Isolation of Brush-Border Membrane from the Rabbit Descending Colon Epithelium

PARTIAL CHARACTERIZATION OF A UNIQUE K⁺-ACTIVATED ATPase

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The mechanisms of ion movement across the apical membrane of the colon have previously been investigated only in intact tissue. To investigate these mechanisms directly, we have undertaken the isolation and characterization of the apical brush-border membrane of the rabbit descending colon. The purification protocol consists of an initial isolation of single epithelial cells after dissociation of the mucosal layer in EDTA, a high pH (8.3), low ionic strength homogenization of the cells, and differential centrifugation and separation of apical membrane from nuclei, and filamentous material on a 7.5% Percoll gradient. A 20-fold enrichment in alkaline phosphatase (an apical membrane enzyme marker) specific activity over the initial homogenate value is observed in the final membrane fraction. This fraction also contains a K⁺-activated, pH 7.8, optimum ATPase (20 times purified over homogenate) with the following properties: 1) low Km (2 x 10⁻⁴ m) for K⁺; 2) resistance to high ionic strength (1 m Tris) solubilization; 3) competitive inhibition by Na⁺ (Kᵢ = 14 mm), no activation by Na⁺; 4) inhibition by orthovanadate (Kᵢ = 40 nm), but no effect of oligomycin (20 µg/ml of protein) or ouabain (10⁻³ m); and 5) a K⁺-sensitive phosphorylated intermediate. These characteristics suggest that this membrane-bound ATPase is distinct from other known ATPases including the Na⁺ + K⁺-ATPase-Na⁺ pump of the basolateral membrane.

The transcellular transport of sodium and potassium across epithelia requires that these cations transverse both the apical (mucosal) and basolateral (serosal) membranes. Results of electrical and isotope flux experiments in high resistance epithelia led Ussing and co-workers (1, 2) to postulate a "leak-pump" mechanism for Na transport. According to their hypothesis, Na ions leak across the apical membrane and are then "pumped" out of the cell across the basolateral membrane. This general model for Na transport continues to be accepted. The basolateral Na⁺ + K⁺-dependent ATPase is the biochemical moiety identified as the "pump." This enzyme mediates the transport of 3 Na⁺ ions out of and 2 K⁺ ions into the cell/ATP hydrolyzed (3, 4). As yet, however, no biochemical counterpart for the apical membrane Na⁺ "leak" pathway has been identified.

Potassium can be either secreted or absorbed across epithelia (5-7). During secretion, K⁺ is "pumped" across the basolateral membrane, a process catalyzed by the Na⁺ + K⁺-dependent ATPase. For most epithelia, the K⁺ "pumped" into the cell is cycled back across a K⁺-permeable basolateral membrane (2). For K⁺-secreting epithelia, the apical membrane is also K⁺-permeable, allowing secretion of part of the K⁺ originally "pumped" into the cell (6, 7). Absorption of K⁺ by epithelia can also be observed (7, 11), particularly in K⁺-depleted animals (7) or when K⁺ secretion is blocked by the serosal application of the cardiac glycoside ouabain (9, 11) which specifically inhibits the basolateral membrane Na⁺, K⁺-ATPase (3, 4). In rat renal distal tubule (9) and turtle urinary bladder (10), K⁺ absorption from the lumen is inhibited by mucosal ouabain, leading to the suggestion that an apical membrane ouabain-sensitive K⁺ pump exists similar to the Na⁺ + K⁺ pump of the basolateral membrane. Serosal ouabain did not inhibit the K⁺ absorption. Thus, not only "leak" pathways for Na⁺ and K⁺ transport but also K⁺ "pumps" have been postulated to reside in the apical membrane of certain vertebrate epithelia (6).

One approach to the direct study of the mechanism and regulation of these transport pathways has been to isolate the apical membranes of Na⁺ and K⁺-transporting epithelia and study their biochemical composition and transport properties in vitro. However, because a suitable enzyme marker has not been described, isolation of apical membranes from distal portions of the intestinal and urinary tracts has not been undertaken. One notable exception is the work of Rodriguez and Edelman (13). These investigators obtained a fraction enriched in apical plasma membrane from the toad urinary bladder using an initial surface radioiodination to mark the apical membrane for monitoring recovery during the purification. No further data, however, describing the composition or functional properties of this fraction have been reported.

