Biosynthesis of the Na₉K-ATPase in Madin-Darby Canine Kidney Cells

ACTIVATION AND CELL SURFACE DELIVERY*

(Received for publication, September 20, 1989)

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Madin-Darby canine kidney cells were used to study events in the postsynthetic processing and cell surface delivery of Na₉K-ATPase. The photoactivatable 2-nitro-5-azidobenzoyl (NAB) derivative of ouabain and an anti-ouabain antibody were employed in experiments designed to determine the time intervals required for newly synthesized Na₉K-ATPase to achieve the capacity to bind ouabain and to arrive at the cell surface. Ouabain-binding capacity was assessed in Madin Darby canine kidney cells which were pulse-labeled with [³⁵S]methionine. At various chase intervals cells were disrupted by probe sonication and the resultant vesicles were permeabilized. Vesicles were incubated with NAB-ouabain and, following UV photolysis, solubilized and subjected to immunoprecipitation with an anti-ouabain antibody. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of immunoprecipitates revealed that newly synthesized Na₉K-ATPase can carry out type II (Mg²⁺ and P₅ supported) ouabain binding throughout the course of its postsynthetic processing. In contrast, the ability to carry out type I (Na⁺, Mg²⁺, and ATP-supported) ouabain binding is not attained until 10 min after the completion of the sodium pump's synthesis. Experiments in which intact pulse-labeled cells were incubated with NAB-ouabain revealed that the Na₉K-ATPase arrives at the cell surface as soon as 50 min after its synthesis. These results suggest that postsynthetic processing is required before the newly synthesized Na₉K-ATPase can display its full repertoire of catalytic functions. This processing seems to be complete prior to the newly synthesized sodium pump's arrival at the cell surface.

* This work was supported in part by National Institutes of Health Grants GM-21714 and GM-07205 and by a Helen Hay Whitney fellowship (to M. J. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The Na₉K-ATPase (or sodium pump) of animal cell plasma membranes plays a critical role in maintaining the transmembrane ion gradients required for cellular homeostasis, electrical excitability, and volume control (1). The primary structure of its constituent polypeptides has recently been determined (for review see Ref. 2). The α, or catalytic, subunit is not glycosylated and spans the bilayer with at least three loops. It possesses an extracellular binding site for its high affinity inhibitor ouabain and an intracellular binding site for ATP, which involves an aspartic acid residue to which the γ-phosphate of the nucleotide is covalently transferred upon enzymatic ATP hydrolysis. The β-subunit is heavily glycosylated and crosses the bilayer at least once. Its relationship to the pump's activity remains unclear.

Recent reports have established that the insertion of both the α- and β-subunits into the membrane of the rough endoplasmic reticulum (RER) is cotranslational as might be expected for transmembrane plasmalemmal proteins (3-5). Assembly of newly synthesized subunits to form the αβ heterodimer appears to be an early posttranslational event that occurs almost certainly in the RER (6). The kinetics and subcellular location of other significant postsynthetic steps are not yet defined. It is not known whether newly synthesized Na₉K-ATPase is capable of performing its catalytic functions ab initio, or only after further processing. This question is significant beyond its immediate relevance to issues of protein processing, since the mechanisms that activate newly synthesized Na₉K-ATPase may be similar to those involved in the regulation of the response of the enzyme to alterations of the intra- or extracellular environment. The site of its functional maturation may further suggest which, if any, subcellular compartment could support transmembrane ion gradients and membrane potentials.

Another issue in need of clarification concerns the time required for newly synthesized Na₉K-ATPase to complete its transit to the cell surface. Previously published reports have measured this interval at between 45 min and 4 h (6, 8-10). Since in many cell types most plasmalemmal proteins reach their final destination within 45 min, a slower delivery time for Na₉K-ATPase might raise questions as to the intracellular pathway it pursues.

