# Solubilization and partial characterization of ouabain-insensitive Na$^+$-ATPase from basolateral plasma membranes of the intestinal epithelial cells.

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**Key words:** Na$^+$-ATPase; polyoxyethylene 9-lauryl ether ($C_{12}E_9$), second sodium pump, epithelial cell, small intestine, furosemide.

**Abstract.** It has been proposed that intestinal sodium transport is mediated by two different active mechanisms: the ouabain-sensitive Na$^+$/K$^+$-ATPase and ouabain-insensitive Na$^+$-ATPase. In order to determine the optimum conditions to solubilize the membrane-bound Na$^+$-ATPase of enterocyte, basolateral plasma membranes were solubilized using different amounts of octyl glucoside (O.G), Tween 20, octaethylene glycol monododecyl ether ($C_{12}E_8$), and polyoxyethylene 9-lauryl ether ($C_{12}E_9$). Solubilized fractions were assayed for protein concentration and ATPase activity and characterized by electrophoresis analysis. Optimal solubilization of Na$^+$-ATPase was obtained after mixing of 1 mg of basolateral plasma membrane with 1.5 mg of $C_{12}E_9$. Under these conditions, $C_{12}E_9$ solubilized over 60% membrane protein and Na$^+$- and Na$^+$/K$^+$- ATPases activities were recovered over 80% in the soluble fraction without inactivation. In addition, when 25 % glycerol and 2 mM ATP were added, the solubilized Na$^+$-ATPase was stable after 3 days at 4°C. The $C_{12}E_9$-solubilized Na$^+$-ATPase presented the following kinetic characteristics: 1) is only stimulated by the Na$^+$ salt, 2) $K_{0.5}$ for Na$^+$= 4.62 ± 0.06 mM, 3) is similarly stimulated by the Na$^+$ salt of different anions, 4) optimal pH= 7.0, 5) inhibited by furosemide (IC$_{50}$= 0.52 ± 0.10 nm). These kinetic properties of the solubilized Na$^+$-ATPase were similar to those described to the native membrane-bound enzyme. This work reports for the first time, solubilization and characterization of a fully active and stable Na$^+$-ATPase from basolateral plasma membranes of enterocyte using $C_{12}E_9$.

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## Solubilización y caracterización de la ATPasa de Na+ insensible a ouabaina a partir de membranas laterobasales de células de intestino delgado.

**Resumen.** Ha sido propuesto que el transporte intestinal del sodio es mediado por dos mecanismos: la ATPasa de Na$^+$/K$^+$, inhibida por ouabaina y la ATPasa de Na$^+$ la cual es insensible a la ouabaina y es inhibida por la furosemida. Con la finalidad de determinar las condiciones óptimas para solubilizar la ATPasa de Na$^+$ del enterocito, membranas plasmáticas laterobasales fueron solubilizadas utilizando diferentes detergentes, octyl glucoside, Tween 20, $C_{12}E_8$ y $C_{12}E_9$. La solubilización de la ATPasa de Na$^+$ y de la ATPasa de Na$^+$/K$^+$ fue óptima después de mezclar 1 mg de membranas con 1,5 mg de $C_{12}E_9$. El $C_{12}E_9$ solubilizó más del 60% de las proteinas de membranas y las ATPasas de Na$^+$ y Na$^+$/K$^+$ fueron recuperadas en un 80% en la fracción soluble. Adicionalmente, cuando glicerol al 25 % y ATP 2 mM fueron utilizados, la ATPasa de Na$^+$ fue estable despues de 3 dias. La ATPasa de Na$^+$ soluble demostró las siguientes características cinéticas: 1) es específicamente estimulada por sales de Na$^+$; 2) $K_{0.5}$ para Na$^+$= 4.62 ± 0.1 mM; 3) es estimulada por todas las sales de Na$^+$, 4) pH óptimo= 7.0; 5) es inhibida por furosemida ($IC_{50}$= 0,52 ± 0,10 nm). Las características cinéticas de la enzima solubilizada fueron similares a las descritas para la forma de la enzima unida a la membrana. Este trabajo demuestra la solubilización y caracterización de la ATPasa Na$^+$ a partir de membranas laterobasales del enterocito usando $C_{12}E_9$.

### INTRODUCTION

In the intestinal epithelial cells, ion transport across the basolateral plasma membranes plays an essential role in the transepithelial sodium movement and in the control of the intracellular environment. Indeed, it has been proposed that intestinal sodium transport is mediated by two active mechanisms: The Na$^+$/K$^+$ pump, that has been characterized at the cellular and molecular level and the second sodium pump that actively transports Na$^+$, accompanied by Cl$^-$ and water. This pump does not require K$^+$ to work, but is inhibited by furosemide (1, 2).