The rabbit descending colon has several advantages as a tissue for isolation and analysis of an apical membrane fraction. Transport across this epithelium has been characterized. The tissue absorbs Na⁺ and Cl⁻ (14) and secretes K⁺ (11, 12) and HCO₃⁻ (14) and under some conditions, absorbs K⁺ (11, 12). Using cytochemical methods, Vengesa and Hopfer (15) have shown that a cysteine-inhibitable alkaline phosphatase is a marker for the apical membrane. Additionally, this simple epithelium contains predominantly a single cell type with a brush-border at the apical surface. These last two features provide both an enzymatic and morphological marker for...
monitoring the apical membrane during isolation. Finally in contrast to other epithelia, relatively large areas of epithelium from a single animal can be obtained for study. In the investigation detailed below, a procedure for isolating the rabbit descending colon epithelial apical membrane is described. In addition, a unique K+-activated ATPase activity has been discovered in this membrane. Partial characterization of this enzyme is reported.

**EXPERIMENTAL PROCEDURES**

**Preparation of Brush-Border Membranes**—Two male New Zealand White rabbits (2 kg) are sacrificed by venous air embolization (50 ml). The descending colons, each 20–30 cm in length, are dissected out, immersed in ice-cold 0.15 M NaCl, and flushed. The tissue is then everted over a glass rod, ligated at one end, distended with ice-cold 30 mm NaCl, 5 mM NaNbEDTA, and 8 mM Hepes/Tris, pH 7.6, and then ligated at the other end. The colons are then incubated for 45 min in 200 ml of the same cold pH 7.6 buffer which is vigorously stirred. The buffer is then collected and the tissue incubated for an additional 45 min in 200 ml of fresh buffer. This second incubation buffer is then combined with the first and the entire 400 ml centrifuged at 500 × g for 10 min at 4 °C. The resulting pellets contain epithelial cells. Approximately 2 × 10^8 cells are routinely recovered and greater than 90% have an identifiable brush border when examined by phase contrast microscopy.

The cell pellet is then divided into two equal portions and each portion suspended in 70 ml of 1 mM NaEDTA, pH 8.3, at 4 °C. The cells are then disrupted by a 17-s treatment at top speed in a Waring Blendor. The resulting homogenate is then subjected to a 750 × g centrifugation for 10 min at 4 °C to sediment brush-borders and other large debris, including nuclear ghosts. Pellets are combined, resuspended in 100 ml of the original homogenization media, and recentrifuged as described above. The second pellet (P2) is then resuspended in 74 ml of the homogenization medium, divided in 2 equal volumes, and 3 ml of Percoll (Pharmacia) are added to each centrifuge tube with gentle suspension for 22 min in a Beckman RC5B centrifuge at 46,000 × g at 4 °C. Five-ml fractions are then aspirated from the gradient; Percoll is removed from each fraction by centrifugation at 200,000 × g for 45 min in a Beckman L8-50 ultracentrifuge, Ti 50 rotor. Material collected on the top of the Percoll pellet is allowed to float up and is collected by aspiration through an 18-gauge needle. Data on the protein content of the various fractions are presented in Table I.

**Enzyme Assays**—Succinate dehydrogenase was assayed according to the method of Pennington (16). Cytochrome oxidase was assayed by the method of Yonetani (17). NADPH cytochrome oxidase was assayed according to the method of Krebs and Henseleit (18). Monooamine oxidase was assayed according to Lismam et al. (19). Acid phosphatase was assayed at pH 5.0 using p-nitrophenyl phosphate as the substrate. DNA was assayed using the procedure of Puzas and Goodman (20). During the development of the fractionation procedure, the protein content of various fractions was assayed by the Lowry procedure (21). After the final procedure was established, the Bradford technique (22) was routinely employed for protein determination. The two methods (using bovine serum albumin as standard) are equivalent for the assay of apical membrane protein.

