# Monosaccharides and Disaccharides Decrease the $K_m$ for Phosphorylation of a Membrane-Bound Enzyme ATPase

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The disaccharides trehalose and sucrose, and to a lesser extent the monosaccharides glucose and fructose, decrease the apparent $K_m$ of the $Ca^{2+}$,$Mg^{2+}$-ATPase of sarcoplasmic reticulum for Pi. This effect is more pronounced at pH 7.4 than at pH 6.2. The enzyme is not phosphorylated by Pi when the temperature of the medium is decreased to 0 °C, but when 1.5 M trehalose or sucrose is present phosphoenzyme formation increases to 0.5 μmol E-P/g protein.

## Introduction

The $Ca^{2+}$,$Mg^{2+}$-ATPase of sarcoplasmic reticulum catalyzes both hydrolysis and synthesis of ATP [1, 2]. The first step of the catalytic cycle in the direction of ATP synthesis is the phosphorylation by Pi of the $Ca^{2+}$-deprived enzyme [1]. This reaction does not require the energy derived from the $Ca^{2+}$ gradient [2, 3]. The phosphorylation reaction is pH- and temperature-dependent. At pH 6.0 the apparent $K_m$ for Pi is about 1.5 mM, and it increases to a value higher then 10 mM when the pH is raised to the physiological range 7.0–7.4 [2]. Maximal levels of phosphoenzyme are obtained above 20 °C and phosphorylation is abolished when the temperature is decreased to 0 °C [4]. The addition of an organic solvent such as dimethyl sulfoxide or glycerol leads to an increase in the apparent affinity of the enzyme for Pi and abolishes both pH and temperature dependence of the phosphorylation reaction [2, 5]. Similar effects are obtained with quarternary methylamines such as trimethylamine-N-oxide and glycine betaine [6].

Trehalose is found in high concentrations in many anhydrobiotic organisms [7], and is responsible for the survival of these organisms after dehydration. Polyols in general can increase the salt tolerance of some organisms and protect the cells from low temperatures [8]. Here we demonstrated that high concentrations of saccharides can lad to changes in the enzymatic properties of the membrane-bound enzyme the $Ca^{2+}$ transporting ATPase of sarcoplasmic reticulum.

*Abbreviations:* MOPS, 4-morpholinepropanesulfonic acid; $Me_2SO$, dimethyl sulfoxide; SRV, sarcoplasmic reticulum vesicles.

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## Materials and Methods

Sarcoplasmic reticulum vesicles were prepared as described by Eletr and Inesi [9]. The phosphorylation reaction was carried out with radioactive Pi for 15 sec, and the amount of phosphoenzyme formed was determined by Millipore filtration [10], the filters were washed 15 times with 5 ml sample of an ice-cold solution containing 0.1 M HCl and 2 mM Pi followed by 15 washes with 5 ml samples of deionized water. $^{32}P$-labeled phosphoenzyme was determined by liquid scintillation counting. In all experiments the pH of the medium was adjusted in the test tube after the addition of all reagents except protein. Carbohydrate solutions were prepared just before use.

## Results

In agreement with a previous report [2], we find that dimethyl sulfoxide reduces the apparent $K_m$ of the ATPase for Pi and reduces the pH dependence of the phosphorylation reaction (Table I) [2]. We now show that trehalose and sucrose activate the phosphorylation reaction, at pH 7.4 decrease the apparent $K_m$ for Pi (Fig. 2 and Table I). A somewhat smaller effects is observed at pH 6.2 (Fig. 1

Table I. Apparent affinity of the $Ca^{2+}$,$Mg^{2+}$-ATPase for Pi. The assay medium contained 50 mM MOPS-Tris (pH 7.4), 10 mM $MgCl_2$, 2 mM EGTA, the $^{32}Pi$ concentrations from 0 to 10 mM, and the additions show in the table. The reaction was started by the addition of 0.3 mg/ml SRV protein and arrested after 15 sec at 35 °C by the addition of ice-cold trichloroacetic acid (10% w/v) containing 1 mM non-radioactive Pi. The $K_m$ was obtained with double-reciprocal plot of the data.

| Additions | Apparent $K_m$ for Pi [mM] pH 6.2 | pH 7.4 |
|---|---|---|
| None | 0.73 | >10.00 |
| Dimethyl sulfoxide 1.6 M | 0.20 | 3.00 |
| Trehalose 1.6 M | 0.40 | 1.60 |
| Sucrose 1.6 M | 0.50 | 2.00 |
| Fructose 3.0 M | 0.30 | 1.50 |
| Glucose 3.0 M | 0.35 | 1.80 |

and Table I). The monosaccharides glucose and fructose can also decrease the apparent $K_m$ for Pi, but the concentration needed to obtain the same effect as trehalose was 3 M (Table I).

