A Model for Active Transport of Sodium and Potassium Ions as Mediated by a Tetrameric Enzyme*

(cell membranes/adenosine triphosphatase/transport kinetics/cation pumping/
protein conformation changes)

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ABSTRACT A new model is proposed for the system that actively transports sodium and potassium ions across animal-cell membranes. The model is based on the physical and chemical properties of transport-associated adenosine triphosphatase (EC 3.6.1.3) and on the kinetics of ion movements mediated by the system. Transport is postulated to occur by internal transfer of cations across a protein tetramer embedded in the cell membrane. The protein tetramer can exist in either of two forms of identical energy; transport occurs as a result of the sequential "flipping" from one conformation to the other. The conformation change results in the interchanging of the affinities of cation-binding sites associated with different subunits of the tetramer, with a concomitant splitting of adenosine triphosphate.

Models for the mechanism of action of the system that actively transports sodium (Na^+) and potassium (K^+) ions across cell membranes have often involved diffusible carriers or proteins undergoing conformational changes (1). We recently put forward a model for glucose transport in human erythrocytes, involving conformational changes within a protein tetramer embedded in the cell membrane (2-4). Similarities in the kinetics of cation and of glucose transport hinted that a similar type of model might explain transport in these two systems. We thus developed a tentative model for Na^+ and K^+ transport, based on accumulated experimental data concerning the behavior of the system as a cation transporter (5-11), as a ($\text{Na} + \text{K}$)-activated ATPase (12-20), and as a K -activated phosphatase (12, 21). Much of the data is indirect, and our model cannot yet account for all the experimental data. Nevertheless, we propose it now because it is an essentially new model and it suggests, therefore, several interesting new experiments.

Physical approach toward the new model

That a tetramer is involved in glucose transport in human erythrocytes was inferred from purely kinetic data (2, 3). The components of the cation active transport system have been isolated. [We assume that in its fundamental features the active transport system for Na^+ and K^+ is the same in all animal tissues and is identical with ($\text{Na} + \text{K}$)-activated ATPase (20), ATP phosphohydrolase (EC 3.6.1.3).]

Kyte (13, 14) purified ($\text{Na} + \text{K}$)-activated ATPase, which

contains two polypeptide chains in equimolar quantities. Tentatively, the protein is of the form $\alpha_n\beta_n$, where α and β are different polypeptide chains and n is a small integer that we proceed to estimate. Only one of the chains can be covalently labeled with phosphate from ATP (14); we call this the α chain. Kyte (13, 14) reports the molecular weights of the α and β chains to be 84,000 and 57,000, respectively. Kyte's results are consistent with those of Uesugi *et al.* (15), who found the two most prominent chains to have apparent molecular weights of 94,000 and 53,000. Thus, the molecular weight of the functional ATPase is about 140,000 n . To determine n , we need the molecular weight of the functional enzyme. Kepner and Macey (12) obtained an average molecular weight for the functional ($\text{Na} + \text{K}$)-activated ATPase of about 250,000. Nakao *et al.* (17) had obtained a value of 500,000, but Kepner and Macey (12) considered that Nakao *et al.* had overestimated the molecular weight. These results suggest that the value of n in $\alpha_n\beta_n$ is certainly greater than unity, with $n = 2$ being the most likely value. Thus, these physical studies are consistent with the tetrameric nature of the cation pump.

Chemical approach toward the new model

We shall draw an analogy between the results of chemical binding studies and the phenomenon of "half of the sites reactivity." Consider alkaline phosphatase (EC 3.1.3.1) from *Escherichia coli*, an α_2 dimer, both chains of which are disposed symmetrically in crystals (22). The binding sites on the two subunits appear identical in the unbound form of the dimer. Nevertheless, one molecule of phosphate binds with high affinity, while a second molecule binds with much reduced affinity, (23). Furthermore, while alkaline phosphatase can bind two molecules of phosphate or ATP per dimer, only one molecule of phosphate from ATP can be retained covalently under physiological conditions (23). Levitzki *et al.* (24) have pointed out that "a significant number of enzymes have now been shown to exhibit the paradox of half-of-the-sites reactivity, in which only one of two apparently identical subunits is expressed in a given reaction." This finding is apparently due to negative cooperativity: binding of substrate at one subunit of an α_2 dimer reduces the affinity at the second subunit.