Using the N-azidobenzoyl (NAB) derivative of ouabain (9, 10) and an antibody directed against ouabain (kindly provided by D. Louvard and B. Rossi, Institut Pasteur, Paris, France), we have developed an assay that allows us to examine several of the issues discussed above. Our experiments were performed on MDCK cells, a polarized epithelial line rich in Na₉K-ATPase. We find that newly synthesized Na₉K-ATPase is only partially active when first synthesized, but appears to become capable of at least some of its characteristic functions within 10 min of synthesis. Delivery to the cell surface occurs with a lag of 45-50 min, which is in good agreement with recently published data gathered from cultured chick neurons (6). We conclude that Na₉K-ATPase undergoes a modification relatively early in its postsynthetic processing that initiates at least some of its catalytic function and that this event

1 The abbreviations used are: RER, rough endoplasmic reticulum; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NAB, N-azide benzoyl; MDCK, Madin-Darby canine kidney cells; BSA, bovine serum albumin; PBS, phosphate-buffered saline.
precedes the arrival of the enzyme at the cell surface. The nature of this modification remains to be elucidated.

**MATERIALS AND METHODS**

**Preparation of Polyclonal Antibody**—Canine kidney Na,K-ATPase was purified according to the Jorgensen method (11). Preparations with specific activities (i.e., 2.9 × 10⁶ units/mg protein), which were injected intradermally into New Zealand White rabbits according to standard immunization procedures, were tested for immunoreactivity to purified Na,K-ATPase by enzyme-linked immunosorbent assays. Boosting and bleeding was carried out at 2-week intervals. The sera were employed in these studies was collected 2 weeks after the third booster injection, and was stored frozen at −20 °C in 0.1 ml aliquots until used.

**Immuno precipitation from Metabolically Labeled MDCK Cells**—MDCK cells (type II, gift of A. Helenius, Yale University) were grown, labeled with [35S]methionine, disrupted by brief sonication, and used to prepare a crude membrane fraction as previously described (20). Membranes obtained from 10⁶ cells were solubilized in 0.5 ml of buffer A (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1% Nonidet P-40). Immunoprecipitation using the anti Na,K-ATPase rabbit serum diluted 1:250 followed by protein A-agarose beads, was carried out as in a previous study (5). Immunoprecipitates were analysed by SDS-PAGE followed by fluorography of the dried gels impregnated with Autorofluor (Autonuclear Diagnostics). Labeling with [3H]NAB-ouabain and immunoprecipitation with the anti-ouabain antibody were as previously described (12).

**Autoradiography of Concanavalin A**—Filtered suspensions were centrifuged at 1.5 × 10⁵ for 140 min at 4 °C in a solution of 1% bovine serum albumin (BSA) (Sigma) in phosphate-buffered saline (PBS), and then blotted for 60 min at 4 °C in PBS-BSA containing 1:400 dilution of either anti-Na,K-ATPase serum or preimmune rabbit serum diluted 1:250 followed by Protein A-agarose beads. The blots were then washed with 5 ml of the same buffer and dissolved in Econofluor (Schuell). The transfers were quenched for 2 h at 4 °C in a solution of 5% H₃PO₄, 1% BSA (for standard immunization procedures. The sera were tested for immunoreactivity to purified Na,K-ATPase by enzyme-linked immunosorbent assays. Boosting and bleeding was carried out at 2-week intervals. The sera were employed in this study (12). Immunoprecipitates were analysed by SDS-PAGE followed by fluorography of the dried gels impregnated with Autorofluor (Autonuclear Diagnostics). Labeling with [3H]NAB-ouabain and immunoprecipitation with the anti-ouabain antibody were as previously described (12).

