Purification and properties of Na⁺, K⁺-ATPase from human kidney and *Torpedo californica*

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Summary

Na⁺, K⁺-ATPases were isolated from human kidney medulla and electric organ of *Torpedo californica* and their enzymatic properties were examined with reference to the sensitivity of the enzyme to ouabain. The specific activity of the purified Na⁺, K⁺-ATPase from human kidney was 93 µmol Pi/mg/h. SDS-polyacrylamide gel electrophoresis revealed that the human enzyme consisted of α and β subunits having molecular weights of approximately 92,000 and 44,000, respectively, as the enzymes from other sources. Kd for ouabain of the human enzyme was estimated to be 1.9 nM, while k₁ and k⁻¹ of the *Torpedo* enzyme in the reaction with ouabain were 7.5 × 10⁻³ s⁻¹ m⁻¹ and 0.21 × 10⁻³ s⁻¹, respectively. Thus it is apparent that the human enzyme is similar to, but the *Torpedo* enzyme is different from, the lamb enzyme with respect to their sensitivity to ouabain.

**Key words:** Na⁺, K⁺-ATPase, human, *Torpedo californica*, ouabain sensitivity, enzymatic properties

Introduction

Na⁺, K⁺-ATPase is considered to be associated not only with the energy-linked movements of sodium and potassium ions but also with the control of calcium fluxes across the cell membrane. Intensive studies on Na⁺, K⁺-ATPases from various sources have been carried out by Schwartz et al. and by other investigators. The enzyme is inhibited by cardiac glycosides and is thought to be the pharmacological receptor for digitalis. Na⁺, K⁺-ATPases isolated from bovine, dog, and lamb are highly sensitive to cardiac glycosides, while those from the kidney or heart of guinea pig and rabbit are less sensitive. The enzyme is also known to consist of a catalytic (α) and a glycoprotein (β) subunit, with both ATP hydrolysis and ouabain-binding sites on the α subunit. The entire amino acid sequences of the α subunits of the lamb kidney and *Torpedo californica* Na⁺, K⁺-ATPases have been deduced from the nucleotide sequences of cDNAs. These studies suggest that the conformational structure of the subunit may contribute to the differences in antigenic properties of the enzymes obtained from different species and even from different tissues.

In the field of pathology, on the other hand, the subcellular distribution of Na⁺, K⁺-ATPase is believed to indicate fairly the functional state of the cell and, therefore, histochemical studies may be very useful to investigate the developing mechanism of a pathological condition, such as seen in ischemic lesions. Taking these into consideration, in the present paper we isolated Na⁺,

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K⁺-ATPases from human kidney and electric organ of *Torpedo californica* and examined the binding properties of these enzymes with ouabain.

**Materials and Methods**

Na⁺, K⁺-ATPases were isolated from the electric organ of *Torpedo californica* (Pacific Bio-Marine Lab. Inc. Calif. U.S.A.) according to the Albers method, and from human kidney according to Lane's method, that was obtained from three autopsy cases after 1 h, 1 h 15 min and 14.5 h of exitus and kept at -80°C until use. The outer medulla of human kidney were removed with scissors and minced in a Waring blender. The tissue was homogenized and centrifuged at 11,000×g and then at 30,000×g. The microsome fraction obtained was treated with NaI and washed with sodium deoxycholate. After two days of dialysis against imidazole-EDTA, the sample was frozen in a dry ice-acetone mixture, and kept in a deep freeze at -80°C.

The activity of the enzyme was followed by a spectrophotometric-coupled enzyme assay. The enzyme was incubated at 37°C with 5 mM Na₂ATP (Boehringer), 5 mM MgCl₂, 100 mM NaCl, 10 mM KCl, 1 mM EDTA, 2 mM phosphoenolpyruvate, 0.4 mM NADH (Sigma), 25 μl of a combined pyruvate kinase/lactate dehydrogenase suspension (Sigma) and 25 mM histidine-HCl (pH 7.4) in a total volume of 2.5 ml. The activity was measured by monitoring the decrease in the absorbance at 340 nm due to the oxidation of NADH. Protein concentration was determined by Lowry's method. The association rate constant (k₁) and the dissociation rate constant (k₋₁) of the reaction of the enzyme from *T. californica* with ouabain were obtained by the millipore filtration method. Dissociation of ouabain from the enzyme was measured by the Chase method. Kd (k₋₁/k₁) of the reaction of the enzyme from human kidney with ouabain was estimated from I₅₀ (the concentration of ouabain needed for 50% inhibition) which is believed to be equal to the value of Kd. I₅₀ was obtained from the inhibition of the enzyme activity by an addition of 40 μl of ouabain at a concentration of 1 × 10⁻⁵ M to 1 × 10⁻⁸ M. The enzyme from human kidney was analyzed by 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

**Results**

Although Na⁺, K⁺-ATPases have been isolated from a number of animal species and studied, relatively little is known about the human enzyme. Table 1 shows the purification of Na⁺, K⁺-ATPase from the microsomal fraction of human kidney. A total amount of 96.5 mg of the enzyme was isolated from approximately 300 g of the outer medulla of human kidney. The ouabain-insensitive ATPase activity of the final preparation (B in Table 1) was estimated to be 3 μmol Pi/mg/h and the ouabain-sensitive ATPase activity (Na⁺, K⁺-ATPase, B−A) was 93 μmol Pi/mg/h.

