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Simple model for the chemical potential change of a transported ion in active transport

(free energy transduction/membrane transport/calcium pump/sodium, potassium pump)

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ABSTRACT **The mechanism for active transport of ions across a membrane probably involves two distinct conformational states of the transport protein, in which the binding sites for the transported ion face opposite sides of the membrane. It is likely that the binding affinity for the ion changes in synchrony with the change in site orientation, such that the affinity is high on the uptake side of the membrane and low on the discharge side. A structural model is proposed for the transmembrane portion of such a protein, based on the known multihelical structure of bacteriorhodopsin. This structure is well adapted to a cyclical alternation between two conformations that differ simultaneously in orientation and binding affinity. No unfolding of the helices or other significant alterations in secondary structure is required. The model is explicitly intended as a hypothetical representation of the E_1 and E_2 states of ATP-driven Na^+,K^+ and Ca^{2+} pumps.**

In active transport and related processes involving osmotic free energy, the transported ion is taken up from one side of a membrane at a chemical potential μ_1 and released on the other side at a much higher (or lower) potential μ_2. In this paper, I propose a simple structural model for a protein molecule designed to catalyze this change in potential.

The proposed model is essentially an elaboration of the allosteric model of Jardetzky (1). Jardetzky assumed that the protein deals with the problem of changing the chemical potential of a transported ion by having binding sites for the ion that differ in affinity when facing opposite sides of the membrane. Appropriate values for the binding constant permit the binding sites for uptake or release of the ions to be about half-saturated at ambient concentrations in the adjacent aqueous media, a desirable property if the protein is to be responsive to changes in ion concentration, for the purpose of concentration regulation or for the synthesis of ATP. If we use the terminology of chemical potentials (2), a difference in binding affinity is equivalent to a difference ($\Delta\mu_b^0$) in the standard potential of the protein-bound ion. If the values are appropriately tailored to the ambient aqueous concentrations, $\Delta\mu_b^0$ would be related to μ_1 and μ_2 as

$$\Delta\mu_b^0 \simeq \mu_1 - \mu_2. \qquad [1]$$

The questions to be asked in designing a structural model for the process then become the following. (*i*) How can a protein have a reaction cycle in which μ_b^0 can alternate between high and low values? (*ii*) How can this alternation be synchronized with a spatial conformational change in which the protein can exchange the transported ion, first with the solution on one side of the membrane and then with the solution on the other side?

Thermodynamic pathway

As Jencks (3) has pointed out, the thermodynamic pathway assumed here, and implicitly by Jardetzky (1), is not a unique requirement for an active transport mechanism. As illustration of this, Jencks (3) has suggested a hypothetical mechanism for proton transport that would not involve a difference in binding affinities. There are, however, sound theoretical reasons in support of the pathway used here, at least for those transport systems that have the physiological function of maintaining the ion activity (a_i) on one side of the membrane at a fixed value. If we assume that ion binding is not the rate-limiting step in the overall transport cycle and that the degree of binding is close to equilibrium during transport, the problem of maintaining constant activity is thermodynamically equivalent to the problem of maintaining constant pH by means of a pH buffer. The buffering capacity of a pH buffer is maximal if the pK of the proton binding site is equal to the pH one wishes to maintain and, likewise, in the present situation, the ability to maintain a_i at a constant level is optimal if the dissociation constant (reciprocal of the binding constant K) is equal to the ion activity to be maintained. In terms of chemical potentials,

$$-RT \ln K = \mu_b^0 - \mu_{aq}^{0'}, \qquad [2]$$

where μ_b^0 is the standard potential for the protein-bound ion and $\mu_{aq}^{0'}$ is the standard potential in aqueous solution, based on a molar concentration scale (2). If the condition $1/K \simeq a_i$ is introduced into the equation for the chemical potential of the ion in the bulk solution,

$$\mu = \mu_{aq}^{0'} + RT \ln a_i, \qquad [3]$$

it follows at once that $\mu \simeq \mu_b^0$. It will be shown in a subsequent paper that this relationship must be closely obeyed for optimal buffering at the steady state of the reaction cycle, even if one does not assume equilibrium at the binding site, and that a similar condition applies to the side of the membrane where the transport protein is not itself the regulatory agent—e.g., the extracellular medium in the case of a Ca^{2+} pump designed to maintain intracellular Ca^{2+} at a very low level. Eq. 1 follows directly from these considerations. There is good experimental evidence that Eq. 1 is in fact applicable to many transport systems. The evidence is especially convincing for the sarcoplasmic reticulum Ca^{2+} pump protein (4). For this protein, $\mu_b^0 - \mu_{aq}^{0'}$ has a value of −9 kcal/mol (1 cal = 4.18 J) when the Ca^{2+} binding site is facing the cytoplasm (conformational state E_1) and a value of about −4 kcal/mol when the binding site is facing the sarcoplasmic reticulum lumen (conformational state E_2). ATP-driven Na^+,K^+ pumps have similar E_1 and E_2 states and a recent estimate (5) gives $\Delta\mu_b^0$ values of 2–4 kcal/mol between uptake and discharge sites for Na^+ and K^+. The difference in $\Delta\mu_b^0$ is smaller than for the Ca^{2+} pump, and the ion concentration gradients maintained by Na^+,K^+ pumps are likewise smaller. Some of these results are based on cooperative equilibria, in which two or more ions are bound simultaneously. Calculation of average chemical potentials for individual bound ions from such data has been described (2).