**RESULTS**

We wished to determine when newly synthesized Na,K-ATPase first becomes competent to split ATP. During the course of its normal active cycling, the Na,K-ATPase passes through a number of biochemically distinguishable conformational states. In the widely accepted Post-Albers model (13, 14) of sodium pump catalysis, at least two of these conformations, E₃P and E₂P, are recognized and immunoprecipitated by antibodies directed against ouabain-binding conformation (i.e., buffer C (4 ml) containing 1 mM NaCl, 4 mM MgCl₂, 2 mM EDTA, 10 mM NaP₄, pH 7.25) containing 1 μM NAB-ouabain. Following a 5-min incubation at 37 °C in the dark, the membranes in each sample were pelleted by centrifugation at 1.5 × 10⁵ for 140 min at 4 °C and the resulting pellets were resuspended by brief sonication either in 50 μl of buffer A with 1% SDS or 50 μl of buffer A with 1% Nonidet P-40. Solubilization and immunoprecipitation with the anti-ouabain and anti-α-subunit antibodies proceeded as in a previous study (12). Immunoprecipitates were analyzed by SDS-PAGE followed by fluorography. Quantitation by densitometric scanning was performed on several exposures of each experiment in order to ensure linearity. This protocol was designed to assess the ouabain-binding competence of newly synthesized [35S]methionine-labeled Na,K-ATPase prior to its delivery to the cell surface. The experiment was repeated at least three times for each condition.

**Ouabain Binding to Sonicated-Permeabilized Cell Membranes**—Aliquots of trypsinized MDCK cells (1.5 × 10⁶) were washed, resuspended, and disrupted (by brief sonication) in 0.15 ml of buffer B (120 mM NaCl, 60 mM imidazole, pH 7.25, 4 mM MgCl₂, 2 mM EDTA, and 1% BSA). Membrane permeabilization was accomplished by addition of SDS (7.5 μl of a 1.3% stock solution) to each aliquot, which, after sitting for 10 min at room temperature, was mixed with 150 μl of the same buffer supplemented with 0 mM Na₂ATP and 0.1 μM [3H]ouabain (18 Ci/mmol, Du Pont-New England Nuclear) (17). After a 10-min incubation at 37 °C, the samples were diluted to 1.0 ml with buffered saline (PBS) and then blotted for 60 min at 4 °C in PBS-BSA containing a 1:400 dilution of either anti-Na,K-ATPase serum or preimmune rabbit serum diluted 1:250 followed by Protein A-agarose beads. The blots were then washed with 5 ml of the same buffer and dissolved in Econofluor (Schuell). The transfers were quenched for 2 h at 4 °C in a solution of 5% H₃PO₄, 1% BSA (for standard immunization procedures. The sera were tested for immunoreactivity to purified Na,K-ATPase by enzyme-linked immunosorbent assays. Boosting and bleeding was carried out at 2-week intervals. The sera were employed in this study (12). Immunoprecipitates were analysed by SDS-PAGE followed by fluorography of the dried gels impregnated with Autorofluor (Autonuclear Diagnostics). Labeling with [3H]NAB-ouabain and immunoprecipitation with the anti-ouabain antibody were as previously described (12).

**Characterization of a Polyclonal Anti-α-subunit Antibody**—In addition to anti-ouabain antibodies, our experiments required the use of an antiserum monospecific for the canine kidney Na,K-ATPase α-subunit. We therefore immunized rabbits with Na,K-ATPase purified from canine kidneys by
the Jorgensen technique. As can be seen in Fig. 1, the resultant antibody immunoprecipitated a single radioactive protein of M, 96,000 from metabolically labeled MDCK cells. No such pattern was observed when nonimmune serum was used (not shown). Preincubation of the anti-Na,K-ATPase antibody with purified sodium pump completely blocked the immunoprecipitation of the 96-kDa polypeptide. Western blot analysis confirmed these immunoprecipitation data (Fig. 2). The antibody recognized a single 96-kDa protein in transfers of MDCK cell membrane proteins. Once again, this polypeptide was not detected when nonimmune serum was employed. The specificity of this antibody is underscored by its ability to immunoprecipitate α-subunit labeled with [3H]NAB-ouabain (Fig. 1). This result confirms the affinity of the antibody for authentic α-subunit since the latter is the only cellular protein that should be labeled by NAB-ouabain. Finally, it has been demonstrated that this antibody partially inhibits Na,K-ATPase activity (16). No immunoreactivity to the Na,K-ATPase β-subunit was observed either by immunoprecipitation or by immunoblotting.