K+-phosphatase was assayed using p-nitrophenyl phosphate as the substrate (23). Duplicate assay tubes contained either 50 mM KCl, 50 mM NaCl, or 50 mM KCl plus 1 mM ouabain. All tubes contained 50 mM Tris-Cl, pH 7.5, 5 mM MgCl₂, and 3 mM substrate, all in 0.5 ml. After adding an aliquot of an appropriate fraction, the tubes were incubated at 37 °C for 20 min before addition of 2 ml of 1 N NaOH. The ouabain-sensitive K+-phosphatase activity was calculated as the difference in substrate hydrolysis in the presence of KCl versus KCl and ouabain. The ouabain-insensitive K+-phosphatase was calculated as the difference in substrate hydrolysis in the presence of KCl and ouabain versus the presence of NaCl. A motor extinction coefficient at 410 nm of 19.4 × 10³ was employed for p-nitrophenol in 1 N NaOH.

Alkaline phosphatase was assayed using the fluorogenic substrate 4-methylumbelliferyl phosphate (23). The assay buffer contained 20 mM glycine-NaOH, pH 9.3, 5 mM MgCl₂, and 1 mM 4-methylumbelliferyl phosphate, all in 1 ml. Protein was added to start the reaction; tubes were incubated at 37 °C for 1 h and the reaction quenched using 1 N NaOH. 4-Methylumbelliforone was used as a standard and the fluorescence recorded at 365–445 nm.

For ATPase assays, fractions were washed in 1 mM Tris/EDTA, pH 7.4, and resedimented. ATPase activity was assayed using Tris/ATP (vanadate-free) as the substrate. The assay medium contained 25 mM Tris-Cl, pH 7.5, 5 mM MgCl₂, 3 mM Tris/ATP, 1 mM ouabain, and various added calcium and magnesium. The reaction was initiated by addition of ATP and quenched with 10% trichlooroacetic acid or 2.5 N HCl. For routine assays, the Fiske and SubbaRowe (24) technique for inorganic phosphate determination was employed. To determine the Km for ATP, the assay described by Penney (25) using malachite green to form a complex with phosphomolybdate was employed. RH-P0 was used as a standard. To obtain the K+ -ATPase activity, Mg²⁺-ATPase activity was subtracted from total activity. To determine the presence of a phosphorylated intermediate, a protocol similar to that described by Saccamani et al. (26) was used. Briefly, 1 mM Tris/EDTA (pH 7.5)-washed brush-border membranes (G2-G4 pool) were phosphorylated on ice in a medium containing 30 mM imidazole-glycylglycine, pH 7.5, 2 mM MgCl₂, 2 μM [γ-³²P]-adenosine-5'-triphosphate (ATP) (2000 cpm/pmol), and 20–40 μg of membrane protein, all in 0.1 ml. To test K⁺ sensitivity, 100 mM KCl was introduced into the medium (addition of 100 mM choline Cl to the reaction mixture had no effect). The reaction was started by the addition of the [γ-³²P]-ATP and stopped at 30 s (labeling had reached steady state by this time) by the addition of 1 ml of cold 10% trichlooroacetic acid containing 5 mM phosphate and 0.3 mM ATP. This solution was filtered (Millipore HAWP 002500 filter) and washed four times with 5 ml of the stop solution. ³²P incorporation was assayed by liquid scintillation counting. Steady state labeling of the membranes was proportional to protein concentration (10–40 μg/ml).

**Electron Microscopy**—Subcellular fractions were fixed in suspension at 4 °C with 1.5% glutaraldehyde in 50 mM sodium cacodylate buffer, pH 7.4, for 30 min and centrifuged at 16,000 × g for 10 min. Pellets were gently rinsed with cacodylate buffer and then postfixed for 2 h on ice with osmium tetroxide in 50 mM sodium barbital-acetate buffer, pH 7.6. Pellets were then rinsed with room temperature buffer and stained en bloc with 0.5% uranyl acetate in barbital-acetate buffer, pH 6.0, for 2 h. After dehydration in an ethanol series, pellets were embedded in Polybed resin (Polysciences, Inc., Warrington, PA), thin sectioned, and stained with uranyl acetate and lead citrate. This material was then photographed in a Philips 201 electron microscope operated at 80 kv.