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Effect of Membrane Potential. In most physiological situations, there is a potential difference across the membrane and electrical work is involved in the transport process in addition to osmotic work. Since Eq. 1 implies only approximate equality between the two sides of the equation, the electrical work term will often have no significant effect. If the membrane potential difference ($\psi_1 - \psi_2$) is large, it can be formally incorporated into the equation simply by replacing the chemical potentials μ_1 and μ_2 by the corresponding electrochemical potentials $\tilde{\mu}_1$ and $\tilde{\mu}_2$. Where $\Delta\mu_b^0$ continues to represent the difference in binding affinity that would be measured in the laboratory in the absence of an electrical potential, we then have

$$\Delta\mu_b^0 \simeq \tilde{\mu}_1 - \tilde{\mu}_2 = \mu_1 - \mu_2 + Z_i F(\psi_1 - \psi_2), \quad [4]$$

where Z_i is the charge on the ion and F is the Faraday constant. Eq. 4 is the appropriate modification of Eq. 1, regardless of which parts of the reaction cycle are most strongly affected by the membrane potential.

Generation of states with differing affinity

The main purpose of this paper is not to justify Eq. 1 or Eq. 4 but to consider how a transport protein might be structurally designed to create a reaction pathway that would satisfy the equation. The way in which sites of different affinity can be generated on the basis of differences in the amino acid side chains (and peptide groups) that constitute the binding site for an ion is shown, using Ca^{2+} as example, in Table 1. Six coordinating groups, including several carboxyl groups, are typical of high-affinity sites. A smaller number (some positions in the coordination sphere of the ion filled by water molecules) would suffice for a low-affinity site. The same principle undoubtedly applies to Na^+ and K^+ binding, but structural information for proteins that bind these ions is not available nor are binding data for model compounds (12) as plentiful as those that exist for Ca^{2+}. Knowledge of H^+ binding, on the other hand, is extensive, and the possibilities for structure-related changes in μ_b^0 that might apply to proton pumps are numerous.

Differing affinities as part of a transport cycle

Reversible protein conformational transitions leading to reversible chemical potential changes of bound ligands are features of many biochemical mechanisms. In some examples (hemoglobin, allosteric enzymes), a rearrangement of subunits within an oligomeric structure is involved. More recently, functionally similar conformational changes have been observed in enzymes consisting of only a single protein subunit. Yeast hexokinase (13), for example, contains two separate structural domains joined by a flexible segment of polypeptide that can serve as a hinge between the two domains. The protein can exist in two conformations, facing portions of the domain surfaces being in close contact in one conformation and separated by solvent in the other conformation. The tight conformation is induced to form by the binding of glucose and, by the principle of linked functions (14), this means that glucose must have a lower chemical potential in the tight form than in the open form.

The hinged structures of hexokinase and similar enzymes (15) belong to water-soluble proteins, and the binding sites are exposed to the same solution in both high- and low-affinity states. It is, however, not difficult to adapt the same principle to the generation of conformational states that have different μ_b^0 values in transport proteins. This can be done, for example, in terms of the one transport protein for which low-resolution structural information is at present available (16), the proton pump molecule bacteriorhodopsin. [Henderson and Unwin (16) have suggested that this structure may be a representative model for many kinds of transport proteins.] The transmembrane portion of bacteriorhodopsin consists of seven more or less parallel helices. The external surface of the assembly is predominantly hydrophobic, and a thread of polar groups runs down the center (17), providing a potential pathway (presumably closed during parts of the reaction cycle) for proton translocation. The seven transmembrane helices are joined by short loops of polypeptide on both sides of the membrane (17) and most of them are long enough to enable them to act as hinges or ball joints permitting the assembly to exist in two distinct conformational states. Amino acid side chains from one helix that would be in close proximity to a site on a second helix in one conformation can be readily imagined as moving away to a greater distance in the other conformation, as is illustrated in Fig. 1. The basic requirement for existence of two states that have different μ_b^0 values is therefore readily met. It should be noted that conformational changes affecting the disposition of

Table 1. Binding data for Ca^{2+} at moderate ionic strength

Ligand	Coordinating groups, no. COO^-	Peptide CO	OH	log K	$\mu_b^0 - \mu_{aq}^{0'}$, kcal/mol
Acetate (6)	1	—	—	0.53	−0.7
Lactate (6)	1	—	1	1.07	−1.5
Glycylglycine (6)	1	1	—	1.0	−1.4
Glutamate (6)	2	—	—	1.43	−2.0
Malate (6)	2	—	1	1.96	−2.7
Citrate (6)	3	—	1	3.50	−4.8
Lysozyme (7)	1 or 2	—	—	1.5	−2.0
Trypsin (8)	2	2	—	3.2	−4.4
Thermolysin (9)	2	1	—	4.5	−6.2
Parvalbumin (10, 11)	4	1	1	9.4	−13.0

Data are for room temperature, except that the result for thermolysin is for 6°C. log K is based on the molar concentration scale and $\mu_{aq}^{0'}$ is the standard potential for aqueous Ca^{2+} on that scale.

