the term ''coupling mechanism'' to refer, not to the rules of coupling governing one particular system, but to the physical basis of these rules in any system: that is, to the underlying processes causing the properties of the coupling protein to be altered in specific ways as the reaction proceeds. The transformations implicit in the rules of coupling, for example those listed above for the scheme in Fig. 1, can involve shifted equilibria or altered rates of conversion, between one carrier state and another, as we have noted. In general, the altered state will be an intermediate on the coupled path, the initial state an intermediate on the uncoupled path. The abruptness of the change in state, which determines the relative importance of the uncoupled reaction, depends on the increment in substrate binding energy as the altered state is formed. As a result, the ratio of coupled to uncoupled turnover rates is a function of binding energy. The relationship may be expressed as follows (Krupka, 1989*a*,*b*, 1990, 1993):

$$Rate_{(\text{coupled})}/Rate_{(\text{uncoupled})} \leqslant K_{(\text{initial state})}/K_{(\text{final state})} \quad (1)$$

$K_{(\text{initial state})}$ and $K_{(\text{final state})}$ are substrate dissociation constants before and after the transformation. According to Eq. 1, the wider the shift in the dissociation constant, the less slippage. Where the controlling step involves catalysis of a change in state, such as the about-face of the carrier in the membrane (C_oST to C_iST in Fig. 1), $K_{(\text{final state})}$ is the virtual dissociation constant in the transition state.

Equation 1, which underlies any rule of coupling, gives the division between coupled and uncoupled paths at each stage in the reaction. The equation holds for equilibrium steps and for catalytic steps. It applies to the two main transport models — a model in which one substrate binding site alternates between the two sides of the membrane, and one in which sites are exposed simultaneously on the two sides. Further, the equation applies to both primary and secondary active transport, because any scheme for secondary active transport, such as Fig. 1, is kinetically equivalent to a corresponding scheme for primary active transport, with the conversion of one of the substrates, S_o to S_i or T_o to T_i, representing not translocation but a chemical reaction such as ATP being hydrolyzed to ADP + Pi (Stein & Honig, 1977; Honig & Stein, 1978). The free energy for uphill movement is supplied by the conversion of the driving substrate from a state of high to a state of low potential. In primary active transport the potential is that of a chemical reaction away from equilibrium and in secondary active transport that of a concentration gradient. As we have seen, it is the linkage between changing states of the carrier and transformations of the substrate that gives rise to coupling: for example, S_o (or ATP) bound to C_o being converted to S_i (or ADP + Pi) bound to C_i. In primary and secondary active transport, Eq. 1 applies to both the driving and driven substrates. The equation also applies to exchange-only transport.

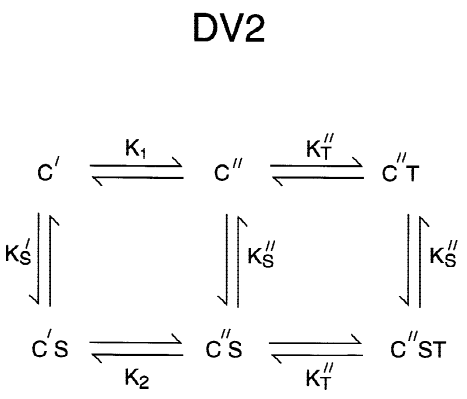

Fig. 2. Reaction scheme to account for an ordered addition of substrates in cotransport. The carrier is postulated to exist as an equilibrium mixture of two states: C' has a binding site for S but not T, and is mobile; C'' has sites for both S and T, and is immobile, but becomes mobile on addition of T. The equilibrium between the two states shifts when S is bound: the equilibrium of the free carrier favors C' ($K_1 = [C']/[C''] \gg 1$), but that of the complex with S favors $C''S$ ($K_2 = [C'S]/[C''S] \ll 1$); therefore, $K''_s \ll K'_s$.

Rate Equations and Coupling Ratios

A scheme for the induction of the proposed carrier transformations is shown in Fig. 2 (Krupka, 1994*b*). The carrier is represented as an equilibrium of two states: C', which is mobile and has a binding site for only one of the substrates (S), and C'', which is immobile and has sites for both substrates (S and T). C' is the more stable form of the free carrier ($K_1 = [C']/[C''] \gg 1$), whereas with substrate S bound, C'' is the more stable ($K_2 = [C'S]/[C''S] \ll 1$); it follows that S is bound much more firmly to C'' than to C' ($K''_s/K'_s = K_2/K_1 \ll 1$). With the second substrate T bound, the immobile carrier form C'' becomes mobile; in effect, the second substrate catalyzes the transition between opposite-facing carrier states.