**RESULTS**

**Preparation of the Apical Membrane Fraction**—The apical membrane of the rabbit descending colon has a brush border similar to that found in the small intestine. However, in contrast to the small intestine where mucosal scrapings have been used successfully as starting material for brush-border membrane isolation, the presence of large amounts of mucus in the crypt cells of the colonic mucosa interfered in the fractionation of whole mucosa homogenates. Collection of surface epithelial cells containing fewer than 10% mucus-secreting cells as outlined in Fig. 1 was developed to isolate the membrane associated with this structure. This procedure has several notable features. One, the starting material consists of isolated epithelial cells. In contrast to the small intestine where mucosal scrapings have been used successfully as starting material for brush-border membrane isolation, the presence of large amounts of mucus in the crypt cells of the colonic mucosa interfered in the fractionation of whole mucosa homogenates. Collection of surface epithelial cells containing fewer than 10% mucus-secreting cells as outlined under “Experimental Procedures” largely avoided the problem of mucus contamination.

Homogenization of epithelial cells using a variety of procedures revealed that the cells were relatively resistant to shear force and once the cells were sheared, cytoplasmic dissolution was not easily achieved. By employing a hypotonic, low ionic strength homogenization media containing EDTA, a high control was achieved. Using Waring blender homogenization (a high shear force procedure) resulted in 100% cell breakage. A number of conditions were tested for effects on cytoplasmic dissolution, the most successful was an alkaline pH (8.3) unbuffered medium. EDTA was required to retain the brush-border structure of the apical membrane and to prevent aggregation.

A washed low speed pellet obtained after two successive centrifugations contained predominantly brush borders (Fig.
Colon Brush-Border Membrane Preparation

**COLON EPITHELIAL CELLS (>90%)**

- 340 ml 1 mM NaEDTA pH 8.3
- Waring Blender 17 sec
- 750 g 10 min

**HOMOGENATE**

- 100 ml 1 mM EDTA pH 8.3
- 750 g 10 min

**P1**

- 7.5% PERCOLL GRADIENT
- 1 mM EDTA pH 8.3
- 48,000 g 22 min

**G1**
**G 2-4**
**G 8**

**G 2-4**
**BRUSH BORDER MEMBRANE FRACTION**

![Diagram of purification scheme](image)

*Fig. 1. Purification scheme for rabbit descending colon brush-border membrane. Details of procedure are described in the text.*

2A). An occasional nuclear ghost was observed. To remove the latter contaminant, a number of density gradient materials were tested. The most successful procedure was Percoll density gradient centrifugation.

By use of a Percoll density gradient, two objectives were achieved: separation of nuclear ghosts from the apical membrane and nearly quantitative removal of filamentous material, primarily actin, from the cytoplasmic surface of the apical membrane. Nuclear ghosts and filamentous material formed an upper band in the Percoll gradient (Fig. 1). Apical membranes, predominantly in vesicular form (Fig. 2B), comprised a lower band in the Percoll gradient (Fig. 1). This latter band was highly enriched for the enzyme markers of the apical membrane (see below). The low ionic strength medium (1 mM EDTA) was responsible for removal of filamentous material subsequent breakdown of the brush-border structure since increasing ionic strength resulted in loss of the band containing apical membranes and creation of a denser band containing intact brush-borders.

The band containing apical membranes was collected in fractions G2 through G4 of the Percoll gradient (Fig. 3). These pooled fractions were highly enriched for alkaline phosphatase, having 25% of the total homogenate activity and representing an approximate 20-fold increase in specific activity over the homogenate value. Alkaline phosphatase had been shown by Vengesa and Hopfer (15) to be a marker for the rabbit colon epithelial cell apical membrane. The alkaline phosphatase of the rabbit colon homogenate and apical membrane fraction is similar to intestinal alkaline phosphatase (23). The colon enzyme has a pH optimum of 8.5-8.75, is inhibited 70% by 10 mM cysteine, and hydrolyzes p-nitrophenyl phosphate, β-glycerol phosphate, and 4-methylumbelliferyl phosphate. With one exception, all other marker en-

*Fig. 2. Structure of isolated fractions. A, electron micrograph of pellet 2; × 6700. Fraction contains primarily brush-borders. B, electron micrograph of pooled gradient fractions 2-4; × 15,000. Fraction contains primarily membrane fragments and vesicles derived from the brush-border.*
zymes tested, cytochrome oxidase, succinate dehydrogenase, monoamine oxidase (mitochondrial markers), NADPH cytochrome c reductase (endoplasmic reticulum and Golgi marker), acid phosphatase (lysosomal marker), and DNA (nuclear marker), had less than 1% of their homogenate total activities represented in the G2-G4 fraction. Succinate dehydrogenase is included in Table I for comparison.