Permeabilization of MDCK Cell Membrane Vesicles—With such means at hand, it became possible to examine the ouabain-binding capacity of newly synthesized Na,K-ATPase at very early stages in its posttranslational processing. It was necessary, therefore, to disrupt the cells in order to render all of their membranous elements equally accessible to the NAB-ouabain added in the bulk solution. Disruption was accomplished efficiently by probe sonication, which produces membrane vesicles, a significant proportion of which are sealed. Since the ouabain-binding site is on the α-subunit’s ectodomain while ATP and P, interact with residues on this protein’s endodomain (1), it was necessary to permeabilize these vesicles with a detergent under conditions which are sufficiently rigorous to allow the penetration of relevant reagents to vesicular lumina but not so harsh as to affect adversely the sodium pump’s enzymatic activity. In the experiment presented in Fig. 3, MDCK cells disrupted by microtip sonication were employed in ouabain binding assays as described under “Materials and Methods.” Equilibrium ouabain binding was measured for intact vesicles as well as for vesicles which had been permeabilized through treatment with 0.065% SDS in the presence of 1% BSA for 10 min at 20 °C. Nonspecific binding was measured by carrying out the experiment in the presence in a 1000-fold excess of nonradiolabeled (Cold) ouabain. Permeabilized vesicles bound approximately twice as much ouabain as untreated vesicles, demonstrating that maximal binding requires the elimination of vesicular latency. (n = 3 for each condition).

FIG. 1. A polyclonal antibody directed against the Na,K-ATPase immunoprecipitates the α-subunit from MDCK cells. Metabolically labeled MDCK cell membranes (prepared and solubilized as described under “Materials and Methods”) were subjected to immunoprecipitation with anti-Na,K-ATPase α-subunit antiserum (lane a) or anti-Na,K-ATPase antibody which had been preincubated for 45 min at 4 °C with 12 μg of purified Na,K-ATPase (lane b). In lane c, MDCK membranes labeled with [3H]NAB-ouabain were subjected to immunoprecipitation with the anti-Na,K-ATPase antibody. Immunoprecipitates were separated by SDS-PAGE and analyzed by fluorography. A 96-kDa band can be seen in immunoprecipitates with the anti-Na,K-ATPase antibody (lane a). The presence of this radiolabeled MDCK protein is prevented by immunocompetition, employing purified Na,K-ATPase (lane b). The anti-Na,K-ATPase antibody’s ability to immunoprecipitate a 96-kDa [3H]NAB-ouabain-labeled polypeptide (lane c) further demonstrates this probe’s capacity to recognize the α-subunit.

FIG. 2. Polyclonal antibody directed against the Na,K-ATPase detects the α-subunit in Western blots of MDCK cell membranes. MDCK cell membranes (prepared under “Materials and Methods”) were separated by SDS-PAGE and transferred to nitrocellulose. Filters were probed with anti-Na,K-ATPase (a) or nonimmune serum (b), followed by 125I-protein A. Washed filters were analyzed by autoradiography. A 96-kDa polypeptide could be visualized when the filter was exposed to anti-Na,K-ATPase but not when nonimmune serum was used.