Introduction of the carrier forms in Fig. 2 into the reaction scheme in Fig. 1 gives the expanded scheme in Fig. 3. An important difference may be noted in the roles of the two substrates: strong binding of S (in C'') can make its dissociation a rate-limiting step, whereas strong binding of T (in the transition state) only increases the rate.

The coupled path for entry, involving transformations of both S and T, leads through f_3 and f_{-1}, while the uncoupled entry of S leads through f_2 and f_{-1}. The less stable the binary complex in the form of $C_o'S$, the less reaction there is through the uncoupled route (in which $C_o'S$ is converted to $C_i'S$); or, put another way, the stronger the binding forces in $C_o''S$ compared with $C_o'S$, and the smaller K_{o2} (equal to $[C_o'S]/[C_o''S]$), the lower the relative rate of uncoupled entry. A further point is that a degree of symmetry is required because coupling has to be tight in both directions; if it were not, coupled entry would be followed by uncoupled exit, a futile cycle that uncouples the system as a whole. Thus, both K_{o2} and K_{i2}, the equilibrium constants for transformations in the outward-facing and inward-facing carrier forms, have to be small.

As usual it may be assumed that the initial substrate complex is at equilibrium with the substrate in solution; that is, the initial binding of the substrate is loose enough for its dissociation to be a fast step, and not rate-limiting. But binding is necessarily stronger in the secondary complex, and rate-limiting dissociation must be allowed for. In Fig. 3, for example, $C_i'S$ is an initial loose complex on the inner surface of the membrane, and $C_i''S$ is the complex derived from it; dissociation of S from $C_i'S$ is fast, while dissociation from $C_i''S$, governed by k_{-Si}'', can be rate-limiting ($K_{Si}'' = k_{-Si}''/k_{Si}''$). Because the equilibria for the two forms of the inner substrate complex are interrelated ($K_{Si}''/K_{Si}' = K_{i2}/K_{i1} \ll 1$), the conversion of $C_i''S$ to $C_i'S$, governed by k_{i2}, can also be a slow step ($[C_i'S]/[C_i''S] = k_{i2}/k_{-i2} = K_{i2} \ll 1$).

The effect of these equilibria on the rate of substrate discharge following the translocation step is apparent from Fig. 3. In a tightly coupled system the equilibria strongly favor $C_i''S$ over $C_i'S$ and C_i'', making $C_i''S$ a comparatively stable intermediate; therefore, the conversion of $C_i''S$ to $C_i'S$ or C_i'' can become rate-limiting if coupling is sufficiently tight. These conversions (one or the other) are obligatory steps in coupled entry. But they are not involved in the uncoupled entry of either substrate: the uncoupled path for S leads from $C_o'S$ to $C_i'S$, bypassing $C_i''S$; the uncoupled path for T leads from $C_o''T$ to $C_i''T$, which unlike $C_i''S$ is not an especially stable intermediate. It is seen that the strong binding of S needed for tight coupling can slow the coupled but not the uncoupled reaction.

The behavior may be described by two rate expressions: one for the maximum rate of coupled entry of S and T, and one for the maximum rate of uncoupled entry of S. The needed expressions can be written directly on the basis of a general kinetic treatment of the carrier model, in which substrate dissociation is not assumed to be a fast step (Devés & Krupka, 1979):

(i) The maximum rate of coupled entry, V_{STo}. The path is through $C_o''ST$ and $C_i''ST$ (f_3); $[S_o]$ and $[T_o]$ are saturating and internal substrates are absent; $K_{o2} \ll 1$ and $K_{o1} \gg 1$, as required for coupling. Ct is the total amount of carrier.

$$V_{Sto} = Ct/\left\{\frac{1}{f_{-1}} + \frac{1}{f_3} + \frac{1 + f_{-3}/f_3}{k_{i2} + k_{-Si}''}\right\} \qquad (2)$$

(ii) The maximum rate of uncoupled entry of S, V_{So}. The path is through $C_o'S$ and $C_i'S$ (f_2); S_o is saturating, and T_o and internal substrates are absent:

$$V_{So} = f_{-1}f_2Ct/\{f_2 + f_{-1}\,(1 + 1/K_{o2})\} \approx f_2\,K_{o2}\,Ct \qquad (3)$$

