Structural Studies of Sodium and Potassium Ion-activated Adenosine Triphosphatase

THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND THE MECHANISM OF ACTIVE TRANSPORT*

(Received for publication, February 20, 1975)

JACK KYTE

From the Department of Chemistry, University of California at San Diego, La Jolla, California 92037

Sodium and potassium ion-activated adenosine triphosphatase is the enzyme responsible for the active transport of sodium and potassium across the plasma membrane. Strophanthinidin, from the external surface of the membrane, and an antibody, from the cytoplasmic surface, bind simultaneously to the large polypeptide subunit of the enzyme. These results demonstrate that this polypeptide chain must span the plasma membrane, having different surfaces exposed on each side.

When (Na\(^+\) + K\(^+\))-ATPase is incubated in the presence of cupric phenanthroline, a reagent which catalyzes the oxidation of cysteine residues to form intermolecular and intramolecular disulfide bonds, a covalent dimer of the larger chains is formed. Several characteristics of this dimerization reaction are consistent with the proposal that at least a noncovalent dimer of large chains exists in the native enzyme.

These conclusions are discussed in the context of a specific description for the molecular mechanism of active transport.

---

EXPERIMENTAL PROCEDURE

Materials

Zonal gradient microsomes are membrane vesicles which are purified from a homogenate of canine renal medulla by zonal gradient fractionation (11). They contain a high concentration of (Na\(^+\) + K\(^+\))-ATPase. Their preparation, chemical composition, and appearance have been previously described (1). Antibodies against a purified preparation of the large chain were raised in rabbits. Antigen preparation, injection, and purification of \(\gamma\)-globulins have been described (1). \(\gamma\)-Globulins from these sera are refered to as anti-large-chain \(\gamma\)-globulin, and they are from the same serum pool that was used in previous work (1). Large chain of (Na\(^+\) + K\(^+\))-ATPase was prepared from salt detergent-extracted microsomes (2) by sodium dodecyl sulfate gel filtration followed by Dowex treatment to remove the detergent (6). The method of Weber and Kuter (12) was slightly modified. Washed Dowex 50-X2 (Bio-Rad) was mixed with 6 M urea, 4 mg of bovine serum albumin/ml of resin, 0.05 M Tris acetate, pH 7.8, and then extensively washed with 6 M urea, 0.06 M Tris acetate, pH 7.8. This equilibrated resin was then immediately mixed with the sodium...
dodecyl sulfate solution of the large chain brought to 6 M in urea. After
10 min of gentle mixing, resin was removed in a disposable syringe,
and heating was followed by air and compression of the plunger.

Medium A, in which either enzyme assay or complement fixation
could be performed, had the composition 10 mM KCl, 130 mM NaCl, 2
mM MgCl2, 1 mM Na2ATP, 0.15 mM CaCl2, 10 mM Tris chloride, pH
7.4. Protein standards for sodium dodecyl sulfate gel electrophoresis
were obtained from the following sources: bovine serum albumin, Miles
Laboratories Inc.; phosphorylase α, Sigma Chemical Co.; Escherichia
coli β-galactosidase, Dr. Irving Zabin, University of California, Los
Angeles; E. coli RNA polymerase, Dr. Robert Dickson, University of
California, San Diego. Strophanthidin and 1,10 phenanthroline were
obtained from Sigma Chemical Co.

Methods

(NA+ + K +)-ATPase assays were performed in the usual manner (2).
Complement fixation was performed as described by Levine and Van
Vunakis (13).

Gel Electrophoresis—Sodium dodecyl sulfate gel electrophoresis
was performed either by the modifications of Fairbanks et al. (14) or
Weber and Osborn (15) of the original procedure of Shapiro et al. (16).
Gels were stained with Coomassie brilliant blue (15), and scanned on a
Gilford recording spectrophotometer with a linear transport chamber.
In order to determine precise mobilities and construct standard curves,
the gels were scanned at 280 nm immediately following removal from
the tubes. In this way, the relative positions of at least one of the
polypeptides and the marker dye could be accurately determined. The
gels were then stained and scanned at 550 nm to locate minor
components such as oligomers. The position of the major polyepitope
on the stained gel could then be used to calculate the absolute
mobilities of all species with respect to the marker dye. Mobilities with
a reproducibility 2% could be routinely obtained.

