Gliding Edge Dislocations in Proteins as a Mechanism for Active Ion Transport

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A mechanism for the translocation of ions across biological membranes is proposed in which transconformational changes occur in the membrane-bound ion pump. In particular, it is suggested that such conformational changes are effected by means of the propagation of crystal defects in the form of edge-dislocation glide.

A fundamental property of living cells is the maintenance of unequal ionic concentrations across the cell membrane. This phenomenon is believed to be largely due to the effect of membrane-bound ion pumps that actively transport ions across the membrane against electrical and chemical gradients, either into or out of the cell. Apart from this general property, ion pumps play specific roles in the physiological functioning of certain specialized tissues, examples being the sarcoplasmic-reticulum Ca^{2+} pump in muscle, and ion pumps in renal tubules and in neural tissue. Although considerable progress has been made in elucidating the process of active ion transport from the enzyme-kinetic and energetic points of view (Askari, 1974; Skou, 1975; Schwartz *et al.*, 1975), little progress has been made in the description of the mechanisms involved in terms of the actual structural changes that must occur in the pump proteins during translocation, even though there has been considerable speculation on the subject.

The object of the present paper is to discuss in more detail a particularly simple mechanism for conformational changes in ion-pump proteins that has been suggested and that could account for active ion transport (Chandler *et al.*, 1977). The mechanism involves the propagation of crystal defects known as edge dislocations through protein helices. The phenomenon of edge-dislocation glide is well known in other branches of physics (Cottrell, 1965; Nabarro, 1967) and, as has been suggested, may well be a fundamental mechanism for conformational changes in proteins generally (Harris *et al.*, 1976). In the present paper, some of the recent knowledge of the structure and properties of membrane ion pumps is

Abbreviations used: Ca^{2+} -ATPase, calcium- and magnesium-ion-dependent adenosine triphosphatase; $(\text{Na}^+, \text{K}^+)\text{-ATPase}$, sodium- and potassium-ion-activated adenosine triphosphatase.

reviewed together with a brief review of the theory of dislocation defects pertaining to transconformational changes in proteins. The way in which these defects can act to provide a mechanism for the ion pump consistent with present knowledge is then described.

Membrane Pumps

Most published work has considered the properties of two specific pumps of great physiological importance, the Ca^{2+} -ATPase pump in the sarcoplasmic reticulum and the $(\text{Na}^+, \text{K}^+)\text{-ATPase}$. The following discussion will be concerned primarily with these two pumps, although the arguments can be applied to other pumps with similar or related molecular structures.

$(\text{Na}^+, \text{K}^+)\text{-ATPase}$

This enzyme is found in the cell membrane (Skou, 1975; Sen & Post, 1964) and its function is to transport Na^+ ions from the interior of the cell to the exterior, and to transport K^+ ions in the reverse direction. Estimates of the number of subunits in the protein vary, but it has been suggested that it consists of two large polypeptide units of mol.wt. 95000, which span the membrane and contain the catalytic site, together with a smaller glycoprotein subunit of 50000 mol.wt. (Perrone *et al.*, 1975; Jørgensen, 1975). Phospholipids appear to be necessary for the functioning of the enzyme, but their identity is not certain (Walker & Wheeler, 1975). One side of the enzyme is exposed to the outer surface of the membrane and contains high-affinity K^+ -binding sites and the site for glycoside binding (Albers *et al.*, 1968; Robinson, 1975). The other side faces the interior of the cell and contains Na^+ -binding sites and an active site for ATP hydrolysis (Kyte, 1975). The detailed structure of the enzyme *in vivo* has yet to be determined, but the membrane-penetrating regions of the protein are

thought to contain hydrophobic amino acid residues in an α -helical array, whereas the portions outside the membrane are thought to contain a large number of hydrophilic groups (Kyte, 1975; Hilden & Hokin, 1975; Schwartz *et al.*, 1975).

The kinetics and energetics of the enzyme have been extensively investigated and the reaction sequence is postulated to be (firstly) binding of Na^+ to the sites on the inner surface of the membrane, resulting in phosphorylation (Post *et al.*, 1975). This is followed by K^+ binding on the outside of the membrane, accompanied by dephosphorylation (Robinson, 1975). Although it is not certain whether Na^+ and K^+ binding occur sequentially or simultaneously, the sigmoidal nature of the kinetic curves indicate a co-operative process occurring between the two ion-binding sites owing to conformational changes (Titus & Hart, 1974).