FIG. 3. [3H]Ouabain binding to MDCK cell membrane vesicles prepared by sonication. MDCK cells were disrupted by sonication and the resultant vesicles were employed in ouabain binding assays as described under “Materials and Methods.” Equilibrium ouabain binding was measured for intact vesicles as well as for vesicles which had been permeabilized through treatment with 0.065% SDS in the presence of 1% BSA for 10 min at 20 °C. Nonspecific binding was measured by carrying out the experiment in the presence in a 1000-fold excess of nonradiolabeled (Cold) ouabain. Permeabilized vesicles bound approximately twice as much ouabain as untreated vesicles, demonstrating that maximal binding requires the elimination of vesicular latency. (n = 3 for each condition).
Thus, the ouabain-binding capacity of Na,K-ATPase residing in detergent/BSA mixture results in near-maximal binding. Thus, the ouabain-binding capacity of Na,K-ATPase residing in intracellular as well as cell surface membranes can be efficaciously probed.

Ouabain Binding by Newly Synthesized Na,K-ATPase—The protocol employed in measuring the ouabain binding competence of newly synthesized Na,K-ATPase is outlined below. Trypsinized MDCK cells in methionine-free Eagle’s minimal essential medium were pulse-labeled for 5 min with [35S]methionine and then chased with unlabeled methionine as described under “Materials and Methods.” Aliquots taken at several chase intervals were disrupted by microtip sonication. The resulting membrane vesicles were permeabilized as described in the legend of Fig. 3 and suspended in a medium containing either (Na+ + Mg2+ + ATP) or (Mg2+ + P). NAB-ouabain was added to each sample, which, after further incubation in the dark for 10 min at 37 °C, was exposed to UV light. The vesicles were then collected by centrifugation (90 min, 1.5 x 10⁶ x g, 4 °C), solubilized, and subjected to immunoprecipitation with the anti-ouabain antibody. It is important to note that only those molecules of Na,K-ATPase synthesized during the pulse contain [35S]methionine, and only those sodium pumps competent to bind ouabain are substrates for the immunoprecipitation.

The data from these experiments are presented in Figs. 4-6. We first examined the ability of newly synthesized Na,K-ATPase to bind NAB-ouabain in the presence of (Na+ + Mg2+)

FIG. 4. Na,K-ATPase can not bind ouabain under Na, Mg, and ATP conditions until 10 min after its synthesis. MDCK cells were metabolically labeled with [35S]methionine and allowed chase periods of up to 20 min as described under “Materials and Methods.” At each chase interval aliquots of cells were disrupted by probe sonication and the resulting vesicles were permeabilized as illustrated in Fig. 3. Vesicles were incubated with NAB-ouabain in the presence of Na+, Mg2+, and ATP and, following UV photolysis, were solubilized and subjected to immunoprecipitation with the anti-ouabain antibody. Immunoprecipitates were separated by SDS-PAGE and processed for fluorography. No radiolabeled α-subunit could be detected in immunoprecipitates from cells harvested at the conclusion of the pulse (0). Fluorographs of immunoprecipitates from cells allowed 10 (10) or 20 (20) min of chase, however, reveal readily detectable α-subunit bands.

Results from these experiments are quantitated in Fig. 5, which compares the amounts of radiolabeled α-subunit (determined by densitometric scanning) in immunoprecipitates from the first and last chase interval. When the experiment is carried out with a 20-min pulse and a 60-min chase, immunoprecipitates prepared from cells subjected to the 60-min chase interval possess -3-4-fold more radiolabeled α-subunit than those prepared from cells harvested at the conclusion of the pulse period. As would be expected, when an anti-α-subunit antibody is employed a ratio of one is obtained, demonstrating that the total amount of radiolabeled α-subunit in the cells does not vary greatly over the course of the chase (Fig. 5). It would appear, therefore, that newly synthesized Na,K-ATPase is not initially competent to bind ouabain under (Na+ + Mg2+ + ATP) conditions, but achieves this capability within 10-20 min of its production.