Although considerable functional evidences indicate the existence of the second sodium pump, and its associated Na$^+$-ATPase (for revision to see 3) as a different mechanism to Na$^+$/K$^+$ pump, and its associated Na$^+$/K$^+$-ATPase, the protein responsible for this transport mechanism has not been identified or isolated.

One of the most important factors limiting our understanding of the Na$^+$- ATPase is the lack of a highly active and stable enzyme, a pre-requisite for structural and functional studies. These limitations rise the necessity to develop a biochemical technique that allows the solubilization and isolation of the Na$^+$-ATPase. In this sense, the

detergents constitute an important tool in the extraction and purification of membrane proteins (4-9). Detergents which can be used for this purpose are broadly categorized into two groups: (i) nonionic (polyoxyethylene glycol or alkyl glucosides) and, in some instances, zwitterionic detergents (e.g. dodecyldimethylamine oxide, lysolecithins), and ii) detergents belonging to the group of bile salts or other steroid-based detergents such as 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfon ate (CHAPS) and digitonin. The foremost challenge in the solubilization of a membrane protein is to obtain a soluble protein in a stable form that retains its original function sodium dodecyl sulfate (SDS) is a detergent that is very much used in the procedures of $Na^+/K^+$-ATPase solubilization, although it partially denatures the enzyme molecule. Other detergents that have also been used to inactivate the enzyme in a concentration-dependent way are cholate and CHAPS (10). Thus 1-2% deoxycholate (DOC) solubilizes 50% of the renal $Na^+/K^+$-ATPase with retention of only half of the original specific activity. In contrast, CHAPS when used at a final concentration of 9 mM solubilized near 25% of the enzyme without loss of specific activity (10).

Another classes of detergents used in $Na^+/K^+$-ATPase solubilization are the O.G and polyoxyethylenes (Triton, Lubrol, Brij, $C_{12}E_8$, Tween). O.G was not efficient for $Na^+/K^+$-ATPase, because it needs long incubation times with the protein, with the consequent inactivation of the enzyme. In contrast, the polyoxyethylenes are good agents for $Na^+/K^+$-ATPase solubilization (10, 11).

A detergent that has been used with great success for membrane protein solubilization is the $C_{12}E_8$ (10, 12-15). Different results showed that $Na^+/K^+$-ATPase, previously treated with SDS, can be solubilized with $C_{12}E_8$ in an active form,

where most of the kinetic and conformational properties of the enzyme are preserved after the solubilization (12, 15).

This work reports for the first time, the solubilization of a fully active and stable $Na^+$-ATPase enzyme. We compared four detergents (O.G, Tween 20, $C_{12}E_8$, and $C_{12}E_9$) and demonstrated that protective conditions and low temperature are essential to maintain activity during solubilization. These findings emphasize the importance of identifying appropriate detergents for $Na^+$-ATPase solubilization and offer promise for the development of rapid and efficient methods to the purification of this enzyme.

## MATERIALS AND METHODS

### Materials

Adenosine triphosphate (Tris salt), ouabain, furosemide, vanadate, sodium chloride, potassium chloride, ethylenediaminetetracetic acid (EDTA), N-2-hydroxyethylpiperazine N'-2-ethanesulfonic acid (HEPES), tris (trishydroxymethyl)-aminomethane (TRIS), SDS, $C_{12}E_8$, $C_{12}E_9$ were obtained from Sigma Chemical Company, St Louis, USA.

### Preparation of basolateral plasma membranes

Basolateral plasma membrane vesicles were obtained as described by del Castillo et al (16). Briefly, guinea-pig small intestinal scrapings were homogenized in a medium containing 250 mM sucrose and 20 mM Tris-HCl (pH 7.2). Basolateral vesicles were isolated by differential centrifugation and application to a Percoll gradient. The final fraction (F-II) containing vesicles was resuspended in the homogenization medium, such that the protein concentration was greater than 4 mg/mL. F-II was used for ATPase assays. It has been previously shown that this fraction contains 60% in-

side-out basolateral plasma membrane vesicles and it is practically free of other membrane contaminants.

