Abundance of the Ca$^{2+}$-pumping ATPase in pig erythrocyte membranes

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The Ca$^{2+}$-pumping ATPase (Ca$^{2+}$-ATPase) was purified from human and pig erythrocyte membranes by calmodulin affinity chromatography in the presence of phosphatidylcholine. The amount of enzyme present in pig erythrocytes is at least 7 times greater than that isolated from human erythrocyte ghosts. However, the properties of the enzyme from the two species are similar in many respects.

INTRODUCTION

The activities of ATP-dependent Ca$^{2+}$ pumps in plasma membranes are fundamental to the messenger functions of Ca$^{2+}$, since these enzymes regulate cytosolic Ca$^{2+}$ concentrations. The Ca$^{2+}$-ATPase from human erythrocytes has been purified and characterized in many laboratories (Gietzen et al., 1980; Niggli et al., 1981; Al-Jobore & Roufogalis, 1981). The activity of the enzyme can be stimulated by calmodulin, acidic phospholipids and unsaturated fatty acids, and by limited proteolysis (Niggli et al., 1981; Steiger & Schatzmann, 1981).

The understanding of the molecular events produced by various effectors on the erythrocyte and other plasma-membrane Ca$^{2+}$-ATPases, as well as the overall mechanism of plasma-membrane Ca$^{2+}$ transport, would require a knowledge of the primary structure of the enzyme. Such studies have been hindered by the paucity of the enzyme, which represents less than 0.1% of the total protein of the human erythrocyte membrane (Gietzen & Kolandt, 1982). As a result, much effort has been put into devising methods for obtaining sufficient quantities of the enzyme for molecular studies (Rosenbery et al., 1981; Gietzen & Kolandt, 1982).

We have previously reported that the specific activity of this enzyme in intact pig erythrocyte membranes is an order of magnitude higher than in some other mammalian erythrocytes examined (Bewaji et al., 1985). In the present study, we have purified the Ca$^{2+}$-ATPase from pig erythrocytes and reconstituted it into liposomes. We report that the enzyme is at least 7 times more abundant in pig than in human erythrocyte membranes.

METHODS

Preparation of proteins

Calmodulin was prepared from bovine brain by the procedure described by Guerini et al. (1984). The Ca$^{2+}$-ATPase was purified from human and pig erythrocyte membranes, in the presence of phosphatidylcholine, essentially as described by Niggli et al. (1981). The pig erythrocyte Ca$^{2+}$-ATPase was reconstituted into asolectin liposomes as described by Niggli et al. (1982).

Assays

ATPase activities were determined at 37 °C as described by Niggli et al. (1981). Protein was determined by

Table 1. Balance sheet for the purification of Ca$^{2+}$-ATPase from human erythrocytes

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Protein yield (%)</th>
<th>Total activity (µmol/min)</th>
<th>Specific activity (µmol/min per mg Ca$^{2+}$-ATPase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No CaM</td>
<td>+CaM</td>
<td>No CaM</td>
<td>+CaM</td>
</tr>
<tr>
<td>1. Ghosts</td>
<td>189</td>
<td>100</td>
<td>1.51</td>
<td>9.45</td>
</tr>
<tr>
<td>2. Triton-X-100-solubilized ghosts</td>
<td>41.6</td>
<td>22</td>
<td>3.74</td>
<td>9.98</td>
</tr>
<tr>
<td>Column:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Peak eluted with Ca$^{2+}$ buffer</td>
<td>35.9</td>
<td>19</td>
<td>1.11</td>
<td>1.29</td>
</tr>
<tr>
<td>4. Peak eluted with EDTA buffer</td>
<td>0.17</td>
<td>0.09</td>
<td>0.44</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Abbreviation used: Ca$^{2+}$-ATPase, Ca$^{2+}$-pumping ATPase.

