Studies on Adenosine Triphosphate Transphosphorylases

HUMAN ISOENZYMES OF ADENYLATE KINASE: ISOLATION AND PHYSICOCHEMICAL COMPARISON OF THE CRYSSTALLINE HUMAN ATP-AMP TRANSPHOSPHORYLASES FROM MUSCLE AND LIVER

(Received for publication, July 6, 1982)

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Procedures are described for the isolation, in crystalline form, of the adenylate kinases from autopsy samples of human muscle and from human liver. Weight average molecular weights were determined by sedimentation equilibrium to be 22,000 (±700) and 25,450 (±160) for the human muscle and liver isoenzymes, respectively. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, their molecular weights were estimated to be 21,700 and 26,500 for the muscle and liver enzymes, respectively. Both isoenzymes are accordingly monomeric proteins in their native state. Amino acid analyses are reported here for the normal human liver, calf liver, and rabbit liver adenylate kinases and compared with the normal human muscle, calf muscle, and rabbit muscle myokinases. The liver types as a group and the muscle types as a group show a great deal of homology, but some distinct differences are evident between the liver and muscle enzyme groups, especially in the number of residues of His, Pro, half-cystine, and the presence of tryptophan in the liver enzymes.

The normal human liver adenylate kinase, as isolated in this report, has proved to be similar in its properties, if not identical, to the adenylate kinase isolated directly from human liver mitochondria (Hamada, M., Sumida, M., Okuda, H., Watanabe, T., Nojima, M., and Kuby, S. A. (1982) J. Biol. Chem. 257, 13120-13128). Therefore, the liver-type adenylate kinase may be considered a crystalline type.

Our preliminary studies, coupled with those reported (e.g. Refs. 2-7), led us to the hypothesis (8) that, at least in mammals, there may be two major forms of isoenzymes of adenylate kinase: the cytoplasmic type, present largely in the skeletal muscle, and the mitochondrial type, as represented by the liver. This idea had prompted us to isolate in crystalline form, the muscle type and the liver type from the same organism, viz. the calf (8). These two isoenzymes differed, for example, in their molecular weights, in their isoelectric points, and in their inhibition by p',p5-di(adenosine-5')pentaphosphate (with their K values almost 2 orders of magnitude larger for the liver type), and most significantly, in their immunological behavior toward a rabbit anti-muscle-type globulin (8). Thus, the anti-muscle enzyme globulin is unreactive as an inhibitor toward the calf liver-type enzyme, but is a powerful inhibitor of the calf muscle-type (or of the human muscle-type (9)). In addition, the liver type showed a unique inhibition by phosphoenolpyruvate and several other distinguishing steady state kinetic features from the calf muscle isoenzyme (10). Recently, we detected an aberrant kinase in the sera from patients with human Duchenne (X-linked) progressive muscular dystrophy (9). This aberrant enzyme appeared in its electrophoretic mobility and heat stability to be similar to that of the normal human liver-type adenylate kinase. Also, the presence of this aberrant liver-type adenylate kinase could be demonstrated by characteristic (for the liver-type) inhibition patterns with p',p5-di(adenosine-5')pentaphosphate, 5,5'-dithiobis(nitrobenzoic acid), and phosphoenolpyruvate. On the other hand, by inhibition titrations with an anti-muscle-type adenylate kinase, these dystrophic sera contained approximately 95-98% muscle-type adenylate kinase. Thus, this aberrant isoenzyme resembles the liver type in a number of properties, but structurally it is a muscle-type, or derived from a muscle type, as shown immunologically by inhibition reactions with an anti-muscle-type adenylate kinase. Recently (11), we have also found that this Duchenne dystrophic adenylate kinase is not inhibited by a specific anti-normal human liver adenylate kinase from rabbit serum, which did inhibit the human liver mitochondrial adenylate kinase (11), but not the human muscle myokinase.

In continuing our systematic comparison with this aberrant Duchenne dystrophic adenylate kinase and with a highly purified human liver mitochondrial adenylate kinase (11), we have also isolated the normal human muscle and normal human liver adenylate kinase from autopsy tissues.

Adenylate kinase from rabbit skeletal muscle was first isolated by Noda and Kuby (22) in crystalline form. Since that time adenylate kinases have also been prepared in either crystalline or apparently homogeneous form from rabbit (3, 22), porcine (24), human (30), and rat (28) muscle, erythrocyte (29), porcine heart (31), bovine (2), calf (8), rat (4, 28), and porcine (5) liver.