## Membrane solubilization

To determine the optimum conditions to solubilize membrane-bound Na$^+$-ATPase, basolateral plasma membranes (1 mg of protein/mL) were solubilized in a buffer containing 8 mM Hepes, 13 mM imidazol, 1mM EDTA, 20 mM Tris-HCl (pH 7.2), 25% glycerol and different amounts of either C$_{12}$E$_8$, octyl glycoside, Tween 20 and C$_{12}$E$_9$. After 30 minutes of incubation at 4°C, non-soluble material was separated by centrifugation at 100.000 × g for 1 hour at 4°C in a Ti50 rotor using a Beckman ultracentrifuge. The clear supernatant and pellet were assayed for protein concentration and ATPase activity.

## ATPase assays

ATPase activities were determined as previously described by del Castillo and Robinson (1). The incubation medium, with a final volume of 0.5 mL, contained 50-100 mM Tris-HCl (pH 7.0 at 37°C), 5 mM MgCl$_2$, and when appropriate, 100 mM NaCl, 20 mM KCl and 2 mM ouabain. The concentration of Tris-HCl was varied to adjust the osmolarity of the medium. The final concentration of protein was between 2 and 20 μg/mL, the range in which ATP hydrolysis was a linear function of the protein concentration. The incubation medium was pre-incubated for 5 min at 37°C and the reaction was initiated by the addition of 2.5 mM ATP-Tris to the mixture. After 10 min of incubation, the reaction was stopped and the Pi liberated was determined following Cariani et al (17). Briefly, at the end of the incubation period the reaction was stopped by adding 1 mL ice-cold solution containing 2.8% ascorbic acid, 0.48% ammonium molybdate, 2.8% SDS and 0.48 N HCl. The tubes were placed on ice. After 10 min, 1.5

mL of a solution of 3.5% arsenite, 2% sodium citrate was added and incubated again at 37°C for 10 min. The developed color was read at 710 nm. K$_2$HPO$_4$ was used as Pi standard. Under these conditions, there was a linear relationship between the orthophosphate liberated and the incubation time. All determinations were run in triplicate. The activity was expressed as nmoles of phosphate liberated per mg of protein per min, after subtraction of a blank obtained on adding membrane suspension only after the reaction was stopped.

The ATPase activity obtained in the presence of Mg$^{2+}$ and ouabain is referred to as Mg$^{2+}$-ATPase. The difference in activity between this Mg$^{2+}$ ATPase and the activity in presence of Mg$^{2+}$, Na$^+$ and ouabain is denoted as the Na$^+$-ATPase. Both, Mg$^{2+}$ and Na$^+$-ATPase are insensitive to ouabain (2). The difference in activity in the presence of Mg$^{2+}$, Na$^+$, K$^+$ and ouabain and the activity determined in the presence of Mg$^{2+}$, Na$^+$ and K$^+$ is referred to as Na$^+$/K$^+$-ATPase.

## SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Santos and Ciancaglini (13). Samples (20 μL) were resuspended in 20 μL of 60 mM Tris-HCl, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol and 0.1% bromophenol blue. They were heated at 70°C for 5 minutes, centrifuged for 1 minute at 14,000 rpm and loaded on a 10% separating gel. Samples were run at 100 mV. Gel was stained with Coomassie blue R-250.

## Stability assay

Basolateral plasma membranes were solubilized with C$_{12}$E$_9$ (1.5 mg) in a buffer containing 8 mM Hepes, 13 mM Imidazol, 1mM EDTA, 20 mM Tris-HCl (pH 7.2), with and without 2 mM ATP and with 25% glycerol. Aliquots of supernatants were removed at zero (within 30-120 min of mixing), 12,

24, 36 and 48 hours to measure the ATPase activity. The native enzyme was incubated in the same buffer without detergent.

### Protein determination

Proteins were determined according to Gaad (18) with bovine serum albumin as a standard.

### Statistical analysis

Results are expressed as means ± standard deviation. The means were compared by one-way variance analysis (ANOVA), taking into account the treatment of experimental groups. The magnitude of differences was evaluated using the multiple comparative Bonferroni test. Each experimental data (n) corresponds to results obtained from different basolateral plasma membrane preparations.

### RESULTS

### ATPase activities in basolateral plasma membrane

To verify the presence of both $Na^+$-ATPase and $Na^+/K^+$-ATPase in basolateral plasma membrane the ATPase activity was measured (Table I). The $Na^+$-ATPase activity increased from $13.67 \pm 1.86$ (homogenate) to $135 \pm 9.82$ nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$ (basolateral plasma membrane) corresponding to an increase of 9.87 times. This activity was measured in the presence

of 2 mM ouabain. The $Na^+/K^+$-ATPase activity increased from $45.32 \pm 5.45$ (homogenate) to $304 \pm 12.34$ nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$ (basolateral plasma membrane), corresponding to an increase of 11.12 times.