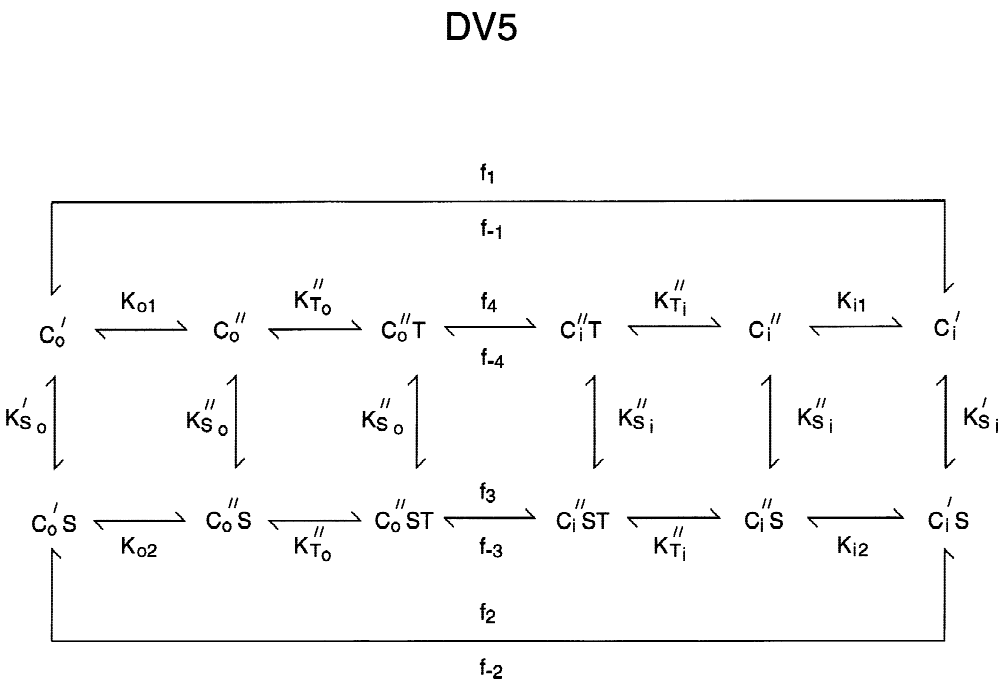

Fig. 3. Expanded cotransport scheme incorporating the two carrier states in Fig. 2 (based on the scheme in Fig. 1). Substrate *S* shifts the equilibrium from the mobile one-site state C' to the immobile two-site state C''; substrate *T*, on adding to C'' catalyses the interconversion of outward-facing and inward-facing forms (the f_3 and f_4 steps). As a result (i) the substrates tend to add in fixed order (*S* first, then *T*), and (ii) the preferred entry path is coupled, via the f_3 and f_{-1} steps, rather than uncoupled, via the f_2 or f_4 steps.

The tightness of coupling is defined as the ratio of coupled to uncoupled rates, the so-called coupling ratio. The coupling ratio for substrate *S*, which is the maximum transport rate of *S* and *T* together relative to the maximum transport rate of *S* in the absence of *T*, may, from Eqs. 2 and 3, be written for two cases.

(i) Dissociation is not rate-limiting:

$$\frac{V_{Sto}}{V_{So}} = \frac{f_{-1} f_3}{f_2 K_{o2} (f_{-1} + f_3)} \tag{4}$$

(ii) Dissociation is rate-limiting:

$$\frac{V_{Sto}}{V_{So}} = \frac{(k_{i2} + k_{-Si}'')}{f_2 K_{o2} (1 + f_{-3}/f_3)} \tag{5}$$

From Eq. 2, low values of k_{i2} and k_{-Si}'' slow the coupled rate; from Eq. 3, a decline in K_{o2}, the counterpart of K_{i2} on the uptake side of the membrane, reduces the uncoupled rate as well. The fall in K_{o2} and K_{i2} will be comparable if the symmetry of the system required for coupling is to be preserved; further, the fall in k_{i2} and k_{-Si}'' is likely to be comparable to the fall in K_{i2} and K_{o2}. We may conclude that Eq. 1 holds only so long as dissociation is a fast step; once dissociation becomes rate-limiting, further increments in binding energy at controlling steps in the reaction not only fail to make coupling more efficient (Eq. 5) but retard the coupled reaction (Eq. 2).

