Uncoupling by Trehalose of Ca^{2+} Transport and ATP Hydrolysis by the Plasma Membrane (Ca^{2+} + Mg^{2+})ATPase of Kidney Tubules

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Trehalose, the disaccharide of glucose, inhibits both initial rate and maximal capacity of ATP-dependent Ca^{2+} transport in inside-out vesicles of basolateral membrane from kidney proximal tubules. This inhibition ($I_{0.5}$ = 60 mM) cannot be attributed to an increase in Ca^{2+} permeability, since the rate of EGTA-stimulated Ca^{2+} efflux from preloaded vesicles is not modified by trehalose. In the presence of 600 mM trehalose, Ca^{2+} uptake was almost completely inhibited, but the Ca^{2+}-stimulated ATPase activity was unaffected; thus trehalose uncouples the Ca^{2+} transport from the ATPase activity. The Ca^{2+} transport inhibition by trehalose is reversible, since the inhibition disappeared when the vesicles were pre-incubated with 600 mM trehalose and then diluted in reaction medium to measure Ca^{2+} accumulation. Other mono- and disaccharides such as glucose, fructose, galactose, sucrose, maltose and lactose were tested but were not so effective as trehalose. The uncoupling of Ca^{2+} transport from hydrolysis can be explained by an interaction of trehalose with the phospholipid environment of the enzyme that induces conformational changes in specific domains of the enzyme so as to impair the coupling process.

Introduction

Trehalose, a non-reducing disaccharide of glucose, is widely distributed among living systems (Sussman and Lingappa, 1959). The survival of anhydrobiotic organisms in a dry state is correlated with the accumulation of high concentrations of trehalose which may reach 20% of the dry weight of the organism (Sussman and Lingappa, 1959). During their rehydration, degradation of this compound is observed (Clegg, 1965; Clegg and Filosa, 1961; Madin and Crowe, 1975; Sussmann and Lingappa, 1959). The protective role of trehalose has been attributed to its capacity for preserving dried membranes (Crowe and Crowe, 1984; Crowe *et al.*, 1983, 1984 a, 1984 b, 1985, 1987; Rudolph and Crowe, 1985), and to its contribution to intracellular osmoregulation (Yancey *et al.*, 1982), but it remains to be determined whether such high trehalose levels have other effects in the cells.

It has been reported that trehalose can preserve the Ca^{2+}/ATP coupling ratio in lyophilized and rehydrated vesicles obtained from sarcoplasmic reticulum of lobster abdominal muscles, when the sugar is present during the lyophilization process. It was proposed that trehalose preserves interactions of the enzyme with the phospholipid bilayer that are needed to avoid leakness and to preserve the coupling ratio (Crowe *et al.*, 1983, 1984 b). On the other hand, trehalose increases the apparent affinity for Pi during the phosphorylation reaction of rabbit sarcoplasmic reticulum (Ca^{2+} + Mg^{2+})ATPase, an observation that shows its capacity to modify the equilibrium between the different conformations of the enzyme (Chini *et al.*, 1991).

The present report shows that trehalose can inhibit ATP-dependent Ca^{2+} transport in vesicles derived from basolateral plasma membranes of kidney proximal tubules. Our results show that trehalose uncouples ATP hydrolysis and Ca^{2+} transport, without increasing the Ca^{2+} permeability of the membrane.

Abbreviations: EGTA, [ethylenebis(oxyethylene)-nitrilo]tetraacetic acid; MOPS, 4-morpholinepropanesulfonic acid; Pi, inorganic orthophosphate; PiK, K_2HPO_4/KH_2PO_4 mixture; Tris, tris(hydroxymethyl)-aminomethane.

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Materials and Methods

Preparation of membrane vesicles

Basolateral membrane vesicles were isolated from sheep kidney proximal tubules by a modification of the Percoll gradient method described by Grassl and Aronson (1986). Compared with the initial homogenate this membrane fraction was enriched 9–12-fold in the specific activity of basolateral membrane marker $(Na^+ + K^+)$ATPase. Protein concentrations were determined using the Folin phenol reagent (Lowry *et al.,* 1951) and bovine serum albumin as a standard.