### Solubilization of $Na^+$-ATPase

We tested four different detergents for their capacity to solubilize the $Na^+$- and the $Na^+/K^+$-ATPases from basolateral plasma membrane. They belong to non-ionic series: O.G, Tween 20, $C_{12}E_8$ and $C_{12}E_9$. O.G and Tween 20 were slightly poorer solubilizing agents and also caused deactivation of the ATPases activities. At the concentration of O.G (1.0 mg), 31% of the total protein was released in the supernatant, but only 12% ($64.86 \pm 4.86$ nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$) of $Na^+$-ATPase activity was recovered. The increase of the ionic strength of the solubilization buffer had not effect on the efficiency of solubilization (data not shown). With Tween 20 at the lower concentration (0.5 mg) only 17% of $Na^+$-ATPase activity ($64.33 \pm 3.61$ nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$) was recovered in the supernatant, for all Tween 20 concentrations, the solubilization was incomplete, since some $Na^+$-ATPase activity was found in pellet (data not shown). The results indicate that $C_{12}E_8$ (1.5 mg/mg of protein) was the best solubilizing agent; it solubilized 70% of the total protein, but inhibits in 50% the $Na^+$-ATPase activity ($108.97 \pm$

### TABLE I
Na+-ATPase AND Na+/K+-ATPase ACTIVITIES FROM INTESTINAL EPITHELIAL CELLS:
ENRICHMENT OBTAINED BY CELLULAR FRACTIONATION

| | $Na^+$-ATPase activity (nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$) | $Na^+/K^+$-ATPase activity (nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$) |
|---|---|---|
| Homogenate | $13.67 \pm 1.86$ | $45.32 \pm 5.45$ |
| Basolateral plasma membrane | $135.04 \pm 9.82$ | $504.03 \pm 12.34$ |
| Enrichment (X) | 9.87 | 11.12 |

Membranes were treated with SDS to open vesicles, as reported before, and the $Na^+/K^+$-ATPase and $Na^+$-ATPase activities were calculated as the difference in Pi relased with and without 2 mM ouabain, respectively. The data are expressed as means ± standard deviations of three different preparations.

8.34 nmol Pi $\times$ mg$^{-1}$ $\times$ min$^{-1}$). In contrast, $C_{12}E_8$ produced an efficient solubilization and preservation of the Na$^+$/K$^+$-ATPase (data not shown).

Finally, $C_{12}E_9$ was evaluated as a solubilizing agent. Fig. 1 (A and B), illustrates the variation of detergent concentration for a fixed protein concentration. The amount of proteins and solubilized ATPases increased proportionally with the increase in detergent concentration, reaching a maximum at a 1.5 mg $C_{12}E_9$/mg protein ratio. Analysis of SDS/PAGE (10%) supports these results (Fig. 1C, lanes 2-4). At protein detergent ratios above 1.5, the Na$^+$- and Na$^+$/K$^+$-ATPases activities in the supernatant decreased, however the amount of solubilized protein remained constant. The decrease in ATPase activity can be interpreted as inactivation by excess of detergent. Under optimal conditions (1.5 mg detergent/mg protein ratio), $C_{12}E_9$ solubilized over 60% membrane protein and Na$^+$- and Na$^+$/K$^+$- ATPases were recovered over 80% in the soluble fraction without inactivation. The Na$^+$- and Na$^+$/K$^+$-ATPase activities of the solubilized enzyme were similar to that detected in the basolateral plasma membrane. All the subsequent experiments were carried out using a relationship detergente/protein of 1.5. When the procedure of solubilzation of the basolateral plasma membranes was carried out in absence of ATP as a protective agent, the activity of Na$^+$-ATPases fell significantly. These results are in agreement with those described by Rabon et al. (19), using other detergents. In consequence, all the solubilization steps were carried out in the presence of 2 mM ATP.