Implications for Coupling

To help appreciate the magnitude of the binding energy shift required for tight coupling, the coupling ratio may be expressed as a function of dissociation constants, as in Eq. 1. From the scheme in Fig. 3, $K_{So}''/K_{So}' = K_{o2}/K_{o1}$. The coupling ratio for *S* (Eqs. 4 and 5) is seen to be *inversely* proportional to K_{o2} ($K_{o2} = [C_o'S]/[C_o''S]$), but because uncoupled transport of *T* is proportional to the concentration of C_o'' the coupling ratio for *T* is directly proportional to K_{o1} ($K_{o1} = [C_o']/[C_o'']$). So, for efficient coupling, K_{o2} should be small and K_{o1} large. And the coupling of the two substrates should be about equally tight, since the uncoupling of either uncouples the system as a whole; consequently, K_{o1} should be roughly the reciprocal of K_{o2}:

$$K_{So}''/K_{So}' = K_{o2}/K_{o1} \approx (K_{o2})^2 \tag{6}$$

Then, from Eq. 4,

$$\frac{V_{Sto}}{V_{So}} \approx \frac{f_{-1} f_3 (K_{So}'/K_{So}'')^{1/2}}{f_2 \quad (f_{-1} + f_3)} \tag{7}$$

DV3

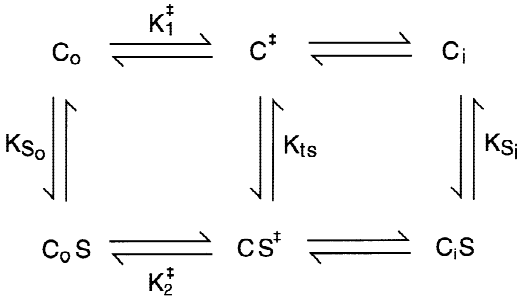

Fig. 4. A carrier mechanism for obligatory exchange in which the substrate plays a catalytic role in the coupling mechanism. The substrate catalyzes the interconversion of the outward-facing and inward-facing states, C_o and C_i, by binding strongly in the transition state, C' and CS'. The free carrier, because the substrate is absent, has low mobility, whereas the substrate complex is mobile.

DV4

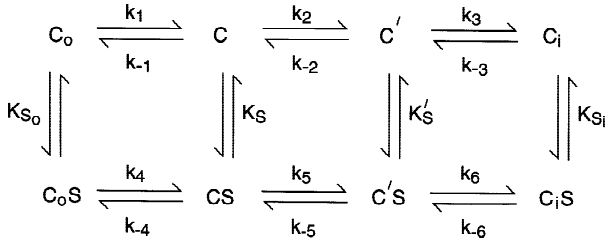

Fig. 5. A carrier mechanism for obligatory exchange in which the substrate stabilizes mobile carrier intermediates. C_o and C_i as well as C_oS and C_iS are immobile, whereas C and C', and CS and $C'S$, are mobile. The equilibrium of the free carrier favors C_o and C_i, while the equilibrium of the substrate complex favours CS and $C'S$ (the substrate is bound more strongly to the intermediate states and therefore shifts the equilibrium in their direction).

Equation 7 calls for a rather large increment in substrate binding energy, since the coupling ratio is related to the square root of the ratio of dissociation constants. The difficulty is that the binding energy generated at a specific site becomes counterproductive once substrate dissociation, which is a function of a dissociation constant, not its square root, becomes rate-limiting. How much these factors limit the efficiency of coupling will have to be decided from experimental measurements of coupling ratios for a variety of systems.

The Tightness of Coupling in Relation to the Coupling Mechanism

The above limitation on tight coupling is avoided if coupling depends on a purely catalytic mechanism, and such a mechanism is possible in exchange-only transport. The coupling here is of two substrates moving across the membrane in opposite directions: the system allows the exchange of the substrates but not the net transport of either. The behavior is explained by the carrier model if the substrate complex, but not the free carrier, is mobile; for exchange depends on the mobility of the complex alone and net transport on the mobility of both the complex and the free carrier. As in the case of the carrier model in Fig. 4, a substrate could directly catalyze the transition between outward-facing and inward-facing carrier states, presumably a conformational change, by binding strongly in the transition-state complex, stabilizing it. Equation 1 holds, with the increment of binding energy being that in forming the transition-state complex. The decomposition of the transition state, unlike an intermediate in the coupled reaction, is necessarily rapid and not rate-limiting. Nevertheless, the coupling cannot be perfect because it depends on a finite increment in binding energy (Krupka, 1989*a*).