Consider now the study of Hegyvary and Post (18), who measured the binding of ATP to membranes. Let R be the ratio of the moles of ATP found bound ionically under non-phosphorylating conditions (i.e., in the absence of Mg) to the

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moles of terminal phosphate incorporated covalently from ATP under standard phosphorylating conditions. With neither Na^+ nor K^+ present, $R = 1$ for ionic binding at a high-affinity site, with the hint of an additional binding at a lower-affinity site. With K^+ added, $R = 2$ for ionic binding at what appeared to be a single population of low-affinity sites, with no binding at sites of high affinity. Thus, twice as many ionic binding sites are expressed under one condition as under another. Further, twice as many ATP molecules can bind ionically to this membrane preparation as can donate at any one time a terminal phosphate group to be stably linked to the enzyme at physiological pH. This result is reminiscent of the behavior of the "half-of-the-sites-reactive" enzymes.

FIG. 1. A plot of the Na^+ - Na^+ exchange data from Fig. 5 of ref. 9. Cells loaded with various concentrations of labeled Na^+ were suspended in a high concentration of Na^+ with no K^+ . v is efflux (mmol/1 erythrocyte per hr) of labeled Na^+ ; S is intracellular concentration (mM) of Na^+ , assuming 70% cell water. Since Na^+ binding is cooperative, we plot data according to $v = [S/(S + K)]^2 V$, where K is the intrinsic dissociation constant of a single binding site and V is the maximum velocity. Concentration K_m (half-saturation constant) at which velocity is half-maximal is then $K_m = 2.42 K$. Intercept on abscissa is $-K$, intercept on ordinate is $K/V^{1/2}$. (a) Ouabain absent. $K_m = 12$ mM and $V = 3.2$ mmol per erythrocyte per hr (transport on inner high-affinity site); $K_m = 130$ and $V = 7.5$ (inner low-affinity site). (b) Ouabain at 0.1 mM. $K_m = 5$ and $V = 1$ (inner high-affinity site); $K_m = 110$ and $V = 4.1$ (inner low-affinity site).

($\text{Na} + \text{K}$)-activated ATPase can function also as a K^+ -activated phosphatase (21), a smaller portion of the enzyme being necessary for activity than for ATPase activity (12). We suggest that the phosphatase activity resides in the α_2 dimer portion of an $\alpha_2\beta_2$ cation pump tetramer. Although all α chains have the intrinsic ability to bind ATP, we postulate that only one chain of a pair can retain terminal phosphate from ATP in a covalent form under physiological conditions. A simple calculation from the data of Uesugi *et al.* (15) shows that the ($\text{Na} + \text{K}$)-activated ATPase preparation contains from 0.4 to 0.6 mol of covalently bound phosphate from ATP for every mol of α chain. Thus, the results of chemical binding studies present an interesting analogy to the properties of other enzymes showing "half-of-the-sites reactivity," and are consistent with the view that the cation transport protein is an $\alpha_2\beta_2$ tetramer.

The transport approach

Most models of active transport assume that during physiological operation of the pump there appear sequentially a high-affinity site for Na^+ on the inside face of the membrane and low-affinity site on the outside. However, recent transport studies performed under nonpumping conditions (i.e., in the absence of external K^+) indicate that high- and low-affinity sites for Na^+ can coexist simultaneously at each face of the membrane. (A high-affinity site for Na^+ has a half-saturation constant, K_m , of about 10 mM; a low-affinity site, a K_m of about 100 mM.)