^{45}Ca uptake

Except when otherwise noted the basic medium contained, in a final volume of 0.5 ml, 30 mM Tris-HCl buffer (pH 8.5), 5 mM ATP, 5 mM $MgCl_2$, 80 mM PiK, 1 mM ouabain, 10 mM NaN_3, 0.1 mM EGTA and 0.05 mM $^{45}CaCl_2$ (10 μM free Ca^{2+}), at 37 °C. Concentrations of trehalose and other modifications in the medium are specified in the corresponding figure legends and tables. ^{45}Ca uptake was started by the addition of membranes (protein concentration 0.2 mg/ml) and stopped by Millipore filtration (Martonosi and Feretos, 1964), using 0.45 μm pore size filters. The ^{45}Ca remaining in the vesicles, after the filters were washed with a cooled solution containing 2 mM $La(NO_3)_3$, 100 mM KCl, and 20 mM MOPS-Tris (pH 7.0), was counted in a liquid scintillation counter.

ATPase activity

ATPase activity was measured in the same conditions as Ca^{2+} uptake, except that the reaction was quenched with acid, followed by adsorption the non-hydrolyzed [γ-^{32}P]ATP on charcoal (Grubmeyer and Penefsky, 1981). Following centrifugation at 4000 × *g* for 30 min, an aliquot of the supernatant was withdrawn to measure the amount of ^{32}Pi released. Spontaneous hydrolysis of [γ-^{32}P]ATP was measured in tubes run in parallel in which the enzyme was added after the acid. The $(Ca^{2+} + Mg^{2+})$ATPase activity was the difference between the ATP hydrolysis measured in the presence and absence of $CaCl_2$ (EGTA 1 μM). ^{32}Pi was counted in a liquid scintillation counter.

Statistical analysis

Experimental data were fitted using the non-linear regression computer program Enzfitter (Elsevier Biosoft). Goodness of fit was assessed by computing reduced chi squares for the fits, as described by Motulsky and Ransnas (1987).

Reagents

ATP, ouabain, phosphoenolpyruvate, pyruvate kinase, EGTA, Tris, trehalose, and the other sugars tested were purchased from Sigma. ^{32}Pi was from the Brazilian Institute of Energy and Nuclear Research and ^{45}Ca was obtained from New England Nuclear. Glass-distilled water deionized by the MilliQ system of resins (Millipore Corp.) was used in the preparation of all solutions. [γ-^{32}P]ATP was prepared as described by Glynn and Chappell (1964).

Results

Measurements of Ca^{2+} uptake by vesicles derived from basolateral membranes of kidney proximal tubules show that the ATP-dependent Ca^{2+} transport is inhibited when 600 mM of trehalose is present in the medium (Fig. 1). This effect is specific for the active Ca^{2+} transport since the ATP-independent Ca^{2+} binding is not modified by the addition of trehalose (data not shown).

Fig. 2 shows that the addition of 600 mM trehalose, a concentration that completely inhibited Ca^{2+} accumulation, does not affect the rate constant for EGTA-induced Ca^{2+} efflux from vesicles preloaded with Ca^{2+} (filled circles). A rapid and complete release of Ca^{2+} from the vesicular lumen is observed on addition of 10 μM A 23187, a Ca^{2+} ionophore. Upon addition of 2 mM EGTA, the efflux of Ca^{2+} can be described by the sum of two exponential functions. The rate constant of the smaller, faster component could not be resolved with the technique employed. The larger, slower component of EGTA-induced Ca^{2+} efflux (k of 0.010 min^{-1}) was not altered by the presence of 600 mM trehalose together with the EGTA. Thus the trehalose effect can not be attributed to an increase in Ca^{2+} permeability.