This report will deal specifically with the isolation of the human adenylate kinase isoenzymes and with a physicochemical comparison of these two normal isoenzymes, and therefore, with the normal human liver mitochondrial enzyme (11) which we feel is essentially identical to this liver enzyme preparation described herein. In a later report, a comparison will be made with the Duchenne dystrophic aberrant adenylate kinase (9). A preliminary report has been presented (12).
Human Adenylate Kinase Isoenzymes

FIG. 1. Molecular weight determination of the crystalline human muscle and liver adenylate kinases by high speed sedimentation equilibrium at 20 °C, according to the method of Yphantis (15), expressed in terms of the logarithm of fringe displacement of micrometers (blank corrected) versus \( R^2 \) (square of the distance in centimeters from the axis of rotation). A, human muscle adenylate kinase in 0.15 M KCl, 10 mM Tris, 0.1 mM dithioerythritol, pH 8.24 (at 25 °C). The time for the data given here is 26 h at 31,400 rpm. Only points with a net displacement of more than 150 μm were used in the calculations. 

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Sedimentation velocity studies at about pH 7 and 20 °C, and 0.16 (7/2), revealed single sedimenting components with \( S_{20, w} \) values of 2.2 and 2.4 S for the human muscle and liver type, respectively, and which may be compared with 2.5 S for the human liver isozone isolated directly from mitochondria (11).

Sedimentation equilibrium studies according to the interferometric method of Yphantis (15) are shown in Fig. 1, A and B, for the muscle and liver enzymes, respectively. \( M_w \), values of 22,000 (±700) and 25,450 (±160) were obtained for the human muscle and liver adenylate kinases, with \( V_{20, c} \) assumed to be 0.734. The muscle type has a tendency to aggregate at the relatively low dithioerythritol concentrations used (0.1 mM) in these long equilibrium runs, as evidenced by the relatively heavy aggregates near the bottom of the cell (Fig. 1A). A value of 25,200 was obtained for the human liver enzyme isolated directly from mitochondria, with use of a \( V_{20, c} \) = 0.73, estimated from its amino acid analysis (11).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (20) revealed the presence of single bands for the human muscle and liver isoenzymes. Estimates of their molecular sizes by this technique are given in Fig. 2 and are as follows: normal human muscle adenylate kinase, 21,700; normal human liver adenylate kinase, 26,500, and identical to the value obtained for the adenylate kinase isolated from human liver mitochondria (11). Therefore, by the techniques of sedimentation equilibrium and sodium dodecyl sulfate-gel electrophoresis, both the native muscle and liver enzymes are monomeric proteins with a single polypeptide chain. The liver enzyme is slightly heavier than the muscle enzyme, an identical finding in the case of the calf isoenzymes (i.e., 21,000 for the calf muscle myokinase, and 25,500 for the calf liver adenylate kinase by sodium dodecyl sulfate-gel electrophoresis (8)).

The amino acid compositions of the liver types from man, calf, and rabbit are compared with the muscle types from man, calf, and rabbit in Table I. The compositions for the rabbit and calf muscle enzymes are taken from their structure.
thiobis(nitrobenzoic acid) in the native state near pH three muscle types measured (man, calf, and rabbit), and both of these cysteinyl residues may be titrated with 5,5'-dithiodipyridine under neutral conditions, but which may be titrated with 4,4'-dithiodipyridine under acidic conditions and thus, under acidic conditions, are exposed for titration (this observation is also identical to that observed in the normal human-liver enzyme isolated directly from mitochondria (11)). The calf liver enzyme, on the other hand, has only 2 half-cystines, which may be readily titrated under acidic conditions with 4,4'-dithiodipyridine. In general, therefore, the muscle types have exposed thiols, whereas the liver types have shielded thiols, which do not appear to be required for enzymatic activity. Finally, the absence of tryptophan in the muscle types and the presence of 1 residue of tryptophan per polypeptide chain of the liver type are very striking. Except for some small differences, which are largely due to differences in the methodology employed, the amino acid compositions of the normal liver adenylate kinase and the adenylate kinase isolated directly from mitochondria (11) are similar.

In conclusion, the normal human liver-type adenylate kinase, isolated from whole liver as described above, has proved to be similar in those properties reported here, if not identical to the normal human liver adenylate kinase isolated directly from mitochondria (11). Therefore, the liver type may be taken as the mitochondrial isoenzyme of adenylate kinase.

REFERENCES


M. Hamada and S. A. Kuby, unpublished observations.
Human Adenylate Kinase Isoenzymes

SUPPLEMENTARY MATERIAL

Adenylate Kinase Isoenzymes

Human isoenzymes of Adenylate Kinase is present and phosphorylated by

L. A. Kuby, G. Fleming, A. Fischer, M. C. Crans, and M. Hamant

I. EXPERIMENTAL PROCEDURES

Materials

Adenylate kinase from rabbit muscle (a-tubulin) was the source of human tissue and stored.