Exchange-only transport, it must be added, need not involve such a simple mechanism. An alternative is shown in Fig. 5, where the carrier exists as an equilibrium mixture of mobile and immobile states. The free carrier is required to be predominantly in the immobile state, with the substrates shifting the equilibrium by adding preferentially to the mobile state. Equation 1 holds, but the increment in binding energy is that in converting an immobile to a mobile substrate complex (Krupka, 1989*a*). Now, sufficiently strong binding in the complex, which produces tight coupling, lowers the dissociation rate and reduces the rate of turnover, limiting the tightness of coupling.

Catalytic mechanisms cannot be solely responsible for coupling in primary and secondary active transport because at some stage in the reaction the substrate is required to stabilize intermediates with altered properties, as in the mechanism in Fig. 1. Stabilized carrier forms, and strong intrinsic binding forces, are found to be involved whether the substrates add in fixed or in random order; they are also involved in a mechanism in which the first substrate sterically blocks carrier movement instead of inducing an immobile carrier form. It appears that the limitations on tight coupling cannot be avoided. Note that catalysis of a carrier transformation could not give rise to the required stable intermediate, because catalysis does not shift an equilibrium.

Experimental Observations

Reliable estimates of coupling ratios have rarely been reported, but the following precise measurements may be cited.

(i) Secondary active transport. The rate of the uncoupled reaction of the Na^+/glucose cotransporter of intestinal epithelium, measured as the Na^+ current in the absence of glucose, is 6–9% of the coupled, i.e., sugar-dependent rate (Parent et al., 1992; Umbach, Coady & Wright, 1990). Thus, the ratio of coupled to uncoupled transport is about 14.

(ii) Primary active transport. The calcium pump of the sarcoplasmic reticulum allows internal calcium to leak out of vesicles in the absence of ATP, ADP or Pi; the leak, therefore, shows the pump working in an uncoupled mode (de Meis, Suzano & Inesi, 1990). The maximum rate of slippage is fast, about the same as that of the coupled reaction. According to the generally accepted E_1E_2 model for the system (Inesi, 1985), the coupled path for calcium exit, the reverse of the normal active uptake path, is as follows. Inorganic phosphate adds to the free inward-facing carrier to form an immobile phosphoryl-carrier derivative, and with addition of internal calcium the complex becomes mobile and moves across the membrane. ADP adds to this outward-facing complex of calcium and phosphate to form ATP, which dissociates, followed by calcium. The free outward-facing carrier then moves inward, continuing the cycle. Clearly, if the exit reaction were perfectly ordered, and followed this path, calcium exit in the absence of Pi and ADP would not be possible. But in fact the path is not absolutely fixed. Calcium has been shown to add not only to the phosphate complex, but, with only about 6 times lower affinity, to the free inward-facing carrier (Jencks et al., 1993). As internal calcium makes the carrier-phosphate complex mobile, the calcium complex of the free carrier is expected to be mobile too, and should move outward at the normal rate, as observed. The mechanism may be illustrated by referring to Fig. 3 (Krupka, 1994*a*). The calcium leak would be through the f_{-4} step, where the second substrate T has added to the two-site conformation of the free carrier C_i''. Thus, addition of the substrate, leading to slippage, depends on the equilibrium between the one-site and two-site conformations C_i' and C_i'', which in a perfectly ordered mechanism absolutely favors C_i', but which here favors C_i' by a factor of only 6. (The scheme in Fig. 3 illustrates the point even though, unlike the E_1E_2 model, it is symmetrically, not asymmetrically, ordered.)

(iii) Exchange-only transport. In the case of the anion exchanger of red cells the ratio of exchange, which is coupled transport, to net transport, which is uncoupled, is about 4×10^4 (Fröhlich, 1984; Fröhlich & King, 1987). This figure is more than a thousand times higher than the figures cited above for primary and secondary active transport systems. The greater efficiency of the exchanger can be explained by a simple catalytic exchange mechanism, as we have seen.