The properties of the Na^+ -binding sites that can appear at the inside face of the membrane can be inferred from Sachs' experiment with human erythrocytes (Fig. 1). Although the dependence of Na^+ efflux on internal Na^+ concentration is greater than first-order, we take this cooperativity into account by plotting, not S/v against S , as for a conventional saturation curve, but $S/v^{1/2}$ against S . The resulting plot (Fig. 1) is fitted best by two straight lines, indicative of the presence of two classes of binding sites, with different affinities for Na^+ . Each class of sites binds more than one Na^+ ion in a cooperative fashion. (If $S/v^{1/2}$ is plotted against S , the same two classes of binding sites are observed.) The analysis in Fig. 1 suggests that, both in the presence and absence of the transport inhibitor ouabain, efflux of labeled Na^+ involves both a high- and a low-affinity site for Na^+ at the inward face of the membrane.

Similarly, the properties of the Na^+ -binding sites at the outward face of the membrane can be inferred from an experiment by Garrahan and Glynn (5). Fresh erythrocytes were suspended in K^+ -free solutions of various Na^+ concentrations. The results of the influx measurement (5) demonstrate the existence of both a high- and low-affinity site for Na^+ at the outer face of the membrane. That the low-affinity site is not merely a simple diffusive leak is shown by the fact that the flux occurring on it was reduced considerably by ouabain. In the efflux measurement (5), as the Na^+ concentration in the external solution was raised from zero, the Na^+ efflux at first dropped to a minimum at about 5 mM and then slowly rose in accordance with a half-saturation constant for a low-affinity site.

Thus, the experiment of Sachs indicates the presence of both a high- and a low-affinity site for Na^+ on the inner face of the membrane, while the experiment of Garrahan and Glynn suggests a similar situation at the outer face. The evidence

indicates the presence of at least four cation binding units per enzyme, consistent with a tetrameric protein.

The tetramer model

An extension of our previous tetramer model (2, 3) to the Na^+ and K^+ transport system can be arrived at as follows: In both systems, transport is postulated to occur by internal transfer of substrate across a protein tetramer embedded in the membrane. For glucose transport (Fig. 2a), each subunit of the tetramer retains its binding affinity for substrate throughout the transport process, while for active transport (Fig. 2b) we now suggest the affinities are switched during each conformational change. The conformation change that alters the position of the binding sites is termed a *transposition*; a change in the nature or affinity of the binding sites is termed a *transformation*. Thus, facilitated glucose transport involves only a succession of transpositions (Fig. 2a), while active cation transport would involve a succession of concerted transposition and transformation events (Fig. 2b).

The cation transport enzyme (Fig. 3) is postulated to be an $\alpha_2\beta_2$ tetrameric protein, asymmetrically disposed in the membrane. The active centers of the α subunits (and perhaps, although not necessarily, the entire α subunits) reside in that half of the membrane that faces the cell interior; those of the β subunits are in the outer half of the membrane. Each subunit has the potential to exist in either a high- or a low-affinity state for the binding of Na^+ ; in the physiological operation of the pump (Fig. 3) each subunit assumes successively such high- and low-affinity states. High affinity for Na^+ (an H_α or H_β site) implies low affinity for K^+ , while low affinity for Na^+ (an L_α or L_β site) implies high affinity for K^+ . The two conformations A and A' are functionally and energetically equivalent.

FIG. 2. Molecular events during transport according to the tetramer model. (a) A transposition. (b) Simultaneous transposition and transformation. *H*, subunit in high-affinity binding state; *L*, low-affinity state. Transport occurs as follows: (i) Substrate molecules bind to sites directed outwards from the tetramer; (ii) they enter the internal cavity after a conformational change; (iii) within the internal cavity substrate molecules redistribute themselves according to the binding affinities; and (iv) after a change back to the original conformation, the substrate molecules emerge into the bathing solutions.

FIG. 3. Model for active transport of Na^+ and K^+ ions as mediated by an $\alpha_2\beta_2$ tetrameric enzyme. *H*, subunit having a high affinity for Na and low affinity for K ; *L*, the converse situation. Each binding site can, intrinsically, be either *H* or *L*. (For simplicity, release of inorganic phosphate from *H* is not shown.) Active transport results from the sequential flipping between the isoenergetic conformations A and A'.

alent. Sequential flipping from one conformation to the other is sufficient to allow active transport to occur.