Cross-linking with Cupric Phenanthroline—The conditions of Steck
(17) were employed to catalyze the cross-linking of the large chains of
(NA+ + K +)-ATPase. Zonal gradient microsomes were dialyzed into 5
mM sodium phosphate, pH 8.0. In most of the experiments, the
incubation was performed at room temperature under air. In order to
follow the kinetics of the cross-linking reaction, however, conditions
were more carefully controlled. The membranes were added to a
reaction vessel, thermostated at 25°, and equilibrated by stirring under
an atmosphere of O2. The reaction was initiated by adding a solution
of 0.6 mM CuSO4, 3 mM 1,10 phenanthroline, 5 mM sodium phosphate,
and 8.0, at 25°. The final protein concentration was 50 to 150 µg/ml.
Zero time samples were immediately withdrawn, and the reaction
vessel flushed with O2 and sealed. At various times, samples were
withdrawn, 0.2 volumes of a 10 mM solution of N-ethylmaleimide was
added, and 3 min later, 0.1 volume of 20% sodium dodecyl sulfate. The
samples were prepared for gel electrophoresis without reduction and
heating. After electrophoresis, the gels were stained with Coomassie
brilliant blue, destained, and scanned. Relative areas of absorbance
were determined by multiplying the height of the large chain peak by
its width at half-height, and dividing by the area at t = 0.

RESULTS

Specificity of the Anti-Large-Chain Antibody—Evidence
has already been presented which demonstrates that the
anti-large-chain antibody is specific for an antigenic site on the
large chain of (NA+ + K +)-ATPase (1). Additional evidence
has been obtained. Large chain was purified to homogeneity by
sodium dodecyl sulfate gel filtration (6), and a scan of a sodium
dodecyl sulfate gel of this preparation is shown in Fig. 1. The
sodium dodecyl sulfate was removed from this protein with
Dowex (12), and it was dialyzed into complement fixation
isodiluent (13). The ability of this large chain to compete with
zonal gradient microsomes for anti-large-chain antibody was
assessed by complement fixation. When increasing amounts of
anti-large-chain γ-globulin are reacted with a constant quan-
tity of zonal gradient microsomes, the per cent complement
fixation increases as antigen-antibody complexes are formed (Fig.
2). If, however, the purified large chain is preincubated with
the anti-large-chain γ-globulin prior to the assay, much more
antibody is required to obtain the same level of complement
fixation (Fig. 2). In other words, a certain fraction of the active
antibody is eliminated due to reaction with the pure large

![Figure 1](http://www.jbc.org/)

**FIG. 1 (left).** Scan of a sodium dodecyl sulfate gel of the large chain of
(NA+ + K +)-ATPase purified by gel filtration in sodium dodecyl
sulfate. Sample was taken from the pool of fractions from a gel
filtration column. Absorbance at 550 nm is recorded as a function of
distance migrated. The direction of electrophoresis was from left to
right. The arrows mark the top of gel and the position of the tracking
dye. The gel was 5.6% in acrylamide, prepared by the method of
Fairbanks et al. (14).

**FIG. 2 (center).** Complement fixation assay of anti-large-chain antibody
against zonal gradient microsomes. The concentration of membranes was
held constant at 90 ng of protein/ml and the amount of anti-large-chain γ-globulin varied as noted in a final volume of 1.2 ml.

It was then assayed, in the usual manner (13), against the zonal
gradient microsomes.

