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Activation of the purified erythrocyte plasma membrane Ca^{2+}-ATPase by organic solvents

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In this report it is shown that organic solvents mimic the stimulatory effects of calmodulin and acidic phospholipids on the erythrocyte plasma membrane Ca^{2+}-ATPase. The solvents used were dimethyl sulfoxide (20%, v/v), glycerol (20%, v/v), ethylene glycol (20%, v/v) and polyethylene glycol (M_r 6000–8000) (10%, w/v). These solvents increased both the affinity for Ca^{2+} and the turnover number of the enzyme. The increase in Ca^{2+} affinity is additive to that achieved with calmodulin. The calcium cooperativity observed in the presence of calmodulin disappears after the addition of dimethyl sulfoxide to the medium. The present data support the proposal that activation of the erythrocyte plasma membrane Ca^{2+}-ATPase is promoted by hydrophobic interactions along the enzyme molecule.

ATPase, Ca^{2+}-; Calmodulin; Organic solvent; Plasma membrane; Hydrophobic interaction; (Human erythrocyte)

1. INTRODUCTION

The Ca^{2+}-pumping ATPase of the erythrocyte plasma membrane [1] can be activated in three different ways: by treatment with calmodulin [2,3], by acidic phospholipids and long-chain polyunsaturated fatty acids [4,5], and by limited proteolysis [6,7]. These treatments promote an increase in both the apparent affinity for Ca^{2+} and the V_{max}. The following data suggest that activation is promoted by hydrophobic interactions along the ATPase molecule: (i) Ca^{2+} binding to calmodulin gives rise to the exposure of hydrophobic domains of the protein [8]. (ii) The effect of calmodulin can be mimicked by certain lipids. The interaction of proteins with lipids involves hydrophobic interactions. (iii) Kosk-Koscika and co-workers [9,10] observed that the stimulation by calmodulin depends on both the concentration of enzyme used and the temperature of the assay medium. These data led the authors to suggest that Ca^{2+} binding to the ATPase is influenced by hydrophobic interactions of binding domains.

Here, it is shown that different organic solvents activate the Ca^{2+}-ATPase purified from erythrocytes. Similar to calmodulin and acidic phospholipids, the organic solvents used promote an increase in both the enzyme affinity for Ca^{2+} and the V_{max}.

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2. MATERIALS AND METHODS

Calmodulin was isolated from bovine brain by phenyl-Sepharose chromatography [11]. Human red cell membranes deficient in calmodulin were prepared from recently outdated human blood [12].

The Ca^{2+}-ATPase was purified using calmodulin affinity chromatography as in [12,13], and stored under N_2 at $-173°C$ in a buffer containing 0.05% Triton X-100, 130 mM KCl, 20 mM Hepes-KOH, pH 7.4, 1 mM $MgCl_2$, 50 μM $CaCl_2$, 2 mM dithiothreitol, 5% glycerol (v/v), and 0.5 mg/ml phosphatidylcholine.

$^{32}P_i$ was obtained from the Brazilian Institute of Atomic

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Energy. [γ-^{32}P]ATP was prepared according to Glynn and Chappell [14].

ATPase activity was assayed by measuring the release of P_i from [γ-^{32}P]ATP at 35°C for 30 min. The reaction was quenched with 2 vols of a suspension of activated charcoal in 0.1 M HCl [15]. After centrifugation aliquots of the supernatant containing $^{32}P_i$ were counted in a liquid scintillation counter.

Free Ca^{2+} concentrations were calculated as described by Fabiato and Fabiato [16], taking into account the concentrations of ATP and $MgCl_2$ in the media and using the dissociation constant of the Ca^{2+}-EGTA complex reported by Schwartzenbach et al. [17].

Protein concentration was determined by the method of Lowry et al. [18,19] using bovine serum albumin as standard.

3. RESULTS

The Ca^{2+}-ATPase was progressively activated by dimethyl sulfoxide concentrations up to 20% (v/v) (fig.1). Activation was abolished and the enzyme inhibited when the solvent concentration was raised from 20 to 50% (fig.1). A small degree of activation was observed in the presence of calmodulin when 5% dimethyl sulfoxide was added, higher concentrations being inhibitory. In the presence of 1.0–10.0 μM Ca^{2+}, essentially the same ATPase activity was observed in the presence of either calmodulin or 20% dimethyl sulfoxide (figs 1,2). In addition to its effect on turnover number, dimethyl sulfoxide promoted an increase in enzyme affinity for Ca^{2+}. This was observed in both the absence and presence of calmodulin (fig.2). The Ca^{2+} concentration required for half-maximal ATPase activity was 0.5 μM in the absence of either calmodulin or organic solvent, decreased to 0.38 and 0.32 μM after addition of either calmodulin or 20% dimethyl sulfoxide and dropped to 0.16 μM when calmodulin and dimethyl sulfoxide were added together to the medium.