The mechanism of inhibition of the ATP-dependent Ca^{2+} transport might be associated with inhibition of the $(Ca^{2+} + Mg^{2+})$ATPase activity. However,

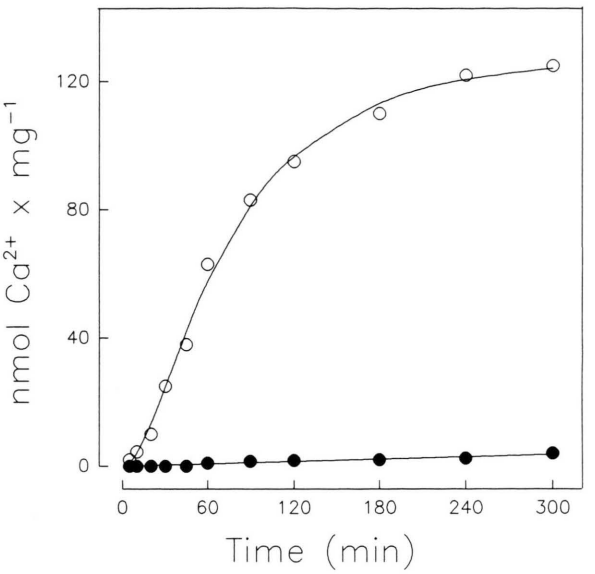

Fig. 1. Time course of Ca^{2+} uptake by basolateral membrane vesicles in absence and presence of trehalose. Assays contained: 30 mM Tris-HCl (pH 8.5), 10 mM NaN_3, 1 mM ouabain, 5 mM $MgCl_2$, 10 μM free Ca^{2+}, 5 mM ATP, 80 mM PiK, 5 mM phospho(enol)pyruvate, 50 μg/ml pyruvate kinase and 200 μg/ml membrane protein. ATP-dependent Ca^{2+} transport was measured in the absence (○) or in the presence of 600 mM trehalose (●). Standard errors were ≤10% of the absolute mean values of eight experiments with different membrane preparations ($n = 8$).

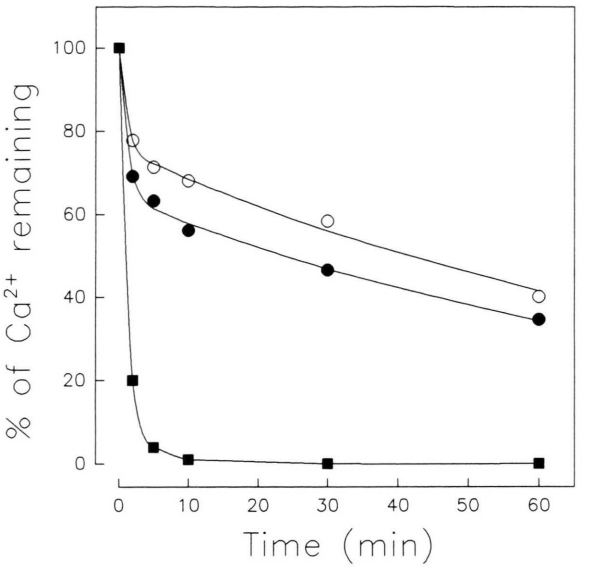

Fig. 2. Time course of Ca^{2+} efflux from preloaded basolateral membrane vesicles in the absence and presence of trehalose. Vesicles were preloaded for 5 h in the experimental conditions described in the legend of Fig. 1. Ca^{2+} remaining in the vesicles was measured at the times indicated on the abscissa after dilution twenty-fold into a medium containing: 2 mM EGTA (○), 2 mM EGTA plus 600 mM trehalose (●) or 2 mM EGTA and 10 μM A 23187 (■). The amount of Ca^{2+} uptaken up after 5 h was 126 ± 12 mmol · mg^{-1} protein ($n = 8$), and standard errors were ≤10% of absolute values.