It has been shown that in aqueous media the phosphorylation reaction is highly dependent on the temperature of the medium [4]. We observed that the phosphoenzyme level obtained at 0 °C is negligible in a purely aqueous medium (0.09 μmol E-P/g protein), but increases significantly after addition of either trehalose or sucrose, respectively 0.49 and 0.53 μmol E-P/g protein.

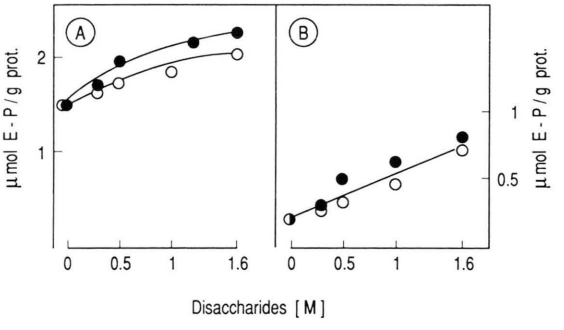


Fig. 1. Effects of trehalose and sucrose on the phosphorylation by Pi. In A the assay medium was 50 mM MOPS-Tris buffer (pH 6.2), 1 mM $^{32}Pi$, 10 mM $MgCl_2$ and 2 mM EGTA. In B the assay medium was the same as in A except that the pH was 7.4. The concentration of sucrose (○) and trehalose (●) given in the figure. The reaction was started by the addition of 0.3 mg/ml SRV protein and arrested after 15 sec at 35 °C by the addition of ice-cold trichloroacetic acid (10% w/v) containing 1 mM non-radioactive Pi.

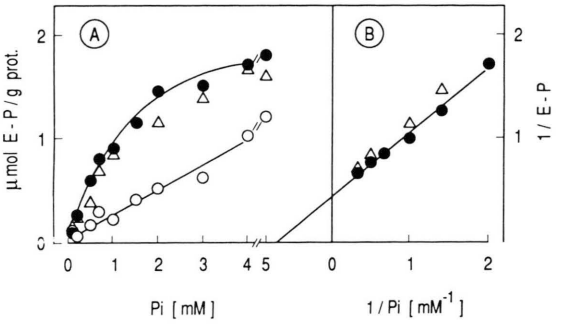


Fig. 2. Effects of trehalose and sucrose on the Pi dependence of the phosphorylation reaction. The assay medium contained 50 mM MOPS-Tris buffer (pH 7.4), 10 mM $MgCl_2$, 2 mM EGTA, 0.3 mg/ml SRV protein, the $^{32}Pi$ concentration show in the abscissa and either no addition (○), 1.6 M trehalose (●) or sucrose (△). Incubation and quenching were as described for Fig. 1. A double-reciprocal plot of the data obtained with trehalose (●) and sucrose (△) is shown in B.

Enzyme phosphorylation by Pi is known to be $Mg^{2+}$-dependent [3] and inhibited by the presence of $Ca^{2+}$ in the medium [2, 3]. These two properties of the phosphoenzyme were unchanged by the presence of trehalose or sucrose (data not shown).

## Discussion

The effects of saccharides reported here are similar to these described for organic solvents and trimethylamines [5, 6, 11]. Equivalent effects on the phosphoenzyme level and on the apparent $K_m$ for Pi are observed for 1.5 M disaccharides or 3.0 M of the monosaccharides (Table I). The hydroxyl groups of sugars are known to have a significant effects on protein and membrane stability [7, 12, 13], and the concentration dependence observed here might be explained by the numbers of hydroxyl groups found on the two classes of sugar molecules. A reduce in temperature dependence of the phosphorylation reaction with naturally occurring osmolytes has not been reported.

It has been reported that sucrose affects the catalytic cycle of the $Ca^{2+}$,$Mg^{2+}$-ATPase from renal plasma membranes, increasing the rate of ATP⇋Pi exchange [14]. These data might be explained by an increase in phosphorylation of the ATPase by Pi, as observed here.

During the catalytic cycle the $Ca^{2+}$,$Mg^{2+}$-ATPase alternates between different conformations,

primarily *E* and **E* [2, 15]. These two conformations have been shown to have different substrate specificities [2]. The enzyme form **E* which is phosphorylated by Pi, is supposed to have a low water activity at the catalytic site [11]. Thus, the major thermodynamic barrier for the phosphorylation reaction is the partitioning of Pi from the assay medium into the hydrophobic environment of the catalytic site [2, 5, 11]. Addition of saccharides might increase the affinity for Pi by shifting the equilibrium towards the **E* form of the enzyme, and also by favoring the partitioning of Pi into the hydrophobic site of **E*. Both of these effects can be explained by a change in water activity of the medium promoted by the presence of sugar.

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