The postulated sequence of events involved in normal transport is as follows. Consider first cation movements on the upper two subunits. (a) In conformation A, Na^+ binds at the H_α subunit and K^+ at L_β . (b) The tetramer flips to state A'; this involves both a transposition and a transformation. (c) In conformation A', ions brought into the internal cavity formed by the upper two subunits will now redistribute and Na^+ and K^+ will bind at the H_β and L_α subunits, respectively. (d) The tetramer flips back to state A, causing Na^+ to be expelled into the external medium, K^+ into the cell interior. (e) The process repeats from step (a). Cation movements on the lower two subunits proceed in an entirely equivalent manner, but 180° out of phase.

The simultaneous changes in the phosphorylation status of the α subunits, which cause the above affinity changes, can be considered in an exactly parallel fashion. Consider first the upper α subunit. (a) In conformation A, ATP binds to H_α . (b) With Na^+ (and Mg^{++} , assumed always present), this subunit is phosphorylated, and simultaneously the tetramer flips to state A', the upper α subunit transforming from H_α to L_α . The K^+ brought into the internal cavity binds to the upper α subunit. (d) With these K^+ ions as cofactors, this upper α subunit dephosphorylates, and simultaneously the tetramer flips back to state A. (e) The process repeats from step (a). Events on the lower α subunit proceed in an entirely equivalent manner, but 180° out of phase. It is important that phosphorylation of one α subunit and dephosphorylation of the other is a concerted process, accounting for the "half-of-the-sites reactivity" finding that under physiological conditions only one phosphate from ATP can be retained covalently by the tetramer at any one time.

Thus, under pumping conditions (Fig. 3), cellular Na^+ ions serve as cofactors for phosphorylation of the α subunit by ATP, whereas K^+ ions in the internal cavity act as cofactors for the transfer of covalently-bound phosphate to water. We suggest that if, in the internal cavity Na^+ rather than K^+ binds to the L_α subunit, then Na^+ will serve instead as a cofactor for the transfer of covalently-bound phosphate to ADP, if this is present. This presumably occurs during the reversal

of the pump (7), with the concomitant synthesis of ATP. Such a postulate would account for the finding (19) that low concentrations (8 mM) of Na^+ are sufficient for complete phosphorylation of (Na + K)-activated ATPase by ATP, whereas much higher concentrations (160 mM) are required for subsequent dephosphorylation by deoxy-ADP. On the tetramer model, dephosphorylation by ADP requires Na^+ in the internal cavity to bind to the L_α site. This binding will occur only if sufficient Na^+ ions are present within the cavity to bind to both H_β and L_α sites. But to have this number of Na^+ ions inside the cavity, both the high- and low-affinity sites must have carried Na^+ into the cavity. Thus, dephosphorylation by ADP requires use of the low-affinity subunits.

During transport under nonpumping conditions, transpositions of the binding sites may not occur, even though transpositions of the tetramer continue (Fig. 2). These transpositions would cause the appearance of binding states that are not seen during active transport; thus the L_α and H_β sites, which under pumping conditions are occluded within the internal cavity (Fig. 3), may be revealed (e.g., Fig. 1).

Several important effects of ouabain are understandable if we postulate that ouabain binds to a β subunit in its L_β binding state, preventing the movement of cations on this subunit. Thus, ouabain inhibits cation pumping only if applied extracellularly; influx of labeled Na^+ on low-affinity sites (i.e., on L_β) is inhibited by ouabain (5), as is stimulation of Na^+ efflux by high concentrations of external Na^+ (5); of the cation fluxes inhibited by ouabain, the influx of K^+ (on L_β) is perhaps that most severely affected (6, 8); K-activated phosphatase activity of the system (K entering the internal cavity by L_β) is abolished by ouabain.