![Figure 2](http://www.jbc.org/)

**FIG. 3 (right).** Adsorption of anti-large-chain antibody by purified
large chain of (NA+ + K +)-ATPase. Percentage of the antibody which
did not bind to zonal gradient microsomes as a result of a preincuba-
tion with the large chain is displayed as a function of the µg of large
chain/µg of γ-globulin in the preincubation mixture. Per cent antibody
loss was calculated, from the data in Fig. 2, by dividing the micrograms
of antibody required to give a certain per cent complement fixed in the
control by the micrograms of antibody required to give the same level
of per cent complement fixed after adsorption and multiplying by 100.
The values for five evenly spaced levels on Fig. 2 were averaged to
obtain the values presented in Fig. 3.
chain. This fraction can be calculated from the curves in Fig. 2, and is presented as a function of the amount of pure large chain preincubated with the anti-large-chain \( \gamma \)-globulin (Fig. 3). It can be seen that greater than 80% of the antibody which reacts with the zonal gradient microsomes can be eliminated by preincubation with purified large chain. These results demonstrate that the antibody bound by native \((Na^+ + K^+)\)-ATPase is directed against an antigenic site on the large chain of the enzyme.

The data displayed in Fig. 2 were obtained with concentrations of \( \gamma \)-globulin and purified large chain less than 6 and 3 \( \mu \)g/ml, respectively. Purified, water-soluble large chain was assayed by complement fixation as an antigen against anti-large-chain \( \gamma \)-globulin (4 \( \mu \)g/ml) between the concentrations of 2.5 \( \mu \)g/ml and 10 ng/ml. In all tubes, less than 10% of the complement was fixed, even though this is the range in which the purified large chain is adsorbing up to 80% of the antibody present, even though this is the range in which the purified large chain is adsorbing up to 80% of the antibody. This is what allows adsorption to be measured by complement fixation. One of several explanations for this behavior is that the purified, water-soluble large chain contains only one antigenic site and is, therefore, not a precipitating antigen. It has already been reported that, at saturation, there is only 0.6 \( \gamma \)-globulin molecule bound to each available large chain in the native enzyme (1), which is consistent with this explanation.

**Simultaneous Binding of Strophanthidin and Anti-Large-Chain Antibody**—The binding of anti-large-chain antibody is assayed by complement fixation, while the binding of strophanthidin is determined kinetically in the presence of the enzyme substrates. The concentrations of substrates present determine whether strophanthidin will bind to the enzyme (18). Therefore, both the complement fixation assay and the ATPase kinetic assay were performed in the same medium (Medium A) so that a direct comparison of results from each procedure could be made. It can be seen (Figs. 4 and 5) that both complement fixation and enzyme turnover occur in this solution.

![Diagram](http://www.jbc.org/)

**Fig. 4 (left).** Complement fixation assay of \((Na^+ + K^+)\)-ATPase saturated with strophanthidin. Zonal gradient microsomes were assayed as an antigen against anti-large-chain \( \gamma \)-globulin (4 \( \mu \)g/ml). The assay was performed in Medium A either in the presence (\( O--O \)) or absence (\( \Delta--\Delta \)) of \( 8 \times 10^{-4} \) \( M \) strophanthidin, a concentration 20 times greater than the apparent \( K \) for this ligand under these conditions (Fig. 5).

**Fig. 5 (right).** Dixon plot for the binding of strophanthidin to \((Na^+ + K^+)\)-ATPase saturated with anti-large-chain antibody. \((Na^+ + K^+)\)-ATPase activity was assayed by following the hydrolysis of the MgATP in Medium A at various strophanthidin concentrations. Anti-large-chain antibody (1.5 eq) was present (\( \Delta--\Delta \)) or absent (\( O--O \)) during the assay.