In contrast, the ability of newly synthesized Na,K-ATPase to bind NAB-ouabain in the presence of Mg2+ and P, did not vary as a function of chase interval. The amount of [35S]-radioactivity in the α-subunit immunoprecipitated by the
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**Fig. 6.** Ouabain binding under Na\(^+\), Mg\(^{2+}\), and ATP conditions by newly synthesized Na,K-ATPase is ATP-dependent. In order to demonstrate that the Na\(^+\), Mg\(^{2+}\), and ATP-supported ouabain-binding capacity observed in Figs. 4 and 5 is actually ATP-dependent, MDCK cells were pulse-labeled for 20 min followed by a 60-min chase period. At 0 (0) and 60 (60) min of chase aliquots of cells were sonicated, exposed to NAB-ouabain and immunoprecipitated with the anti-ouabain antibody as described in Fig. 4. Omission of ATP from the ouabain labeling incubation results in the loss of the anti-ouabain immunoprecipitable radiolabeled α-subunit band. Inclusion of 1 mM ouabain during the anti-ouabain immunoprecipitation step also results in the absence of detectable α-subunit in the fluorographs.

ANTI-OUABAIN ANTIBODY

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**DISCUSSION**

Following their synthesis on polyribosomes that become bound to the membranes of the rough endoplasmic reticulum, the α- and β-subunits of Na,K-ATPase must be transported through a network of membrane delimited compartments to their site of final functional residence on the cell surface (18). During its transport, the sodium pump subunits must undergo several modifications, some of which are characteristic of most transmembrane proteins, while others are probably restricted to the Na,K-ATPase and its close molecular relatives. The list of sodium pump-specific processing steps is highlighted by the assembly of the α- and β-subunits to form the holoenzyme and the initiation of the various components of the enzymatic activity of the Na,K-ATPase.

To explore the kinetics of these various steps we have reached the cell surface and thus become accessible to NAB-ouabain in the incubation medium should be substrates for the immunoprecipitation by the antiouabain antibody.

The densitometric quantitation of the data from a representative experiment are presented in Fig. 7. No newly synthesized α-subunit could be detected in antiouabain immunoprecipitates from cells harvested immediately after the pulse. Immunoprecipitates from cells subjected to 30 min of chase, however, revealed a faintly visible α-subunit band when analyzed by fluorography. The quantity of radioactivity migrating with this band increased dramatically with increasing chase time until the 120-min time point. These results suggest that the cohort of α-subunits produced during the pulse period begins to arrive at the cell surface ~50 min after its biosynthesis. Its delivery to the cell surface appears to continue throughout a 90-min span, and is completed within 120 min of the cessation of the pulse. The data presented in Fig. 7 reveal that arrival of newly synthesized Na,K-ATPase at the cell surface occurs with a half-time of ~75 min.

**Fig. 7.** Delivery of newly synthesized Na,K-ATPase α-subunit to the MDCK cell surface. MDCK cells were metabolically labeled during a 20-min pulse followed by a 150-min chase. At each chase interval, aliquots of cells were surface-labeled with NAB-ouabain followed by photolysis, anti-ouabain immunoprecipitation, SDS-PAGE, and fluorography as described under "Materials and Methods." The amount of radioactivity present in the α-subunit bands was quantitated by densitometry scanning and the data from a representative experiment (normalized to the quantity of α-subunit detected at the 90-min time point) are presented above. Radiolabeled α-subunit is first visualized in the anti-ouabain immunoprecipitates after 30 min of chase and continues to accumulate for 90 min (○). No radiolabeled α-subunit is detected in immunoprecipitates performed in the presence of 1 mM ouabain (●).
developed a procedure in which NAB-ouabain is used to examine the functional characteristics of newly synthesized Na,K-ATPase. Since ouabain binds preferentially to the E2P conformation of the sodium pump (15), NAB-ouabain binding can be used to probe the conformations assumed by the Na,K-ATPase throughout the course of its posttranslational processing and transit to the cell surface. It would appear that, under conditions that drive the mature sodium pump into its E2P conformation without requiring the enzymatic hydrolysis of ATP, newly synthesized Na,K-ATPase binds NAB-ouabain and thus becomes susceptible to immunoprecipitation with an antiouabain antibody. The quantity of pulse-labeled Na,K-ATPase that binds NAB-ouabain under these conditions does not change over the course of a 60-min chase. The data are markedly different, however, when NAB-ouabain binding is carried out under conditions demanding that the sodium pump catalytically split ATP and pass through the E2P state prior to arriving in the ouabain-sensitive E2P conformer. Sodium pumps examined immediately after their synthesis appear incapable of assuming the ouabain binding conformation in the presence of (Na' + Mg2+ + ATP). After 10-20 min of posttranslational processing, however, they are fully capable of binding NAB-ouabain and do so in a strictly ATP-dependent manner. It must be noted that, while ATP is clearly required for this binding, we have not actually demonstrated that the binding is linked to ATP hydrolysis. Although these data do not prove that the sodium pump is fully functional within 10-20 min after synthesis, they are certainly consistent with such a presumption.