SDS-polyacrylamide gel electrophoresis showed two protein bands at molecular weights of about 92,000 and 44,000, indicating that the human enzyme consisted of two kinds of subunits, α and β, like the enzymes from other species (Fig. 1). One protein band with a molecular weight of 150,000 seen in Fig. 1 is presumably the undissociated enzyme.

Table 2 shows the kinetic constants measured with Na⁺, K⁺-ATPase from human kidney and *Torpedo californica* for binding of ouabain, in which the data previously reported for the enzymes
Table 1 Purification of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase from human kidney

<table>
<thead>
<tr>
<th></th>
<th>Specific Activity (\mu mol Pi/mg h)</th>
<th>Protein Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. (\textminus)</td>
<td>B. (\textplus) Ouabain</td>
</tr>
<tr>
<td>1. Homogenized microsomes</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>2. After NaI treatment</td>
<td>64</td>
<td>9</td>
</tr>
<tr>
<td>3. After dialysis</td>
<td>96</td>
<td>3</td>
</tr>
</tbody>
</table>

*40 \mu l of 1\times10\textsuperscript{-4} M ouabain was added.

Table 2 Ouabain sensitivities of Na\textsuperscript{+}, K\textsuperscript{+}-ATPases from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>(k_1) (s\textsuperscript{-1} \times 10\textsuperscript{-3})</th>
<th>(k_{-1}) (s\textsuperscript{-1} \times 10\textsuperscript{3})</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torpedo (california)</td>
<td>7.5</td>
<td>0.21</td>
<td>28.0\textsuperscript{*}</td>
</tr>
<tr>
<td>Human kidney</td>
<td>--</td>
<td>--</td>
<td>1.9\textsuperscript{**}</td>
</tr>
<tr>
<td>Lamb kidney</td>
<td>45.4</td>
<td>0.0642</td>
<td>1.41</td>
</tr>
<tr>
<td>Dog skeletal muscle</td>
<td>56.3</td>
<td>1.85</td>
<td>33</td>
</tr>
<tr>
<td>Guinea pig kidney</td>
<td>17.5</td>
<td>2.54</td>
<td>150</td>
</tr>
</tbody>
</table>

\(k_1\), the association rate constant,
\(k_{-1}\), the dissociation rate constant,
\(*\)Calculated from Kd=\(k_{-1}/k_1\),
\(**\)Deduced from \(I_{50}\)

*Ref. 3, **Ref. 11

Fig. 1 SDS-polyacrylamide gel electrophoresis of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase from human kidney
The \(\alpha\beta, \alpha\) and \(\beta\) bands correspond to molecular weights of approximately 150,000, 92,000 and 46,000, respectively.

from other sources are also given for comparison. As seen in Table 2, for the Torpedo Na\textsuperscript{+}, K\textsuperscript{+}-ATPase the rate of association, \(k_1\), was estimated to be \(7.5 \times 10\textsuperscript{-3}\) s\textsuperscript{-1}m\textsuperscript{-1} and the rate of dissociation, \(k_{-1}\), was \(0.21 \times 10\textsuperscript{3}\) s\textsuperscript{-1}. A Kd of 28.0 nM was calculated from these constants (Kd= \(k_{-1}/k_1\)). On the other hand, Kd for the human enzyme was 1.9 nM as deduced from the value of \(I_{50}\).

**Discussion**

There is a considerable variation between species in the sensitivity of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase to cardiac glycosides, which inhibit the enzyme. It is known that Na\textsuperscript{+}, K\textsuperscript{+}-ATPase prepared from human, bovine heart, dog skeletal muscle and lamb kidney medulla are highly sensitive to ouabain, while those from kidney or heart of guinea pig and rabbit are less sensitive and those from rat submaxillary gland, heart and kidney are insensitive to it\textsuperscript{3}. The sensitivity of the enzyme from Torpedo \(california\) to ouabain, however, has not been well documented so far.

It is apparent from the present result that Kd for the human Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is very close to that for the lamb enzyme, indicating that the human enzyme is highly sensitive to ouabain like the lamb enzyme. In contrast, Kd for the Torpedo Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is in the same order of
magnitude as that for the dog skeletal muscle enzyme, suggesting that the Torpedo enzyme is less sensitive to ouabain than the lamb and human enzymes. This consideration is also supported by the fact that $k_{-1}$, which is believed to be a major determinant of the affinity of Na$^+$, K$^+$-ATPase for ouabain$^{13}$, of the Torpedo enzyme is not so small as that for the lamb enzyme.

Previously the entire amino acid sequences of the subunits of the Na$^+$, K$^+$-ATPase from lamb (1,016 amino acids) and Torpedo (1,020 amino acids) were determined by cDNA cloning$^{2,7}$ and there is an 87% homology found between the sequences of the two enzymes. In addition, in the region from the position 306 to 316, which is speculated to be the binding site for ouabain$^{7}$, only a glutamic acid at the position 307 in the lamb enzyme is replaced to glycine in the Torpedo enzyme. Thus it is of some interest that only one amino acid substitution in this region causes a considerable difference in the sensitivity of the enzyme.

An antigenic site residing in the $\alpha$ subunit of the enzyme may be involved in the regulation of the catalytic activity and also may reflect the binding property of the enzyme with ouabain$^{8}$. In this respect, the monoclonal antibody of Na$^+$, K$^+$-ATPase may be a powerful tool not only for examining the structural features of the enzyme but also for analyzing the etiological roles of the enzyme in pathological conditions.

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References


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