When anti-large-chain \( \gamma \)-globulin is assayed against zonal gradient microsomes by complement fixation in Medium A, the addition of strophanthidin at a concentration of \( 8 \times 10^{-4} \) \( M \) (20 times \( K \)) does not affect the reaction between antibody and antigen (Fig. 4). When the apparent dissociation constant of strophanthidin is determined kinetically (18), using Medium A as the assay mixture, the addition of 1.5 equivalents of anti-large-chain antibody had no effect (Fig. 5). The \( V _{\text{max}} \) of the enzyme in the presence of antibody was 1.1 \( \pm \) 0.1 times the \( V _{\text{max}} \) in its absence. It has already been demonstrated that anti-large-chain antibody does not alter the turnover rate of the enzyme (1).

Since the presence of neither strophanthidin nor anti-large-chain antibody at greater than saturating concentrations alters the binding of the other, both must bind simultaneously and independently to the enzyme.

**Cross-linking of \((Na^+ + K^+)\)-ATPase with Cupric Phenanthroline**—Zonal gradient microsomes (80 to 190 \( \mu \)g/ml, final concentration) were incubated at 25\(^\circ\) in a buffer containing 0.25 \( M \) CuSO\(_4\), 1.3 \( M \) I,10 phenanthroline, 5 \( mM \) sodium phosphate, pH 8.0 (17). Samples were withdrawn, 0.2 volume of 10 \( mM \) N-ethylmaleimide was added to prevent further oxidation and disulfide interchange, and, 3 min later, 0.1 volume of 20% sodium dodecyl sulfate was used to denature the protein. These samples were then submitted to sodium dodecyl sulfate gel electrophoresis. It can be seen in Fig. 6 that the large chain of \((Na^+ + K^+)\)-ATPase (Fig. 6, d) gradually disappears while a new protein of higher molecular weight (Fig. 6, c) appears. The other polypeptides present in the sample are unaffected by the incubation with cross-linking agents.

The higher molecular weight protein (Component c), a product of the reaction catalyzed by cupric phenanthroline, is a covalent dimer of two large chains. The mobilities of several standard proteins were determined under the same conditions of sample preparation and electrophoresis. From these results, the standard curve shown in Fig. 7 was constructed. The mobility of the dimers and trimers of bovine serum albumin and muscle phosphorylase were included since the protein in question is also a covalently bonded oligomer. It can be seen that the mobilities of the standard proteins (15, 19, 20) fall on two lines, perhaps because of differences in the number of intramolecular disulfide bonds. In any case, the mobility of the cross-linked product (Component c) corresponds to an apparent molecular weight twice that of the apparent molecular weight of the monomeric large chain, regardless of which curve is used in the calculation. One of the cupric phenanthroline reaction mixtures of zonal gradient microsomes was denatured and submitted to sodium dodecyl sulfate gel electrophoresis. The region of the gel in which the covalent dimer is located was sliced out, and 25 \( \mu \)l of 0.2 \( mM \) dithiothreitol were added (17). The slice stood at 25\(^\circ\) for 16 hours under \( N_{\text{2}} \), was placed on top of a fresh gel, and the reduced protein was rerun. This gel was stained and scanned (Fig. 8). It can be seen that Component c, the high molecular weight product of the cross-linking reaction, can be cleaved by reduction to yield only large chain monomer. Notice that a small amount of the dimer remains.

When \((Na^+ + K^+)\)-ATPase, still within the native membrane, is incubated with cupric phenanthroline, a covalent dimer, formed from two large chains, is produced. No protein appears on the gel in the regions where a covalent tetramer or trimer is expected to run (Fig. 6, a and b, respectively). The amount of large chain lost is always greater than the amount of dimer present, and material accumulates at the top of the gel.
FIG. 6. Scans of sodium dodecyl sulfate gels of (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase cross-linked in the presence of cupric phenanthroline. Zonal gradient microsomes and cross-linking reagents were mixed so that the final concentration was 90 \mu g/ml of protein. Forty-microgram samples were removed, denatured, and run on 3.25% polyacrylamide gels prepared by the method of Fairbanks et al. (14). Gel A is of a sample taken immediately. Gel B is of a sample incubated for 15 min at 25\textdegree C under air. Gel C is of a sample incubated for 15 min at 25\textdegree C under \textsuperscript{18}O. The \textsuperscript{18}O hastens the cross-linking reaction. Absorbance at 550 nm is displayed as a function of distance migrated, and a, b, c, and d mark the predicted positions of tetramer, trimer, dimer, and monomer of the large chain, respectively. Direction of electrophoresis is from left to right; arrows mark top of the gel and position of the tracking dye.