The only significant contaminant appeared to be fragments of basolateral membrane, detected by the ouabain-sensitive K\(^+\)-phosphatase activity, a catalytic activity of the Na\(^+\)+K\(^+-\)ATPase. Yields of this enzyme activity in the pool of fractions G2 through G4 averaged 2% of the homogenate value in contrast to 25% for the yield of alkaline phosphatase (Table I). In addition, the peak of basolateral enzyme activity was generally observed one fraction higher (G1 or G2) in the Percoll gradient than the fraction containing the highest yield of apical membranes (G2 or G3) (Fig. 3).

**Ouabain-insensitive K\(^+\)-dependent Phosphohydrolase**—In addition to alkaline phosphatase and the ouabain-sensitive K\(^+\)-phosphatase, a third membrane-bound phosphohydrolase was detected in homogenates of rabbit colon epithelial cells. This hydrolytic activity was discovered when it was observed that not all of the K\(^+\)-dependent phosphohydrolase activity could be inhibited by the addition of 1 mM ouabain to the assay medium. This enzyme activity, an ouabain-insensitive K\(^+\)-phosphatase, represented 37% of the total K\(^+\)-phosphatase activity of the homogenate (Table I). Furthermore, using the fractionation scheme described previously, this enzyme activity co-purified with alkaline phosphatase, the enzyme marker for the apical membrane. In Fig. 3, the distributions of these two enzymes as well as that of ouabain-sensitive K\(^+\)-phosphatase and succinate dehydrogenase in a 7.5% Percoll gradient are compared. The co-distribution of the ouabain-insensitive K\(^+\)-phosphatase and alkaline phosphatase is apparent. The other two enzyme markers distributed very differently; ouabain-sensitive K\(^+\)-phosphatase activity was maximal in G1 and G2, succinate dehydrogenase activity in fractions G6 and G7.

The ouabain-insensitive K\(^+\)-phosphatase activity did not appear to be the result of the conversion of an ouabain-sensitive K\(^+\)-phosphatase to an ouabain-insensitive form of the enzyme. Recoveries for both enzymes through the entire fractionation approached 100% (Table I). In addition, assays of both enzymes for all fractions, performed at 37\(^\circ\)C, were linear with respect to time over a 20-min period and were proportional to enzyme concentration. Finally, this K\(^+\)-dependent phosphohydrolase appeared to be tightly associated with the apical membranes since exposure to high ionic strength caused minimal activity to be detected in fractions G6 and G7.

**Table I**  
**Analysis of fractions encountered in purification of rabbit colon brush border membrane**

Data for the homogenate, the washed low speed pellet (P-2), and the pooled 7.5% (P-2) Percoll gradient fractions 2-4 are presented. Total activity = micromoles h\(^{-1}\) total volume. Specific activity = micromoles mg\(^{-1}\) h\(^{-1}\). Relative specific activity = specific activity fraction / specific activity homogenate. Recovery = recovery totaled over the entire fractionation. Other enzyme activities, cytochrome oxidase, monoamine oxidase, NADPH cytochrome c reductase, and acid phosphatase, and DNA all had relative specific activity less than 1.0 in the G fraction pool 2-4 and are not included. Data are presented from three consecutive preparations and are expressed as the mean ± S. D.

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<th>Homogenate</th>
<th>Pellet 2</th>
<th>G fraction pool 2-4</th>
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<tr>
<td>Protein (mg)</td>
<td>Recovery</td>
<td>131.2 ± 4.1</td>
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<td></td>
<td>Ouabain-sensitive K(^+)-Phosphatase</td>
<td>Total activity</td>
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<td>Alkaline phosphatase</td>
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strength buffer (1.0 M Tris-Cl) did not dissociate the enzyme from the sedimentable membrane.