## Kinetics characterization
## of the solubilized Na$^+$-ATPase

In order to determine the functional integrity of the $C_{12}E_9$ solubilized Na$^+$-ATPase some kinetics studies were per-
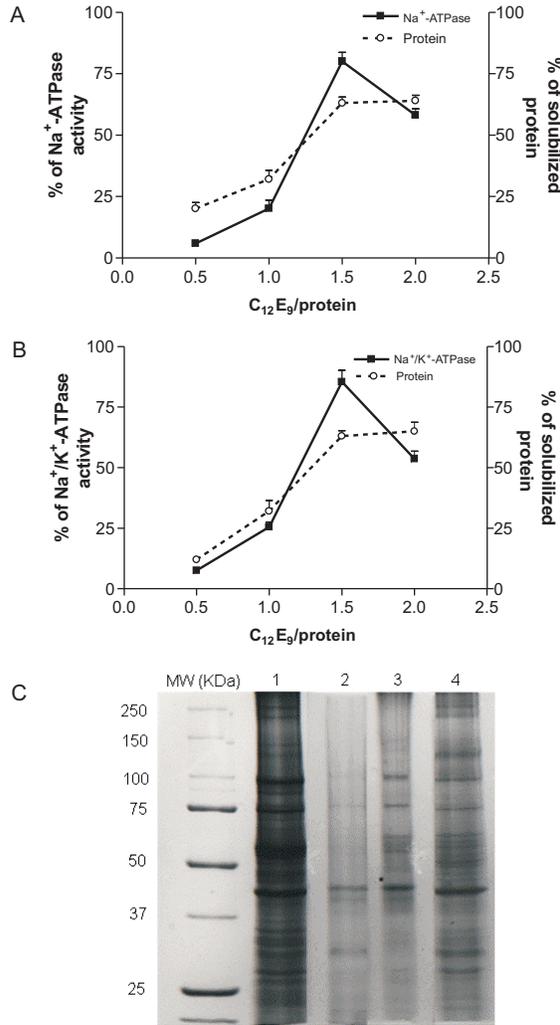


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Fig. 1.   Solubilization of membrane-bound Na$^+$-ATPase and Na$^+$/K$^+$-ATPase by $C_{12}E_9$. Basolateral plasma membranes were solubilized with varying concentrations of $C_{12}E_9$, ultracentrifuged and the supernatant was assayed for (A) Na$^+$-ATPase and (B) Na$^+$/K$^+$-ATPase (■) activities and protein content (○) as described under experimental procedures. Activities are expressed as a percent of activities measured in the basolateral plasma membrane in absence of detergent. (C) SDS-PAGE: Samples (20 $\mu$L) from each supernatant were run in a 10% polyacrylamide gel at 100 mV. Gel was stained with coomassie. The data are expressed as means ± standard deviations of three different preparations.

formed. The enzyme was incubated in the presence of 100 mM of the chloride salt of the different monovalent cations. The results are shown in the Fig. 2. As already shown for membrane-bound enzyme (2), the cation stimulation was specific for Na$^+$. The optimal Na$^+$ concentration, as show in Fig. 3, is around 100 mM and the apparent $K_{0.5}$ was 4.62 mM. In addition, Fig. 4 shows that Na$^+$ stimulation was independent of Na$^+$-accompanying anion. This observation is similar to that made with basolateral plasma membrane (2). Fig. 5 shows the effect of pH on the Na$^+$-ATPase activity. The optimal pH was about 7.0. In the Fig. 6, the ouabain-insensitive Na$^+$-ATPase activity was progressively inhibited by furosemide (0.002 to 2 mM) in a dose-dependent manner. Furosemide at 2 mM inhibits the Na$^+$-ATPase activity by 85%. The concentration of furosemide that produces half-maximum inhibition ($IC_{50}$), calculated through fitting of experimental data was $0.52 \pm 0.10$ mM.

**Stability of solubilized Na$^+$-ATPase activity**

When a detergent/protein ratio of 1.5 was used with no ATPase-protective agent, the activity of the soluble Na$^+$-ATPase clearly decreased (Fig. 7). This inactivation was lower in the presence of 2 mM ATP. When 25 % glycerol was added, the solubilized enzyme was stable after 3 days at 4°C. These results indicate that the samples stored in presence of ATP and glycerol in all times showed only small losses of Na$^+$-ATPase activity and demonstrated the long term stability of this enzyme activity under our experimental conditions.