These observations suggest that the dimer is an intermediate in a reaction which leads subsequently to highly polymerized protein.

The cross-linking reaction catalyzed by cupric phenanthroline could occur by one of two processes. The large chain of the enzyme could exist as a monomer in the native membrane, and covalent dimerization would be due to the collision of two molecules of the enzyme as a result of translational diffusion. On the other hand, a noncovalent dimer of the large chains could be a part of the native enzyme complex and collision would not be required for cross-linking to occur. The large chain disappears in a process which is kinetically first order (Fig. 9). This result is consistent with the prior existence of a noncovalent dimer. It is also consistent with a mechanism requiring collision, provided that the collision follows the rate-limiting step in the reaction.

DISCUSSION

It has been proposed that (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase, in order to catalyze the active transport of sodium and potassium, spans the plasma membrane and forms a channel across it through which the cations pass, in and out of the cell (1, 9, 10, 21). This pathway for the ions lies through the center of the enzyme molecule, is formed as a result of the juxtaposition of two of the subunit polypeptides of the enzyme, and is located in the space between them, much like the channel which runs along the b, 2-fold axis in the hemoglobin molecule (22). The enzyme alternates between two conformational states during turnover which differ from each other only as the result of minor structural alterations in the geometry of the channel. In one conformation, a sodium-specific site, which can be approached only from the inside of the cell, is found in the center of the channel. In the other conformation, the specificity and orientation of this cation binding site have been altered so that it is specific for potassium and can only be approached from the environment. During the conformational changes, each cation remains on the site, subsequently finds itself bound to an unfavorable location, and departs into the medium on the side of the plasma membrane opposite to the one from which it originally came. The transition from the first conformational state to the second occurs only when sodium ions are occupying the cation site and MgATP is bound to the hydrolytic site. Whether phosphorylation of the protein precedes or follows this transition is undecided (23). The transition from the second conformational state to the first occurs only when potassium ions are occupying the cation site, and is accompanied by dephosphorylation of the enzyme. In this manner, the hydrolysis of MgATP is obligatorily coupled to the movement of the cations.

It has been shown that the large chain and small chain of (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase are associated in a specific complex. They can be covalently cross-linked under conditions where no other cross-linked products are observed (6). The enzyme, when purified from several different species (2-5), always contains both large and small chains. Both the phosphorylated interme...
which a reduced sample of the large chain dimer has been run. The weight for sodium dodecyl sulfate gels, 5% in acrylamide, prepared by volume of 20% sodium dodecyl sulfate. Mobilities were determined as described in the text. Standards were: 1, phosphorylase trimer; 2, phosphorylase dimer; 3, bovine serum albumin trimer; 4 and 5, $\beta$-RNA polymerase; 6, $\beta$-glactosidase; 7, bovine serum albumin dimer; 8, phosphorylase; 9, $\alpha$-RNA polymerase; 10, bovine serum albumin (15, 19, 20). The mobility of the large chain monomer is designated as $\alpha_1$; that of the large chain dimer, as $\alpha_2$.