To further characterize this ouabain-insensitive phosphohydrolyase activity, its substrate specificity was examined. A number of phosphorylated substrates including ATP, GTP, CTP, and UTP were examined. All assays were performed on the G2 through G4 fraction of the Percoll gradient. In addition, the assay medium contained 1 mM ouabain to minimize interference by any contaminating Na+ + K+ ATPase.

The K+-stimulated phosphohydrolase is highly specific for ATP as a substrate since in the presence of 0.1 mM KCl, ATP was hydrolyzed at least 3 times faster than any of the nucleotide triphosphates and in the presence of 50 mM KCl, only ATP was hydrolyzed. Of other substrates tested, p-nitrophenyl phosphate can also be hydrolyzed by the K+-activated phosphohydrolase activity.

The apical membrane K+-dependent ATPase is not alkaline phosphatase. The pH profile of the ouabain-insensitive K+-ATPase showed a broad pH maximum between pH 7 and pH 8. The K+-dependent phosphohydrolase will not cleave β-glycerol 3-phosphate, a substrate for the alkaline phosphatase, and cysteine does not inhibit the K+-dependent phosphohydrolase. Furthermore, the ouabain-insensitive K+-dependent phosphohydrolase activity is not detectable in an homogenate of rabbit small intestine (data not shown), a tissue in which the alkaline phosphatase specific activity is 100-fold greater than in the rabbit descending colon.

The K+-ATPase is not the mitochondrial inner membrane ATPase. Less than 1% of the homogenate activity of the mitochondrial inner membrane enzyme markers, succinate dehydrogenase and cytochrome oxidase, were found in the apical membrane fraction. Also, inhibitors of the mitochondrial ATPase showed no effect of the K+-dependent ATPase activity. A small (less than 10% of total activity) effect of these inhibitors on the basolateral Mg2+-ATPase activity in the fraction G2-G4 pool was noted.

The K+-ATPase is not the activity of the basolateral membrane Na+ + K+-ATPase. As noted previously, recoveries of both enzyme activities over all cell fractions approached 100% (Table I), ruling out conversion of the basolateral membrane enzyme activity to an ouabain-insensitive form. Varying the sodium ion concentration in the presence or absence of K+ did not stimulate the ouabain-insensitive ATPase activity. Thus, this K+-ATPase shows no requirement for Na+, a defining characteristic of the basolateral membrane Na+ + K+-ATPase (3, 4).

Three characteristics of the apical membrane ouabain-insensitive K+-ATPase do, however, bear a strong resemblance to known properties of the Na+ + K+-ATPase. One is the effect of monovalent cations on ATPase activity. The external cation binding and activating site of the Na+ + K+-dependent ATPase has a high apparent affinity for K+ and related cations NH4+ and Rb+. Sodium ions and the related cation Li+ both competitively inhibit the binding of K+ and the subsequent hydrolysis of the phosphorylated intermediate. Similarly, the ouabain-insensitive K+-ATPase found in the colon apical membrane was extremely sensitive to K+, K0.5 = 0.18 ± 0.01 mM (the same as for the Na+ + K+-ATPase) (27). In addition, the activation of this ouabain-insensitive ATPase by K+ was competitively inhibited by Na+ with an apparent K0.5 of 13.7 ± 1.0 mM. Other cations tested fell into two categories, activating Rb+, NH4 or competitively inhibiting Li+. A second similarity between the two ATPases was the effect of orthovanadate. Orthovanadate strongly inhibits the ouabain-insensitive K+-ATPase with a K0.5 of 40 nM, a value strikingly similar to that found for orthovanadate inhibition of the Na+ + K+-ATPase (28). This inhibitor has significant effects, as well, on Mg2+-ATPase of the apical membrane but only when higher concentrations (greater than 6 x 10^-6 M) are employed. Finally, Ca2+ inhibits the K+-ATPase (K = 0.3 mM) but has no effect on Mg2+-ATPase activity, a finding similar to the observed effect of Ca2+ on the Na+ + K+-ATPase (3, 4). The K+ ATPase activity was not altered by 1 μM valinomycin or 5 μM gramicidin. Using the pH-sensitive fluorescent probe quinacrine, no K+ + ATP-dependent pH gradient was detected in vesicles of the G2-G4 fraction. Finally, a phosphorylated intermediate of the K+-ATPase was detected. Under steady state conditions, phosphorylation of the apical membrane fraction from γ-32P]ATP required only Mg2+ but not Na+ (56.9 ± 17.5 pmol of P/μg of protein, x ± S. D., n = 5). Preincubation with 50 mM KCl or the addition of 50 mM KCl after steady state had been reached decreased the phosphorylated intermediate to a new steady state level (25 ± 12.6 pmol of P/μg of protein, x ± S. D., n = 5). The phosphorylation of the membrane in the presence of Mg2+ and the effect of K+ were unchanged in the presence of 1 mM ouabain.