Immediately testable predictions of our model are: (a) transport experiments in K^+ -free and in Na^+ -free systems will display kinetic anomalies like those found for glucose transport in human erythrocytes (4); (b) many additional parallels will be found between properties of the cation pump enzyme and other "half-of-the-sites reactive" enzymes (24); (c) a method might be developed capable of detecting the occlusion of cations (19) within the internal cavity of the tetramer; (d) the β subunit will combine with cardiac glycosides and derivatives that are site-directed irreversible inhibitors (16); (e) if some residual ATPase or phosphatase activity can be found in α_2 dimers free of β chains, it will be insensitive to ouabain.

From the model (Fig. 3), the two conformations of the protein involved in the energy-transducing process are exactly isoenergetic, which may be important for at least two reasons. For rapid rates of energy transduction, the conformations of the protein must not differ much in free energy lest the protein be trapped in the more stable conformation and

the number of transitions decrease. For efficient energy transduction, none of the hydrolysis energy of the high-energy phosphate bond need be stored in the conformation of a stable intermediate, presumably a wasteful process. Indeed, with isoenergetic conformations, the hydrolysis free energy can be directly converted to cation pumping, with minimal losses.

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1. Caldwell, P. C. (1970) in *Membranes and Ion Transport*, ed. Bittar, E. E. (John Wiley & Sons, London), Vol. I, pp. 433-461.
2. Lieb, W. R. & Stein, W. D. (1970) *Biophys. J.* **10**, 585-609.
3. Lieb, W. R. & Stein, W. D. (1971) *Nature New Biol.* **230**, 108-109.
4. Lieb, W. R. & Stein, W. D. (1972) *Biochem. Biophys. Acta* **265**, 187-201.
5. Garrahan, P. J. & Glynn, I. M. (1967) *J. Physiol.* **192**, 159-174.
6. Garrahan, P. J. & Glynn, I. M. (1967) *J. Physiol.* **192**, 189-216.
7. Garrahan, P. J. & Glynn, I. M. (1967) *J. Physiol.* **192**, 237-256.
8. Glynn, I. M., Lew, V. L. & Lüthi, U. (1970) *J. Physiol.* **207**, 371-391.
9. Sachs, J. R. (1970) *J. Gen. Physiol.* **56**, 322-341.
10. Sachs, J. R. (1971) *J. Gen. Physiol.* **57**, 259-282.
11. Caldwell, P. C. & Keynes, R. D. (1959) *J. Physiol.* **148**, 8P.
12. Kepner, G. R. & Macey, R. I. (1968) *Biochem. Biophys. Acta* **163**, 188-203.
13. Kyte, J. (1971) *J. Biol. Chem.* **246**, 4157-4165.
14. Kyte, J. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1259-1265.
15. Uesugi, S., Dulak, N., Dixon, J. F., Hexum, T. D., Dahl, J. L., Perdue, J. F. & Hokin, L. E. (1971) *J. Biol. Chem.* **246**, 531-543.
16. Hokin, L. E. (1969) *J. Gen. Physiol.* **54**, 327S-342S.
17. Nakao, H., Nagano, K., Nakao, T., Mizuno, Y., Tashida, Y., Fujita, M., Maeda, H. & Matsudaira, H. (1967) *Biochem. Biophys. Res. Commun.* **29**, 588-592.
18. Hegyvary, C. & Post, R. L. (1971) *J. Biol. Chem.* **246**, 5234-5240.
19. Post, R. L. & Kume, S. (1971) *Proc. Int. Union Physiol. Sci.* **8**, 84-85.
20. Bonting, S. L. (1970) in *Membranes and Ion Transport*, ed. Bittar, E. E. (John Wiley & Sons, London), Vol. I, pp. 257-363.
21. Rega, A. F., Pouchan, M. I. & Garrahan, P. J. (1970) *Science* **167**, 55-56.
22. Hanson, A., Applebury, M. L., Coleman, J. E. & Wyckoff, H. W. (1970) *J. Biol. Chem.* **245**, 4975-4976.
23. Lazdunski, M., Pettitclerc, C., Chappolet, D. & Lazdunski, C. (1971) *Eur. J. Biochem.* **20**, 124-139.
24. Levitzki, A., Stallcup, W. B. & Koshland, D. E., Jr. (1971) *Biochemistry* **10**, 3371-3378.