The stoichiometry of the enzyme reaction has been shown to involve the movement of two K^+ ions into the cell for every three Na^+ ions out for the hydrolysis of one molecule of ATP, each event thus requiring an energy input of 38 kJ (9 kcal)/mol (Whittam & Ager, 1965; Hilden & Hokin, 1975). The reaction is essentially a vectorial chemical reaction, but it can, however, be reversed under extreme non-physiological conditions of ion-concentration gradient, the reverse reaction producing ATP from ADP.

Ca^{2+} -ATPase

This enzyme, which constitutes about two-thirds of the protein content of the sarcoplasmic reticulum, was first purified by MacLennan (1970), who established that it has a total mol.wt. of about 400 000 and consists of polypeptide-chain subunits with a proteolipid component (Malan *et al.*, 1976). It is believed that each polypeptide unit has two membrane-spanning regions containing largely hydrophobic residues (Rizzolo *et al.*, 1976), and it is estimated that there are three or four polypeptide chains per molecule of the enzyme (Tanford & Reynolds, 1976). There is one specific ATP-binding site and two Ca^{2+} -binding sites per phosphorylation site (Meissner & Fleischer, 1973). The amino acid sequence of the ATP-binding site has been determined, and the site consists of a tripeptide that is similar to that in (Na^+ , K^+)-ATPase (Bastide *et al.*, 1973).

Both (Na^+ , K^+)-ATPase and Ca^{2+} -ATPase have been shown to be capable of transporting ions when the purified proteins are reconstituted in artificial membranes (Hilden & Hokin, 1975; Warring *et al.*, 1974).

Translocation Models

Numerous models have been proposed to explain the translocation of ions across cell membranes, but,

in the light of more recent experimental findings, many of these are now mainly of historical interest. The most important of the later experimental findings is that by Kyte (1975), who showed that fixation of the transmembrane portion of (Na^+ , K^+)-ATPase both internally with antibodies and externally with cardiac glycosides did not affect the function of the enzyme. This indicates that any conformational changes involved in the translocation process must be small, thus eliminating models involving large conformational changes such as the mobile steady-state carrier model (Caldwell, 1968), the flip-flop model (Repke *et al.*, 1974) and a swivelling model (Charnock & Opit, 1969).

Among other models, Jardetzky (1966) proposed a model that involved two movable polypeptide chains capable of undergoing allosteric reorganization and thereby permitting transport of ions in the intermolecular cleft between the polypeptide chains. Kyte (1975) has also developed a similar model for intermolecular transport. In contrast with these, Shamoo (1974) has discussed a model in which the ions are transported within the helical coils of polypeptides. The present dislocation model uses the main features of these other models, namely either intramolecular transport or intermolecular transport, and suggests that the formation of the necessary ion-containing bulges is due to the glide of edge-dislocation defects.

Dislocations in Helical Proteins

Crystal defects, of which the edge dislocation is only one example, are widespread in Nature and have been observed in a wide variety of materials. In particular, dislocation theory has been very successful in describing the inelastic properties of metals (Cottrell, 1965; Nabarro, 1967) and has been extended to describe the mechanical properties of organic polymers (Predecki & Statton, 1967). In materials that have a three-dimensional lattice structure, such as metals, dislocations are line defects and their propagation through the lattice does not produce any conformational changes. Protein helices, however, are examples of cylindrical crystals and may be represented in terms of a two-dimensional lattice. In such lattices, edge dislocations are point defects and their propagation can give rise to conformational changes. The theory of edge dislocations in cylindrical structures such as viral tail sheaths, together with the way in which such defects change the structure, has been previously described (Harris & Scriven, 1973). The theory, which will be briefly reviewed below, may equally well be applied to the study of protein helices (Harris *et al.*, 1976).

The plane-lattice representation of a protein helix may be formed by imagining the cylinder as a roller, inking in equivalent lattice points, in this case the α -carbon atoms, and printing these points on a plane.

The resulting plane lattice is illustrated in Fig. 1. The basic lattice is described by the vectors $\mathbf{a}_1$ and $\mathbf{a}_2$, $\mathbf{a}_1$ representing the hydrogen-bond and $\mathbf{a}_2$ the covalent-bond sense of the lattice. Particular cylindrical forms of the crystal are represented by vectors such as $\mathbf{C}_3$ etc. which represent circumferential lines on the cylinder such that the two lattice points connected by $\mathbf{C}_n$ on the plane-lattice representation are identical points on the rolled-up cylinder. In forming the cylindrical crystal from the plane lattice, the convention is adopted that vectors $\mathbf{C}_n$ are clockwise-directed lines on the cylinder when the cylinder is viewed in the direction of the normal vector $\mathbf{z}$. Thus a crystal represented by vector $\mathbf{C}_3$ is a right-handed helix and is, in fact, the α -helix. Left-handed helices may equally well be formed from the plane lattice, but the discussion below will be limited to right-handed helices.