### DISCUSSION

In the present study, the existence of Na$^+$-ATPase was confirmed in basolateral plasma membrane of small intestinal epithelial cells. Two active Na$^+$-stimulated
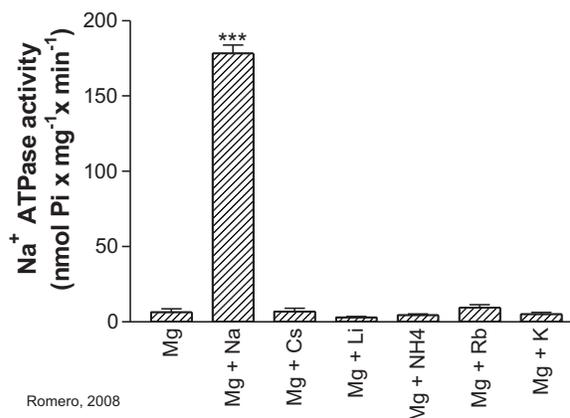


Fig. 2. Effect of different cations on solubilized Na$^+$-ATPase activity. The ATPase activity was measured as described in Section 2 and expressed in nmol Pi liberated per min per mg protein. The cations were added as chloride salts. Assays were performed in the presence of 2 mM ouabain, Mg$^{2+}$ 5mM, cations, 100 mM. All experiments were done in triplicate. The values are expressed as the means ± standard deviations of five different preparations. *** p < 0.001.



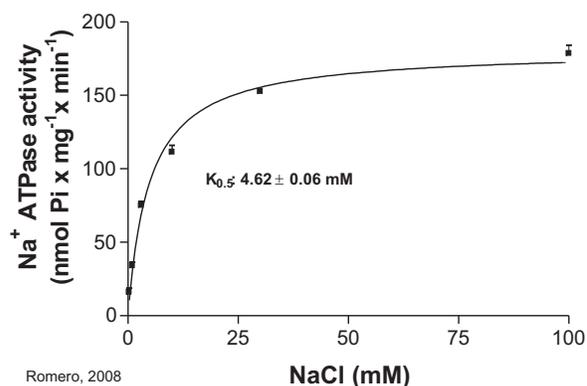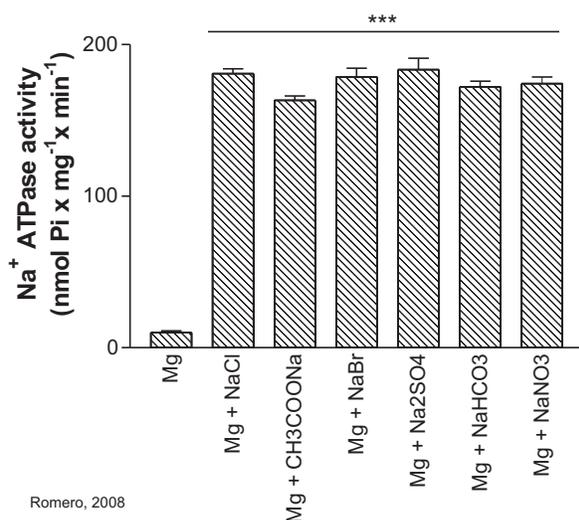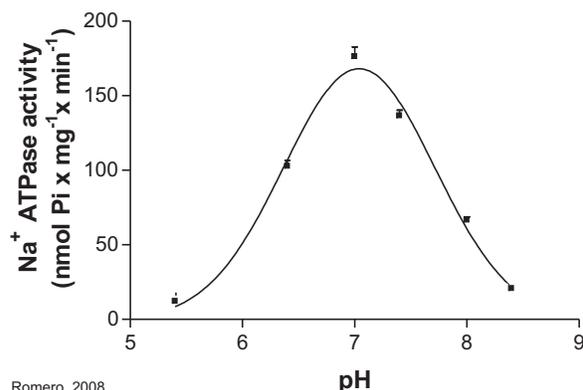Fig. 3. Effect of different sodium concentrations on the solubilized Na$^+$- ATPase. The ATPase activity was measured as described in Section 2 and expressed in nmol Pi liberated per min per mg protein. Assays were performed in the presence of 2 mM ouabain, Mg$^{2+}$ 5mM, cations, 100 mM. All experiments were done in triplicate. The values are expressed as means ± standard deviations of five different preparations. The kinetic parameters were calculated by the following equation: $v = V0\, Ki\, /Ki + [I]$.
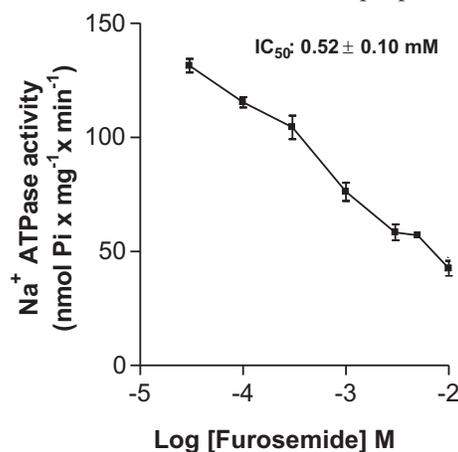
Fig. 4.   Effect of different anions on the solubili-
zed Na$^+$- ATPase. The ATPase activity
was measured as described in Section 2
and expressed in nmol Pi liberated per
min per mg protein. The anions were
added as sodium salts. Assays were per-
formed in the presence of 2 mM
ouabain, Mg$^{2+}$ 5mM, anions, 100 mM.
All experiments were done in triplicate.
The values are expressed as means ±
standard deviations of five different
preparations. *** $p < 0.001$.