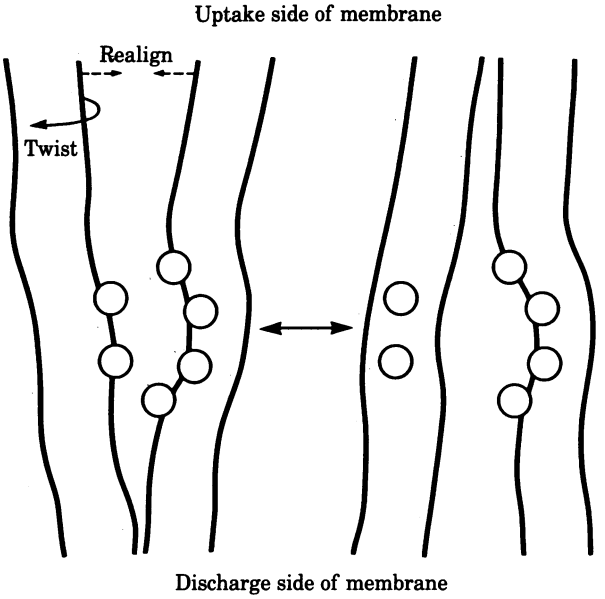

FIG. 1. Schematic illustration of how a change in μ_b^0 might be coupled to a change in accessibility of the binding site from one side of a membrane to the other. The circles represent amino acid side chains and peptide groups that constitute the binding site for the ion, and they are visualized as attached to helical transmembrane segments of the protein molecule. Only two segments are shown, but the overall structure is assumed to be an assembly of six or more such segments, as observed in bacteriorhodopsin (16).

amino acid side chains have been experimentally observed in bacteriorhodopsin itself (18).

The model illustrated in Fig. 1 is more attractive for the generation of differences in μ_b^0 values in transport proteins than a hinged domain (hexokinase-like) model, not only because it is based on the known structure of an actual transmembrane protein, but also because it readily permits the change in μ_b^0 to be accompanied by a simultaneous change in the accessibility of the ion binding site from one side of the membrane to the other. I thus suggest that Fig. 1 can serve as a conceptual model for the E_1 and E_2 states of ATP-driven Na^+,K^+ and Ca^{2+} pumps (3, 4, 19). Both these pumps transport more than one ion per reaction cycle, a property readily accommodated by the model since the structure shown in the figure could be duplicated several times in a multihelical assembly. In the case of Na^+,K^+ pumps, the model could apply equally well for simultaneous or sequential transport of Na^+ and K^+ ions.

In active transport and related processes, the change in chemical potential of the transported ion is of course necessarily coupled to a chemical process such as ATP hydrolysis or synthesis. The thermodynamics and kinetics of such coupling have been discussed by Jencks (3). In terms of Fig. 1, what is required is that some other part of the protein molecule (probably a part of the protein that extends into the cytoplasm) must undergo an appropriate conformational change in synchrony with the change affecting the transmembrane portion. This does not require postulation of a new principle. Protein structures are generally tightly coordinated, so that no one part can change its conformation independently of the whole. What might be characterized as "action at a distance" is a natural consequence. In hemoglobin (20), for example, there are only two conformational states, which simultaneously alter the oxygen affinities of all four heme iron atoms, despite the fact that the iron atoms are separated from each other by 24 Å or more. Moreover, changes in the pK values of critical acidic groups occur as part of the same transition.

Discussion of the model

The model proposed here is crude, but it is more explicit than the model of Jardetzky (1) and models that have been discussed in relationship to ion translocation alone (21, 22). Fig. 1 has the advantage of accounting for *both* translocation and change in chemical potential occurring in synchrony, and it is readily adapted to the transport of more than one ion per reaction cycle. It indicates no obvious need for an oligomeric structure, a topic of recent debate (21, 22), nor does it require that the obligatory conformational change, without which active transport can hardly be conceived to occur at all (3), be a major one. The conformational rearrangement envisaged by Fig. 1 does not involve a significant change in secondary structure, a prediction that is experimentally testable. For example, CD spectra of the E_1 and E_2 conformations of Na^+,K^+ and Ca^{2+} pumps would be expected to be very similar.

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