Vectors such as $\mathbf{C}_n$ are referred to as the characteristic lattice vectors and may be written:

$$\mathbf{C}_n = n_1 \mathbf{a}_1 + n_2 \mathbf{a}_2$$

where n_1 and n_2 are integers.

A particular topological form may therefore be described by the integers n_1 and n_2 , where n_1 refers to the number of polypeptide chains in the helix and n_2 refers to the hydrogen-bond topology. In the discussion below, the protein helices contain only one chain, and $n_1 = 1$ in each case. The integer n_2 can be used to describe the particular type of helix, the helix being referred to as an (n_2)-helix (Harris *et al.*, 1976). This alternative notation for older usage (Hopfinger, 1973) will be used below and, in terms of this notation, the α -helix is a (3)-helix, the 3_{10} -helix is a (2)-helix and the π -helix is a (4)-helix.

There are three operations that can be performed on protein helices containing one polypeptide chain

Fig. 1. Plane-lattice representation of polypeptide helices
For details, see the text.

(Harris *et al.*, 1978), of which the topological contraction is of interest as far as the present ion-translocation model is concerned. Topological contractions involve a change in the value of n_2 and thus a change in the form of the helix. Such contractions can be carried out as illustrated in Fig. 1 by adding or subtracting vectors $\mathbf{b}$ to the characteristic vector:

$$\mathbf{C}_{n+1} = \mathbf{C}_n + \mathbf{b}$$

Adding or subtracting vectors $\mathbf{b}$ to the characteristic vector is formally equivalent to passing an edge dislocation through the lattice, and $\mathbf{b}$ is known as the Burgers vector of the dislocation.

Edge dislocations may easily be incorporated into the structure of protein helices and may be of two types, the O-dislocations, which are essentially non-hydrogen-bonded C=O groups in the main chain, and H-dislocations, which are non-hydrogen-bonded N-H groups. The structure of a dislocation in a polypeptide helix is illustrated in Fig. 2 and, in this case, is an H-dislocation at the junction between a (3)-helix and a (2)-helix. Such a defect may easily propagate through the helix by a process of breaking and re-forming one hydrogen bond at a time. For the dislocation of Fig. 2 to propagate towards the top of the Figure, the hydrogen bond labelled A is broken and the O atom forms a hydrogen bond with the original dislocation, leaving the H atom at A as the dislocation. Continuing the process moves the dislocation through the lattice and, in so doing, converts the remaining (3)-helical structure into a (2)-helix. If the dislocation were to propagate in the reverse direction by breaking the hydrogen bond at B and continuing through the lattice, the (2)-helical region would be converted into (3)-helix. Dislocation glide thus constitutes a simple mechanism for effecting conformational changes in proteins involving only localized disruptions in the structure, which can propagate as waves of slip in the helix.

Although no direct experimental evidence for the existence of dislocation processes in proteins has so far been obtained, indirect evidence that such processes are energetically favourable may be obtained from empirical conformational-energy maps. Conventionally, conformational-energy maps are presented as energy contours plotted in conformation space described by ϕ and ψ , where ϕ and ψ are the dihedral angles of rotation about the N-C and C-N bonds respectively of neighbouring peptide units. Plotted in this way, low-energy transformation pathways are not readily apparent. However, if such energy maps are re-plotted in terms of helical parameters n and h , where n is the number of peptide residues per turn of the helix and h is the distance between neighbouring α -carbon atoms measured along the axis of the helix, a different pattern emerges, as shown in Fig. 3. Helical parameters are more

Fig. 2. Edge dislocation between a (3)-helix and a (2)-helix (scale model)

The dislocation (arrowed) is represented by the symbol $\perp$. For further details, see the text.

directly related to the plane-lattice representation as illustrated in Fig. 4 than are the dihedral angles of rotation normally used. Fig. 3 shows a portion of an empirical conformational-energy map derived by Brant *et al.* (1967) re-plotted in terms of helical