**DISCUSSION**

The procedure for the isolation of the apical membrane of the rabbit descending colon epithelium described in this study is both simple, rapid (requiring 6 h to completion), and reproducible. Based on marker enzyme recovery, an approximate 25% yield of the apical brush-border membrane is obtained with a 20-fold purification over the homogenate. There is some contamination of the final apical membrane fraction. In particular, basolateral membrane (Table I), junctional complexes and aggregates of filaments (Fig. 2B) appear in small quantity in the fraction G2-G4 pool. Since small segments of basolateral membrane were often seen attached to brush-border structures (Fig. 2A), contamination of the final fraction by basolateral membrane was expected. Attempts to further improve the purity of this final apical membrane fraction have resulted in very low yields of apical membrane and are not reported.

Purification of apical membranes from epithelia such as the renal distal tubule, urinary bladder, and colon is difficult primarily because of the small amounts of starting material and paucity of marker enzymes for the apical membrane. Rodriguez and Edelman (13), using surface radioiodination as an apical membrane marker, did achieve a 10-fold purification of this membrane fraction, but further work will be needed (other marker enzymes, electron microscopy) to assess removal of other contaminating membranes.

In addition to alkaline phosphatase, the apical membrane of the rabbit colon contains a K+-dependent phosphohydrolase. Evidence for the localization of the latter enzyme activity in the apical membrane is the co-purification of this enzyme with alkaline phosphatase and with morphologically identifiable brush-borders. A more definitive conclusion as to its location will have to await electron microscopic cytochemical localization.

The K+-ATPase has two features which clearly distinguish it from the ubiquitous Na+ + K+-ATPase. Na+ is not required for either ATPase activity or formation of a phosphorylated intermediate and ouabain does not inhibit the enzyme. All other features (inhibition by Na+, Ca2+, and vanadate; K+-mediated dephosphorylation) are similar to those of the Na+ + K+-ATPase (3, 4, 27, 28).

On the other hand, the colon K+-ATPase is thus far enzymatically indistinguishable from the K+-ATPase of the gastric parietal cell tubulovesicular membrane (28, 29) with one potential difference. Recent studies on the hog gastric K+-ATPase indicate a biphasic dependence on K+ concentration. K+...
initially stimulates the rate of ATP hydrolysis at low concentrations but at concentrations higher than 5-10 mM, K+ inhibits enzyme activity (30, 31). Kinetic studies on phosphorylation-dephosphorylation of the hog gastric K+-ATPase have shown high concentrations of K+ inhibit phosphorylation (30, 31). An earlier study in bull frog parietal cells revealed no inhibitory effects of high K+ concentration on K+-ATPase activity (32). Although the colon K+-ATPase is not inhibited by high K+ concentrations, further study of the phosphorylated intermediate of this enzyme is needed to resolve this point. Finally, the gastric K+-ATPase catalyzes the secretion of protons in an electroneutral exchange for K+ across the apical surface of the parietal cell (33, 34). There is no evidence to indicate protons are secreted by the colon (14, 35, 36).

The apical membrane K+-ATPase described here may play a role in the K+ absorption observed in the rabbit colon. Inhibition of K+ secretion in the rabbit colon by serosal addition of ouabain reveals an absorptive net flux of K+ from mucosa to serosa (11, 12). This flux requires metabolic energy.2 Further study of the relationship between the apical membrane K+-ATPase and the absorption of K+ by the rabbit colon will await the development of a specific inhibitor of the ATPase as well as the characterization of the ion transport properties of colon apical membrane vesicles.

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