Fig. 8 (center). Scan of a sodium dodecyl sulfate gel, 5% in acrylamide, prepared by the method of Weber and Osborn (15), on which a reduced sample of the large chain dimer has been run. The large chain dimer was cut out of a sodium dodecyl sulfate gel of cross-linked zonal gradient microsomes, dithiothreitol was added to the slice, and it stood under N$_2$ overnight. That slice and its liquid were diate (3, 7) and the cardiac glycoside site (8), however, are on the large chain of the enzyme. Calcium ion-activated adenine triphosphatase, an enzyme very similar to (Na$^+$ + K$^+$)-ATPase, contains only large chains in the functional unit (24). The significance of the small chain, which is definitely a stoichiometric component of the enzyme complex, to the function of active transport is, therefore, not clear. It can, however, be demonstrated that the large chain of (Na$^+$ + K$^+$)-ATPase, by itself, possesses many of the structural features described in the above proposal. If that description of the mechanism of (Na$^+$ + K$^+$)-ATPase is accurate, then several predictions can be made. Many of these predictions have been experimentally demonstrated.

1. (Na$^+$ + K$^+$)-ATPase is a protein which spans the plasma membrane, having surfaces exposed to both the cytoplasm and the environment simultaneously. Strophanthinidin, the aglycone of several cardiac glycosides, binds to (Na$^+$ + K$^+$)-ATPase only on the external surface of the plasma membrane (25). At least a portion of the cardiac glycoside site is composed of amino acids in the large chain of the enzyme (8). The antibody prepared against the large chain binds specifically to an antigen site, on the large chain of the enzyme (Fig. 2), which is located on the cytoplasmic surface of the plasma membrane (1). Both anti-large-chain antibody, at the cytoplasmic surface of the membrane, and strophanthinidin, at the external surface, can bind simultaneously and independently to the large chain of (Na$^+$ + K$^+$)-ATPase (Figs. 4 and 5). It follows that the large chain of the enzyme must span the membrane, having unique surfaces exposed to each side of the membrane simultaneously. It has been pointed out (6) that if the large chain were folded as a sphere, its diameter would be 75 A, large enough to span the bilayer.

2. (Na$^+$ + K$^+$)-ATPase does not rotate at any significant rate about an axis parallel to the plane of the membrane. It has been shown that the activity of the enzyme is unchanged when a molecule of anti-large-chain antibody is bound to the large chain (1). Such a ligand would certainly hinder a transmembrane rotation of the large chain. Thermodynamic arguments have also been presented which suggest that the rates of rotation of integral proteins, about an axis parallel to the plane of the membrane, must be exceedingly slow (26). Experimental observations have been uniformly consistent with this prediction (28-30). The latter two classes. It has been shown, however, that the vesicles to which the antibody binds are actually more active than those to which it does not (1). Furthermore, all active enzyme molecules bind strophanthinidin since there is almost no ATPase activity in its presence. For these reasons, it is clear that the enzyme molecules which bind antibody also bind strophanthinidin. Those active enzyme molecules, however, which are located in the right side out, sealed vesicles, do not bind antibody but contribute to the kinetic experiment displayed in Fig. 5. Nevertheless, at least 75% of the active enzyme molecules in this experiment bind strophanthinidin as avidly in the presence of antibody as in its absence.

*It has recently been shown that the purple membrane protein, which is isolated from Halobacterium halobium and which is responsible for the active transport of protons out of this cell, does not diffuse through or rotate across the membrane during a transport cycle (31).
3. (Na\(^+\) + K\(^+\))-ATPase is oriented in the membrane of the cell so that each enzyme molecule has a unique outside surface and a unique inside surface, and every enzyme molecule has the same orientation at all times. Anti-large-chain antibody binds only to the cytoplasmic side of membrane vesicles containing (Na\(^+\) + K\(^+\))-ATPase (1). Therefore, the antigenic sites on all large chains must be on the same side of the membrane. This structural asymmetry results in the functional asymmetry that the enzyme displays. Sodium acts as a substrate only from the inside of the cell (32, 33); potassium, only from the outside (32, 33); MgATP, only from the inside (32, 34); and strophanthidin, as an inhibitor, only from the outside (25). This structural asymmetry is possible only because rotation of the large chain about an axis parallel to the membrane does not occur.