ATPases were identified: a potassium-de-
pendent, ouabain-sensitive Na$^+$/K$^+$-ATPase
and a potassium-independent, ouabain-in-
sensitive Na$^+$-ATPase. These results are in
agreement with the ones described by Del
Castillo et al (2). Although the precise bio-
chemical function of Na$^+$-ATPase and its ex-
act physiological role in mammalian intesti-
nal cells remains unraveled, it has been pro-
posed an important role of this enzyme on
extracellular volume control by the fine
tuning of renal sodium excretion, being a
target for autacoids and hormonal selective
regulation in proximal tubule cells (20-25).
In this way, the isolation of the Na$^+$-ATPase
as being a different entity from the Na$^+$/K$^+$-
ATPase is extremely important for the com-
prehension of its physiological role in
transepithelial sodium transport and in the
control of the intracellular environment.

Fig. 5.   Effect of pH of the incubation medium
on solubilized Na$^+$- ATPase activity. The
ATPase activity was measured as de-
scribed in Section 2 and expressed in
nmol Pi liberated per min per mg pro-
tein. The buffer used for obtaining the
different pH values: Acetate, pH 5;
Tris-HCl, pH 6-9.0. Incubation medium
contained. MgCl$_2$, 5 mM; NaCl 100 mM;
KCl 20 mM; 2 mM ouabain. All experi-
ments were done in triplicate. The val-
ues are expressed as means ± standard
deviations of five different preparations.

Fig. 6.   Effect of increasing concentrations of
furosemide on solubilized Na$^+$-ATPase ac-
tivity. The ATPase activity was measured
as described in Section 2 and expressed
in nmol Pi liberated per min per mg pro-
tein. Incubation medium contained
MgCl$_2$, 5 mM; NaCl 100 mM and different
concentrations of furosemide. All experi-
ments were done in triplicate. The values
are expressed as means ± standard devia-
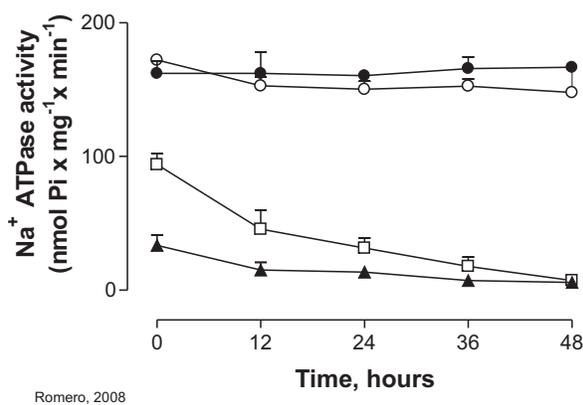tions of five different preparations.

Fig. 7. Time course of solubilized $Na^+$- ATPase activity from basolateral plasma membranes. Basolateral plasma membrane were solubilized with $C_{12}E_9$ (1.5 mg) in in a buffer containing 8 mM Hepes, 13 mM Imidazol, 1mM EDTA, 20 mM Tris-HCl (pH 7.2), without (▲) and with (☐) 2 mM ATP and with 2 mM ATP and 25% glycerol (○). Aliquots of supernatants were removed at various times to measure the ATPase activity. The native enzyme was incubated in the same buffer without detergent (●). The ATPase activity was measured as described in Section 2 and expressed in nmol Pi liberated per min per mg protein. All experiments were done in triplicate. The values are expressed as means ± standard deviations of five different preparations.

Detergent solubilization is a crucial step in the characterization, isolation and purification of an integral membrane (26, 27). This report compared the solubilization of $Na^+$-ATPase by different detergents and reactants. The ability of a range of detergents such O.G, Tween 20, $C_{12}E_8$ y $C_{12}E_9$ were examined. The results show that $Na^+$-ATPase was solubilized with all detergents used. The O.G and Tween 20 have been useful in the study of many membrane proteins (27, 28); however, after treatment of basolateral plasma membranes with these detergents, up to 40% of activity of intestinal $Na^+$-ATPase was recovered in the supernatant with retention of only half of the original specific activity. In addition, O.G was not efficient for $Na^+/K^+$-ATPase solubilization because it inactivates the enzyme (data not shown). Identical results were obtained with rabbit kidney membrane (29). A plausible explanation for the instability and low activity of $Na^+$-ATPase in the presence of long chain detergents, with a bulky head group, is that these detergents are unable to establish adequate contact with the hydrophobic region, due to steric restrictions caused by the bulky head group.