The maximal levels of pulse-labeled Na,K-ATPase that bind NAB-ouabain, thereby becoming immunoprecipitable with anti-ouabain antibodies, are consistently ~3-fold larger when NAB-ouabain is introduced under (Na' + Mg2+ + ATP) (catalytic) versus (Mg2+ + P) (noncatalytic) conditions (data not shown). Since mature Na,K-ATPase is capable of binding ouabain in both circumstances, one would expect that, following the completion of posttranslational processing, equal quantities of sodium pump should be detectable in the two NAB-ouabain binding regimens. The discrepancy mentioned above is probably not due to the maturation state of the enzyme, since it persists throughout 60 min of chase. A likely explanation can be found in the susceptibility of ouabain binding to the presence of monovalent cations: the reaction is severalfold more sensitive to millimolar concentrations of K+ in the presence of (Mg2+ + P) than in the presence of (Na' + Mg2+ + ATP) (22, 23). Although our binding experiments are performed in nominally K+-free solutions, substantial quantities of K+ are released into the binding medium when the MDCK cells are lysed. We have performed flame photometric measurements which indicate that, following disruption, the binding medium could contain in excess of 5 mM K+. We have found that this K+ concentration is sufficient to depress Mg2+- and P2-dependent ouabain binding to MDCK cell membranes ~3-fold without exerting a similarly drastic effect on the Na+, Mg2+, and ATP stimulated reaction (data not shown).

Several groups have measured the time required for newly synthesized Na,K-ATPase to complete its transit to the cell surface. Using HeLa cells and a heavy isotope-dilution technique, Pollack et al. (7) derived an estimate of 4.5 h for this interval. Karin and Cook (4) estimated a 3.4-h transit time in HTC hepatoma cells. Churchill and Hokin (8) found that approximately 2 h are required for pulse-labeled Na,K-ATPase to become detectable in a plasma membrane fraction prepared from the eel electroplax. Since the vast majority of membrane proteins studied to date arrive at the plasma mem-
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chase, however, they find that the enzyme can produce the full range of proteolytic peptide fragments characteristic of the mature sodium pump. Furthermore, a similar "conformational maturation" has been demonstrated for the newly synthesized α subunit of the acetylcholine receptor in BC3H1 cells, which possesses some but not all of the toxin binding properties characteristic of its mature conformation (21).

The nature of these putative activating modifications remains entirely unknown. It is clear, however, that Na,K-ATPase can acquire ATP-sensitivity prior to arriving at the cell surface. These findings imply that during the last 20-30 min of its intracellular transit time, the Na,K-ATPase might be capable of functioning in the regulation of [Na⁺] and [K⁺] in certain intracellular compartments. Determining the steps of the process by which the Na,K-ATPase is turned on may provide valuable information about the biochemical mechanisms involved in the activation of the sodium pump and shed light on its homeostatic regulation.

Acknowledgments—We wish to acknowledge the helpful discussions of Dr. Z. D. J. Smith and the photographic assistance of Anne Curley-Whitehouse.

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