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Table 2. Balance sheet for the purification of Ca\(^{2+}\)-ATPase from pig erythrocytes

Experimental conditions are as stated in the legend to Table 1.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Protein yield (%)</th>
<th>Ca(^{2+})-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total activity ((\mu)mol/min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No CaM</td>
</tr>
<tr>
<td>1. Ghosts</td>
<td>500</td>
<td>100</td>
<td>10.5</td>
</tr>
<tr>
<td>2. Triton-X-100-solubilized ghosts</td>
<td>145.5</td>
<td>29.1</td>
<td>56.7</td>
</tr>
<tr>
<td>Column: 3. Peak eluted with Ca(^{2+}) buffer</td>
<td>138</td>
<td>27.6</td>
<td>9.25</td>
</tr>
<tr>
<td>4. Peak eluted with EDTA buffer</td>
<td>3.6</td>
<td>0.72</td>
<td>16.02</td>
</tr>
</tbody>
</table>

the procedure of Lowry et al. (1951), with bovine serum albumin as standard. Where there are interfering materials such as Heps, dithiothreitol and Triton X-100, the protein was first precipitated with deoxycholate and trichloroacetic acid as described by Bensadoun & Weinstein (1976).

**SDS/polyacrylamide-gel electrophoresis**

The electrophoretic system used was that described by Laemmli (1970). Samples (50 µl) containing about 20 µg of erythrocyte ghost protein or 6–7 µg of the purified Ca\(^{2+}\)-ATPase were mixed with 25 µl of a buffer containing 30 mM-sodium phosphate, pH 7.0, 30% (v/v) glycerol, 7.5% (w/v) SDS, 10 mM-dithiothreitol and 0.05% (w/v) Bromophenol Blue as tracking dye, boiled for 3 min and applied to the gels; 10%–polyacrylamide gels were routinely used. The gels were stained with Coomassie Brilliant Blue R-250 and destained with 7.5% acetic acid/20% methanol (both v/v).

**RESULTS AND DISCUSSION**

The balance sheets for the purification of Ca\(^{2+}\)-ATPase from human and pig erythrocyte membranes are shown in Tables 1 and 2. These results clearly demonstrate that the enzyme is more abundant in pig than in human erythrocyte membranes. The yield of the enzyme from human erythrocyte membranes is 0.09% of the total membrane proteins, compared with 0.7% from pig erythrocytes. Fig. 1 also shows a Coomassie Blue-stained polyacrylamide gel of the enzyme from pig erythrocyte membranes at various stages of purification. On SDS/10%–polyacrylamide slab gel, the enzyme shows a major band of \(M_f\) approx. 140000. A minor band, representing about 6% of the total proteins, is usually present at a position corresponding to \(M_f\) 90000. It is not clear whether the minor band is a subunit of the ATPase or a product of proteolytic degradation of the enzyme. After storage of the enzyme for a few days at \(-80^\circ\)C it usually forms aggregates, probably dimers, which could not be dissociated in the presence of SDS.

In other respects, the properties of the pig erythrocyte Ca\(^{2+}\)-ATPase are similar to those of the human enzyme. It is highly sensitive to inhibition by orthovanadate; the inhibition constant, determined by a Dixon plot (Fig. 2), is 1.6 ± 0.2 µM. The purified enzyme, when reconstituted into asolectin liposomes, transports Ca\(^{2+}\) into the vesicles with an initial rate of 1.43 ± 0.11 \(\mu\)mol/min per mg of protein (Fig. 3). The steady-state rate of ATP hydrolysis was 3.14 ± 0.08 \(\mu\)mol/min per mg of protein. This was stimulated about 4-fold after the addition of A23187 (Fig. 4).

The abundance of the Ca\(^{2+}\)-ATPase in pig erythrocyte membranes reported here looks promising, because it
Fig. 2. Dixon plot for the inhibition of purified pig erythrocyte Ca\textsuperscript{2+}-ATPase by vanadate

ATP hydrolysis was measured at 37 °C with the coupled enzyme assay described by Niggli et al. (1981).

Fig. 3. Ca\textsuperscript{2+} transport by the purified Ca\textsuperscript{2+}-ATPase from pig erythrocytes after reconstitution into asolectin liposomes

Ca\textsuperscript{2+} transport was measured at 37 °C with a Ca\textsuperscript{2+}-selective electrode. The system was calibrated after completion of the uptake by the addition of 10 nmol of CaCl\textsubscript{2}.

Fig. 4. ATPase activity of the purified pig erythrocyte Ca\textsuperscript{2+}-ATPase after reconstitution into asolectin liposomes

ATP hydrolysis was measured at 37 °C by the coupled enzyme assay described by Niggli et al. (1981). The absorbance difference between 366 and 550 nm was monitored continuously in an Aminco dual-wavelength spectrophotometer.

REFERENCES