it was found that the ATP hydrolysis stimulated by micromolar Ca^{2+} concentrations and measured under the same conditions as Ca^{2+} transport is not inhibited by trehalose (Fig. 3, squares). In the same figure it is shown that the inhibitory effect of trehalose is dose-dependent, with an $I_{0.5}$ of 60 mM (circles). The uncoupling effect of trehalose on ATP-dependent Ca^{2+} transport is reversible. The inhibition of Ca^{2+} uptake disappears if the vesicles are diluted in a trehalose-free medium after incubation for 60 min in the presence of 600 mM trehalose (data not shown).

Several other carbohydrates were tested for their ability to inhibit ATP-dependent Ca^{2+} transport, but none was so effective as trehalose. Only lactose, maltose and trehalose had significantly inhibited the activity (Table I).

Discussion

The role of trehalose in biological systems has been attributed to its ability to preserve the structural and functional integrity of biological membranes in dry conditions (Crowe and Crowe, 1984; Crowe *et al.*, 1983, 1984a, 1984b, 1985, 1987; Rudolph and Crowe, 1985). This phenomenon is related to the occurrence of a strong interaction between polar groups of phospholipids and hydroxyl groups of trehalose (Crowe and Crowe, 1984; Crowe *et al.*, 1984a).

In renal plasma membranes the uncoupling of ATP-dependent Ca^{2+} transport and ATP hydrolysis (Fig. 3) may reflect an interaction of trehalose with the enzyme's phospholipid environment. The Ca^{2+} transport/ATP hydrolysis coupling ratio of the (Ca^{2+} + Mg^{2+})ATPase from sarcoplasmic reticulum is affected by the lipid composition of the membrane (Navarro *et al.*, 1984). Duramycin, a polypeptide antibiotic that modifies phospholipid-protein interactions in sarcoplasmic reticulum vesicles, inhibits the ATP-dependent Ca^{2+} uptake without affecting the hydrolysis of ATP or the permeability of the membrane (Navarro *et al.*, 1985). The tre-

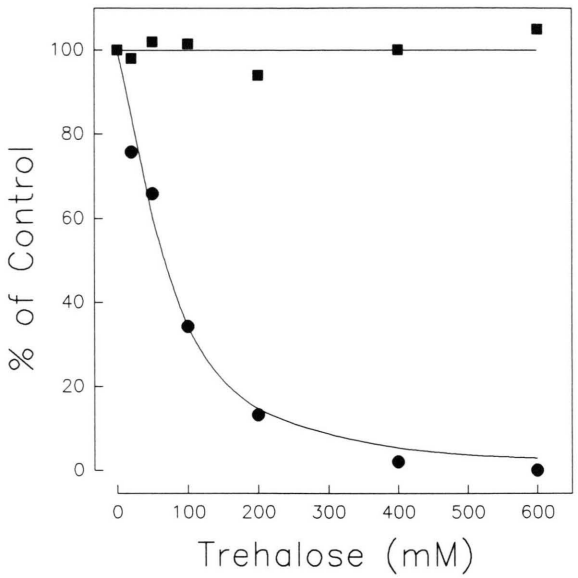

Fig. 3. Uncoupling by trehalose of ATP-dependent Ca^{2+} uptake and $(Ca^{2+}+Mg^{2+})$ATPase activity. ATP-dependent Ca^{2+} uptake (●) and $(Ca^{2+}+Mg^{2+})$ATPase activity (■) were measured after 30 min at 37 °C in the same conditions indicated under Materials and Methods (10 μM ionized Ca^{2+}), in the presence of trehalose concentrations shown on the abscissa. The maximal amount of Ca^{2+} uptaken up under these conditions was 28 ± 2 nmol · mg^{-1} protein ($n = 8$), and the velocity of ATP hydrolysis in the absence of trehalose was 26 ± 2 nmol · mg^{-1} · min^{-1}. ATP hydrolysis in the absence of Ca^{2+} (1 mM EGTA) were constant for all trehalose concentrations. Standard errors were ≤10% of absolute values.