Fig. 3. Part of the conformational energy map near the (4)-, (3)- and (2)-helices plotted in terms of height per turn of helix (h) against number of residues per turn of helix (n)

Fig. 4. Plane-lattice representation showing the relationship between height per residue (h) and number of residues per turn (n)

parameters by using the relationships (Fraser & MacRae, 1974):

$$\begin{aligned}\cos\left(\frac{1}{2}t\right) &= -0.817 \sin \frac{1}{2}(\phi + \psi) + 0.045 \sin \frac{1}{2}(\phi + \psi) \\ h \sin\left(\frac{1}{2}t\right) &= 2.966 \cos \frac{1}{2}(\phi + \psi) + 0.663 \cos \frac{1}{2}(\phi + \psi)\end{aligned}$$

where t , the unit twist = $360/n$

From Fig. 3 it is clear that there is a low-energy pathway between (4)-, (3)- and (2)-helical configurations. The energy well is very steep-sided in other directions and it is thus unlikely that other transformation paths will be followed. The low-energy transformation path is precisely that which a dislocation-induced conformational-change process would be expected to follow.

Ion Transport by Dislocation

The mechanism for ion transport being proposed suggests that dislocation processes such as those described above can produce conformational changes in the membrane-penetrating helical portions of

EXPLANATION OF PLATE I

Bulge formed in (3)-helix by a small region of (4)-helix bounded by an O-dislocation (⌞) and an H-dislocation (⌞)

EXPLANATION OF PLATE 2

Space formed between two (3)-helical polypeptide chains by short regions of (2)-helix
The space is formed by two H-dislocations (**I**) and two O-dislocations (**T**).

transport proteins and produce bulges that can accommodate ions. Dislocation glide then causes the bulges to propagate through the structure, transporting the ions across the membrane. In assemblies of (3)-helical proteins there are two ways in which such bulges can be formed, the first being to form a short region of (4)-helix to provide a space within the helix and the second being to form regions of (2)-helix to provide a space between chains.

Intramolecular transport

Construction of a space-filling model indicates that there is sufficient space inside a (4)-helix to fit a sphere of approx. 0.2 nm diameter and thus sufficient space to accommodate either a Na^+ or a Ca^{2+} ion (but not a K^+ ion, which is too large). For a (3)-helical protein with the *N*-terminal group on the inside of the membrane, a (4)-helix can be nucleated from the internal surface by propagation of an O-dislocation [propagation of an H-dislocation from the internal surface would nucleate a (2)-helix]. The O-dislocation would tend to have a small negative charge associated with it, which would exert an attractive force on a positive ion, which could then enter the space in the core of the (4)-helix. With respect to the lower-energy (3)-helix, the (4)-helix is unstable and would be expected to revert back to the original structure as soon as possible. This it can easily do as, after the ion has entered the helix, an H-dislocation propagated from the internal surface would convert the (4)-helix into a (3)-helix. The ion would thus be contained in a (4)-helix bulge in the normal (3)-helix, and glide of the two dislocations would cause the bulge to move across the membrane.

Plate 1 shows a skeletal model of a (3)-helix polypeptide containing a (4)-helical bulge bounded by an O-dislocation and an H-dislocation. To form a suitable-sized bulge to fit either a Na^+ or a Ca^{2+} ion, only three peptide units need to be in the (4)-helical configuration. Ion translocation by this means therefore requires only small, localized conformational changes, and the easy reversion of the structure back to the original leaves the protein available for further transport.

An ion channel operating in this manner is essentially a one-way channel. For an ion to be transported in the reverse direction intramolecularly, the initial dislocation from the external surface converting the (3)-helix into a (4)-helix would have to be an H-dislocation, which would tend to have a small positive charge and therefore make it less likely to transport cations. This mechanism could therefore explain the pumping of Na^+ out of the cell by $(\text{Na}^+, \text{K}^+)\text{-ATPase}$ and the pumping of Ca^{2+} into the sarcoplasmic reticulum by $\text{Ca}^{2+}\text{-ATPase}$, although in the latter case, for intramolecular transport, the *N*-terminal would have to be on the external surface of the membrane.