Coupling Under Physiological Conditions

Rigorous selection pressure is likely to have made coupling in organisms as tight as possible, since any avoidable waste of metabolic energy would be a disadvantage. In this light the rather loose coupling of the calcium and sugar transport systems could represent the most advantageous tradeoff between efficiency and rate that can be achieved in primary and secondary active transport.

But this much slippage, if multiplied by the many coupled vectorial systems in a cell, seems excessively wasteful, and it is possible that under physiological conditions there is less slippage than individual coupling mechanisms allow. It can be shown that because of the expected differences in the concentrations of the driving and driven substrates, outside and inside, slippage in an ordered mechanism may be minimized if the driving substrate is first on and first off (Krupka, 1993). The problem is complicated by the fact that from another point of view the reverse of this order is preferred: loading and unloading the carrier are facilitated, and the transport rates increased, if the driving substrate is last on and last off (Stein, 1986). Again, there is a conflict between coupling efficiency and the turnover rate. Circumstances may decide the relative merits of different arrangements, and indeed various mechanisms are found in nature. To what extent slippage is curtailed in individual systems is yet to be determined. The calcium pump, which was described above, provides one example. In the presence of phosphate ion the inward-facing carrier will exist mainly as the phosphate complex, which is on the coupled path and therefore does not contribute to an uncoupled calcium leak; and there would be little of the free carrier, which is responsible for slippage.

References

de Meis, L., Suzano, V.A., Inesi, G.J. 1990. Functional interactions of catalytic site and transmembrane channel in the sarcoplasmic reticulum ATPase. *J. Biol. Chem.* **265:**18845–18851

Devés, R., Krupka, R.M. 1979. A general kinetic analysis of transport. Tests of the carrier model based on predicted relations among experimental parameters. *Biochim. Biophys. Acta* **556:**533–547

Fröhlich, O. 1984. Relative contributions of the slippage and tunnelling mechanisms to anion net efflux from human erythrocytes. *J. Gen. Physiol.* **84:**877–893

Fröhlich, O., King, P.A. 1987. Mechanisms of anion net transport in human erythrocytes. *J. Gen. Physiol.* **90:**6*a*

Honig, W., Stein, W.D. 1978. Design principles for active transport systems. *J. Theoret. Biol.* **75:**299–305

Inesi, G. 1985. Mechanism of calcium transport. *Annu. Rev. Physiol.* **47:**573–601

Jencks, W.P., Yang, T., Peisach, D., Myung, J. 1993. Calcium ATPase of sarcoplasmic reticulum has four binding sites for calcium. *Biochemistry* **32:**7030–7034

Knowles, J.R., Albery, W.J. 1977. Perfection in Enzyme catalysis: the energetics of triose phosphate isomerase. *Acc. Chem. Res.* **10:**105–111

Krupka, R.M. 1989*a*. Role of substrate binding forces in exchange-only transport systems: I. Transition-state theory. *J. Membrane Biol.* **109:**151–158

Krupka, R.M. 1989*b*. Role of substrate binding forces in exchange-only transport systems: II. Implications for the mechanism of the anion exchanger of red cells. *J. Membrane Biol.* **109:**159–171

Krupka, R.M. 1990. Expression of substrate specificity in facilitated transport systems. *J. Membrane Biol.* **117:**69–78

Krupka, R.M. 1993. Coupling mechanisms in active transport systems. *Biochim. Biophys. Acta* **1183:**105–113

Krupka, R.M. 1994*a*. Interpreting the effects of site-directed mutagenesis on active transport systems. *Biochim. Biophys. Acta* **1193:**165–178

Krupka, R.M. 1994*b*. The application of vectorial coupling theory to the calcium pump. *Biochim. Biophys. Acta* **1193:**179–185

Parent, L., Supplisson, S., Loo, D.D.F., Wright, E.M. 1992. Electrogenic properties of the cloned Na^+/glucose cotransporter: I. Voltage clamp studies. *J. Membrane. Biol.* **125:**49–62

Stein, W.D. 1986. Transport and Diffusion Across Cell Membranes. pp. 400–404. Academic press, Orlando

Stein, W.D., Honig, W. 1977. Models for active transport of cations. The steady-state analysis. *Mol. Cell. Biochem.* **15:**27–44

Umbach, J.A., Coady, M.J., Wright, E.M. 1990. Intestinal Na^+/glucose cotransporter expressed in *Xenopus* oocytes is electrogenic. *Biophys. J.* **57:**1217–1224