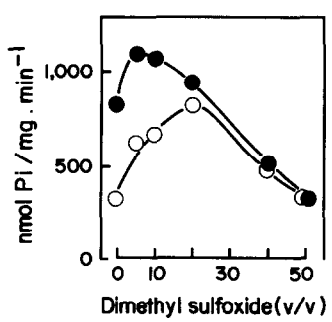

Fig.1. Stimulation of Ca^{2+}-ATPase activity by dimethyl sulfoxide. Composition of the assay medium was 100 mM Mops-Tris buffer (pH 7.4), 100 mM KCl, 10 mM $MgCl_2$, 0.2 mM [γ-^{32}P]ATP, 1 mM EGTA and 0.926 mM $CaCl_2$. The calculated free Ca^{2+} concentration was 1 μM. The reaction was started by the addition of enzyme to a final concentration of 2 μg per ml and quenched after 30 min at 35°C. (○) Without calmodulin. (●) Plus 4 μg calmodulin per ml.

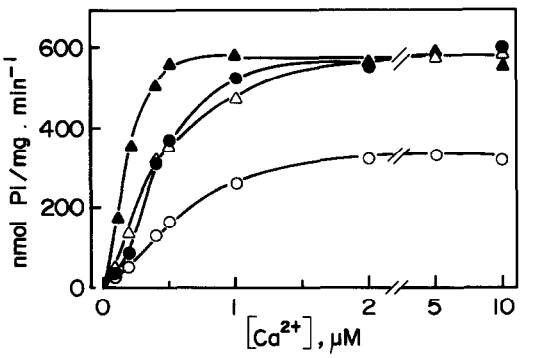

Fig.2. Effect of dimethyl sulfoxide on enzyme affinity for Ca^{2+}. Composition of assay media and experimental conditions as in fig.1. (○) No additions; (●) with calmodulin, 4 μg per ml; (△) with dimethyl sulfoxide, 20% (v/v); (▲) with calmodulin, 4 μg per ml, and dimethyl sulfoxide, 20% (v/v).

The Ca^{2+} cooperativity observed in the presence of calmodulin [20–22] disappeared after the addition of dimethyl sulfoxide. Under the experimental conditions of fig.2, the Hill coefficient determined in the presence of calmodulin was found to vary between 1.3 and 1.4 and decreased to unity after the addition of dimethyl sulfoxide to the medium.

Table 1

Effect of different organic solvents

Solvent	ATPase activity (nmol P_i/mg protein per min)			
	Without calmodulin		Plus calmodulin	
	Ca^{2+} concentration			
	0.2 μM	5 μM	0.2 μM	5 μM
H_2O	39	334	96	596
Dimethyl sulfoxide, 20% (v/v)	139	586	368	598
Glycerol, 20% (v/v)	217	882	838	905
Ethylene glycol, 20% (v/v)	214	1097	430	1181
Polyethylene glycol, 20% (w/v) (M_r 6000–8000)	200	1135	357	1097

Assay medium composition and experimental conditions were as described in fig.1

Other organic solvents such as glycerol, ethylene glycol and polyethylene glycol (table 1) were also able to stimulate the erythrocyte plasma membrane Ca^{2+}-ATPase. These solvents also increased the apparent affinity of the enzyme for Ca^{2+} in a manner similar to that observed with dimethyl sulfoxide.

4. DISCUSSION

In this report it is shown that organic solvents mimic the stimulatory effects of calmodulin and acidic phospholipids on the erythrocyte plasma membrane Ca^{2+}-ATPase. Acidic phospholipids are known to increase the enzyme's affinity for Ca^{2+} to a greater extent than that attained with calmodulin [4,23,24]. The Ca^{2+} cooperativity observed in the presence of calmodulin is abolished with acidic phospholipids. These two effects can also be evoked by using organic solvents (fig.2). A common feature of the various organic solvents used is that they decrease the water activity. The similarity between the effects obtained with organic solvents and acidic phospholipids supports the proposal that activation of the ATPase is promoted by hydrophobic interactions along the enzyme molecule [8,9]. A model describing the interaction of calmodulin and acidic phospholipids with the Ca^{2+}-ATPase has been presented elsewhere [13,25,26].

The effects of organic solvents on the erythrocyte plasma membrane Ca^{2+}-ATPase described here differ from those previously described for the Ca^{2+}-ATPase of sarcoplasmic reticulum. For the latter enzyme, the Ca^{2+} affinity is not modified and the ATPase activity is inhibited by concentrations of dimethyl sulfoxide that maximally activate the erythrocyte enzyme [27].

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