Intermolecular transport

In an assembly of two or more helices with the *N*-terminal groups on the internal surface, cations can be transported from the external surface in spaces between the helices to the cell's interior. To create an intermolecular space, the helix diameters would have to be decreased, and this can be done by nucleating O-dislocations on the external surface, which have the effect of converting the (3)-helices into (2)-helices. The small negative charges associated with such dislocations would attract cations such as K^+ into the intermolecular space, and these ions could be transported as the dislocations propagate. The structure can then easily revert back to the (3)-helix by propagation of following H-dislocations once an ion has entered the channel. Plate 2 is a skeletal model showing an intramolecular space formed between two helices by short regions of (2)-helix. Sufficient intermolecular space to accommodate a K^+ ion can be formed if each chain has only three peptide units in the higher-energy (2)-helix conformation.

The intermolecular transport mechanism is, in a way, more complex than intramolecular transport, since, at some instants, one or more of the dislocations involved will be at a position in the helix remote from the ion being transported. However, the space between helices could be maintained by the presence of the ion itself and also by any pinning effect of the surrounding membrane. In addition, the presence of dislocations in cylindrical crystals produces kinks (Harris & Scriven, 1973), which could tend to keep the ion channel open.

Energy Estimates

In the light of present knowledge of the structure of ion-pump proteins, it is not possible to make estimates of the energy requirements of the present model in detail. However, from conformational-energy maps such as that of Fig. 3, it is possible to obtain a rough estimate of the energy required to maintain the necessary non-equilibrium bulges in the helix. Most conformational-energy maps, including that in Fig. 3, consider the energies of dipeptide units or polypeptides in isolation and do not consider any effects caused by the surrounding media. Consequently such estimates give rather large values for the energy of the dislocation process compared with those obtained experimentally. For a membrane protein, the effects of the surrounding aqueous medium, the lipid membrane and the included ion would be expected considerably to influence the energy requirements. No energy estimates have been made accounting for all the relevant factors, but estimates have been presented for the conformational energies of dipeptides in water (Hopfinger, 1973), and these were used to estimate the bulge energy without accounting for membrane or ion effects.

For the three helical forms of interest, the approximate energies are: (4)-helix, -25.5 kJ/mol per residue; (3)-helix, -27.2 kJ/mol per residue; (2)-helix, -26.0 kJ/mol per residue. One (4)-helix bulge of sufficient size to accommodate an Na^+ or Ca^{2+} ion and having three peptide units in the (4)-helix conformation will thus have an energy approx. 5.1 kJ/mol greater than that of the (3)-helix. Similarly, a channel formed between two helices consisting of three (2)-helical residues per chain will have an energy approx. 7.2 kJ/mol greater than the (3)-helix.

For $(\text{Na}^+, \text{K}^+)\text{-ATPase}$, the energy involved in forming the channels to transport three Na^+ ions out of and two K^+ ions into the cell is approx. 29.7 kJ/mol which compared favourably with the experimentally determined value of 38 kJ/mol (Whittam & Ager, 1965).

The above energy estimates can only be regarded as rough order-of-magnitude indications, because factors such as membrane effects, the energy required to bind the ions initially, the energy to initiate dislocation movement etc. are not accounted for. However, they do indicate that the proposed dislocation translocation mechanism may be feasible from an energetic point of view.

Discussion

The present model for ion translocation is intended only to suggest a mechanism whereby ions can be transported through the membrane-penetrating regions of pump proteins and does not attempt to explain a mechanism for many other important features of such pumps, such as how the ions or ATP are bound, ion selectivity or how the initial dislocations are propagated. It is likely that these features will not be fully explained until more information about the precise structure of such proteins becomes available. The present model is, however, consistent with available data and, in particular, can explain how it is possible to translocate ions in normally closed channels with only small, localized conformational changes in the proteins. The possible role played by such defects is also of interest for other reasons. Firstly, such defects are by no means unique in Nature, and many fundamental properties of a wide variety of materials, both inorganic and organic, may be ascribed to some form of structural irregularity. Secondly, defect-induced conformational changes in proteins may be a quite general method whereby conformational changes may be induced, and may therefore be a universal mechanism that could be used to explain many other physical and chemical properties of proteins, including conformational changes occurring during protein folding and denaturation.

Although no direct experimental evidence is available that defect-induced conformational-change processes occur in proteins, it is clear from protein

structure that the defects do exist. For example, many amino acid residues can affect the hydrogen-bond topology. Proline residues covalently bond back on to the main chain and consequently disrupt the hydrogen bonding, producing edge dislocations. Similarly some of the polar amino acid residues can form hydrogen-bonds with the main chain and thereby form dislocations. Such residues are generally referred to as helix-breaking residues (Anfinsen & Scheraga, 1975), but they could equally well be classified as dislocation-forming residues.

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