halose-induced uncoupling of ATP hydrolysis and Ca^{2+} transport reported in this paper for renal plasma membranes may be related to the fact that trehalose can decrease membrane mobility by its interaction with membrane phospholipids (Crowe and Crowe, 1988). Since the Ca^{2+} binding site in different Ca^{2+}-ATPases is located in a hydrophobic region (Carafoli, 1991, 1992; Clarke *et al.*, 1989; Pick and Racker, 1979; Shull and Greeb, 1988), interactions between trehalose and the phospholipids around this domain might impair the enzyme conformational changes associated with cation translocation.

Disaccharides interact with phospholipids and proteins (Arakawa and Timasheff, 1982; Back *et al.*, 1979; Carpenter and Crowe, 1989; Crowe and Crowe, 1984; Crowe *et al.*, 1984 a, 1987; Giles and McKay, 1962; Lakshmi and Nandi, 1976; Lee and Timasheff, 1981) increasing the hydrophobic interactions between non-polar portions of proteins (Back *et al.*, 1979; Lakshmi and Nandi, 1976) and reducing the partitioning of hydrophobic groups from an aqueous to a non-polar environment (Back *et al.*, 1979). These carbohydrates also interact with water, forming hydrogen bonds that decrease water activity (Crowe and Crowe, 1984; Somero, 1986). However, the uncoupling of Ca^{2+} transport and ATP hydrolysis cannot be explained by a decrease in water activity around the enzyme, since other carbohydrates were not nearly so effective (Table I). The ability to uncouple transport from hydrolysis to different extents may be related with some of the physical properties of these carbohydrates (Duda and Stevens, 1990), which are also thought to endow trehalose with the ability to be the most effective carbohydrate on preserving structure and function of some membranes (Crowe *et al.*, 1987).

Table I. Effects of several carbohydrates on ATP-dependent Ca^{2+} uptake. ATP-dependent Ca^{2+} uptake at 30 min was measured in the experimental conditions described in the legend of Fig. 1. The absolute value for 100% Ca^{2+} uptake was 28 ± 2 nmol · mg^{-1} protein ($n = 8$), and standard errors were in all cases ≤10% of absolute values.

Carbohydrate [600 mM]	Ca^{2+} uptake [% of control uptake]
Fructose	95.8
Glucose	92.5
Galactose	89.5
Sucrose	82.3
Maltose	48.0
Lactose	19.5
Trehalose	5.0

The uncoupling promoted by trehalose suggests that in systems where trehalose occurs, this solute can regulate physiological processes. In other systems that are able to accumulate polyols, but not trehalose, polyols modify catalytic properties of ion transporting enzymes. Recently, it has been shown that carbohydrates accelerate the ATP ↔ Pi exchange reaction catalyzed by renal plasma membrane $(Ca^{2+}+Mg^{2+})$ATPase (Vieyra *et al.*, 1989, 1991) and increase the level of phosphorylation by Pi in the $(Ca^{2+}+Mg^{2+})$ATPase from sarcoplasmic reticulum (Chini *et al.*, 1991). These data suggest that trehalose may modulate ion transport in some cells when it is present in high concentrations.

The mechanism that concentrates the urine to an osmolality several times that of plasma results in high concentrations of solutes. In rabbits during antidiuresis sorbitol and inositol are present at high concentrations in the inner medulla but not in urine suggesting that these polyols play a significant role in the maintenance of intracellular osmotic balance (Bagnasco *et al.*, 1986). Although there is no trehalose in kidney tissues, the uncoupling of the Ca^{2+} pump promoted by this polyol demonstrates a modulation by an osmolyte at high concentration, and supports the thesis that the physiological osmolytes present at high concentrations under specific physiological and pathological conditions could be involved in the modulation of several biochemical pathways.

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