4. (Na\(^+\) + K\(^+\))-ATPase, in its native, membrane-bound state, is a subunit aggregate, which contains at least a dimer of the large chain polypeptide. When zonal gradient microsomes, which are membrane vesicles directly isolated from a homogenate of renal medulla (1, 11), are incubated in the presence of cupric phenanthroline, it is found that the large chain of (Na\(^+\) + K\(^+\))-ATPase is preferentially polymerized by a reaction in which the only identifiable intermediate is a covalent \(\alpha_2\) dimer. The first order kinetics of the process (Fig. 9) is consistent with the existence of a noncovalent dimer of large chains as a component of the native enzyme. If the cross-linking were the result of collision of monomers in a random fashion, one would have expected other polypeptides to disappear at approximately the same rate, as they were cross-linked to themselves or to the large chain, the predominant polypeptide. This, however, does not occur. When (Na\(^+\) + K\(^+\))-ATPase is examined by other methods of cross-linking, such as dimethylsuberimidate (6), glutaraldehyde, or light (8), the only covalent product observed is the covalent \(\alpha_2\) dimer of one large chain and one small chain. If the cross-linking of the large chain to form a covalent \(\alpha_2\) dimer, which occurs when the enzyme is incubated with cupric phenanthroline, were a random event, an \(\alpha_2\) dimer should also form during these other cross-linking reactions. If, however, the large chain exists in the native enzyme as a dimer, it is not surprising to observe different behavior with different reagents, since cross-linking within a molecular complex depends upon the proper orientation of the amino acid side chains with which the reagent reacts.

It has been observed that, although there is one cardiac glycoside binding site on each large chain in the purified enzyme, only 40% of the large chains can be phosphorylated under optimal conditions (18). This half-the-sites reactivity would require that the large chains be present in the native complex as at least a dimer.

5. (Na\(^+\) + K\(^+\))-ATPase can exist in two distinct conformational states. Although the exact sequence of events is still in doubt (22), it is certain that the enzyme can exist in a phosphorylated and an unphosphorylated form. Phosphorylation requires binding of sodium ion, and dephosphorylation requires the binding of potassium ion (37). The enzyme passes consecutively through at least these two kinetic states during turnover (38). Several lines of evidence demonstrate that the conformation of the enzyme changes when it is phosphorylated. The phosphorylated form binds cardiac glycosides about 100 times more tightly than the unphosphorylated form (18). The binding site for these ligands is on the opposite side of the membrane from the active site (25, 32, 34). Information must be transferred between these sites via a conformational change in the protein. There are sulfhydryl groups on the enzyme which react with N-ethylmaleimide more extensively when the enzyme is phosphorylated than when it is dephosphorylated (39). Since this alteration in the reactivity of the enzyme probably occurs at a location removed from the active site, it could be the result of an over-all conformational change upon phosphorylation.

6. (Na\(^+\) + K\(^+\))-ATPase must have a kinetic mechanism which is ping-pong with respect to the alkali cation substrates. Although it was pointed out above that each cation is necessary for one of the conformational transitions, the model proposed further requires that only one type of cation binds to the enzyme at any time. Cleland (40) has demonstrated that product inhibition experiments can be used to determine the order of product release from an enzyme. The competitive product inhibition which each cation displays with respect to the other cation acting as a substrate (41-43) requires that each cation leave the enzyme as a product before the other can bind as a substrate.

This discussion has presented several pieces of evidence which are consistent with the mechanism of active transport described above. It is proposed as a working hypothesis which will suggest further experiments.

Acknowledgments—I would like to thank Dr. S. J. Singer and Patricia Boggus. I would like to thank Dr. Theodore Steck and Dr. Kuan Wong for encouraging me to use cupric phenanthroline as a cross-linking agent.

REFERENCES

Structural studies of sodium and potassium ion-activated adenosine triphosphatase. The relationship between molecular structure and the mechanism of active transport.

J Kyte


Access the most updated version of this article at http://www.jbc.org/content/250/18/7443

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/18/7443.full.html#ref-list-1