A detergent that has been used with great success in $Na^+/K^+$-ATPase and $Ca^{+2}$-ATPase solubilization is $C_{12}E_8$ (10, 12-15, 30-32). It has been shown that $Na^+/K^+$-ATPase, previously treated with SDS, can be solubilized with $C_{12}E_8$ in active form, where most of the kinetic and conformational properties of the enzyme are preserved after solubilization.

In this study, $C_{12}E_9$ was better for preserving $Na^+$-ATPase activity than $C_{12}E_8$. The $C_{12}E_9$ (1.5 mg detergent/1.0 mg protein) allows the solubilization of both $Na^+$- and $Na^+/K^+$-ATPase without inactivation, as showed by 80% recovery of total activity after solubilzation. Thus, the detergent with an intermediate chain length (C12) and a polyoxyethyleneglycol head group ($C_{12}E_9$) are optimal for the solubilization of $Na^+$- and $Na^+/K^+$-ATPases.

In order to determine the integrity of $C_{12}E_9$-solubilized $Na^+$-ATPase some kinetic studies were examined. As shown (Figs. 2-6), detergent solubilization had no effect on the binding affinity of $Na^+$-ATPase since $K_{0.5}$ for $Na^+$ in membrane and solubilized ATPase were practically identical (2). The $K_{0.5}$ for $Na^+$ is in agreement with that found for the $Na^+$-ATPase of other organisms (33) and is different from that observed in dog and sheep kidney outer medulla $Na^+/K^+$-ATPase, measured in the ab-

sence of $K^+$ (34, 35). The soluble intestinal $Na^+$-ATPase is only stimulated by $Na^+$ and is indifferently stimulated by different sodium salts. It is known that $K^+$ acts as an activator of mammalian $Na^+/K^+$-ATPase activity with a $K_{0.5}$ of approximately around 1.9 mM (36). In this work, KCl up to 100 mM did not change the $Na^+$-ATPase activity of small intestinal epithelial cells (Fig. 2). The apparent optimum pH for $Na^+$-ATPase was 7.0, similar to that reported for the membrane-bound $Na^+$-ATPase (2). The effect of some $Na^+$-ATPase inhibitors showed that solubilized enzyme is inhibited by 3 mM furosemide, as described by del Castillo et al (2). Additionally, the enzyme activity was totally inhibited by 3 $\mu$M vanadate (data not shown), a specific P-type ATPase inhibitor (37-39).

Different buffers were tested for their ability to preserve the $Na^+$-ATPase activity in the soluble fraction. By trial and error, it was determined that the use of a buffer containing glycerol (25%), 1.5 mg/mL $C_{12}E_9$, 2 mM ATP, 8 mM Imidazol, 13 mM Hepes results in recovery of 70% or more of the $Na^+$-ATPase activity in the supernatant following ultracentrifugation. The presence of ATP during the incubation of $Na^+$-ATPase-rich membrane fraction with $C_{12}E_9$ is recommended, because this substrate protects the enzyme against inactivation. In reference to the treatment temperature, solubilization at low temperature has been reported to reduce protein aggregation (40). We used 4°C as the temperature of solubilization. Under these conditions, the stability of the $C_{12}E_9$-protein preparations was sufficient for further purification. Nonetheless, the ability of this buffer to maintain the $Na^+$-ATPase activity represents an important advancement for future analysis and purification of the enzyme. Most importantly, the ATPase activity appears to be relatively stable in this buffer for up to 48 hours at 4°C. Such stabilization will allow future experiments to focus on conventional means of membrane protein purification.

In summary, this work provides a rapid and efficient solubilization procedure for $Na^+$-ATPase from basolateral plasma membranes of small intestinal epithelial cells. In particular, several properties of the solubilized $Na^+$-ATPase with regard to solubility and stability are presented. The functional data suggest that the $C_{12}E_9$-solubilized $Na^+$-ATPase preserves the main characteristic observed for the membrane-bound ATPase such as specific stimulation by $Na^+$ and inhibition by furosemide. Current studies are aimed at purification of the $Na^+$-ATPase enzyme.

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