1. EXPERIMENTAL PROCEDURES

Materials

Human Adenylate Kinase Isoenzymes

Isoenzymes from muscle and liver

5 S. A. Kuby, G. Fleming, unpublished observations.
FIGURE 1A

Fig. 1 (A) The photomicrographs were obtained with a Zeiss phase-contrast microscope.

A. Crystals of ATP-AMP-creophosphorylase (adenylate kinase) from human muscle (amp; D).

TABLE 1 (A)
Purification of normal human muscle ATP-AMP-creophosphorylase

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Initial As</th>
<th>Final As</th>
<th>% Recovery</th>
<th>As (units)</th>
<th>Recovery of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Supernatant from 100% homogenate</td>
<td>2,470</td>
<td>26,900</td>
<td>155,800</td>
<td>5.26</td>
<td>(1)</td>
</tr>
<tr>
<td>II.</td>
<td>Acid denaturation of liver protein</td>
<td>2,450</td>
<td>26,300</td>
<td>151,000</td>
<td>19.8</td>
<td>3.4</td>
</tr>
<tr>
<td>III.</td>
<td>De fractionation</td>
<td>149</td>
<td>2,230</td>
<td>59,800</td>
<td>26.7</td>
<td>4.4</td>
</tr>
<tr>
<td>IV.</td>
<td>Sucrose density centrifugation</td>
<td>71</td>
<td>1,620</td>
<td>55,100</td>
<td>33.9</td>
<td>5.9</td>
</tr>
<tr>
<td>V.</td>
<td>Phenolcellulose chromatography (pH 8.0) followed by concentration with saturated (NH₄)₂SO₄</td>
<td>64</td>
<td>75.0</td>
<td>54,000</td>
<td>611</td>
<td>106</td>
</tr>
<tr>
<td>VI.</td>
<td>Sephacryl S-200 filtration, followed by concentration with saturated (NH₄)₂SO₄</td>
<td>3.0</td>
<td>50.0</td>
<td>41,500</td>
<td>825</td>
<td>145</td>
</tr>
<tr>
<td>VII.</td>
<td>Crystallization</td>
<td>1.6</td>
<td>12.8</td>
<td>20,700</td>
<td>1620</td>
<td>281</td>
</tr>
<tr>
<td>VIII.</td>
<td>Crystals</td>
<td>0.85</td>
<td>9.79</td>
<td>13,300</td>
<td>1370</td>
<td>235</td>
</tr>
<tr>
<td>IX.</td>
<td>Mother liquor</td>
<td>1.0</td>
<td>3.8</td>
<td>5,010</td>
<td>1,630</td>
<td>290</td>
</tr>
</tbody>
</table>

*One unit = 1 molecules by spectrophotometric (coupled-enzyme) procedure. See Kubo et al., Arch. Biochem. Biophys., (1951) 182, 34-52.

TABLE 1 (B)
Purification of normal human liver ATP-AMP-creophosphorylase

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Initial As</th>
<th>Final As</th>
<th>% Recovery</th>
<th>As (units)</th>
<th>Recovery of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Supernatant from homogenate</td>
<td>13,000</td>
<td>156,000</td>
<td>146,000</td>
<td>0.16</td>
<td>(1)</td>
</tr>
<tr>
<td>II.</td>
<td>De fractionation</td>
<td>2,200</td>
<td>26,300</td>
<td>151,000</td>
<td>13.0</td>
<td>1.4</td>
</tr>
<tr>
<td>III.</td>
<td>(NH₄)₂SO₄ Fractionation</td>
<td>580</td>
<td>50,000</td>
<td>17,000</td>
<td>0.31</td>
<td>1.8</td>
</tr>
<tr>
<td>IV.</td>
<td>Acid denaturation of liver protein followed by precipitation with (NH₄)₂SO₄ and dialysis</td>
<td>300</td>
<td>3,900</td>
<td>15,000</td>
<td>1.6</td>
<td>10</td>
</tr>
<tr>
<td>V.</td>
<td>High capacity DEAE cellulose chromatography (pH 0.75-3.5)</td>
<td>214</td>
<td>4,900</td>
<td>9,700</td>
<td>2.4</td>
<td>15</td>
</tr>
<tr>
<td>VI.</td>
<td>High capacity phenolcellulose chromatography, followed by precipitation with saturated (NH₄)₂SO₄</td>
<td>20</td>
<td>150</td>
<td>6,100</td>
<td>14.2</td>
<td>88.6</td>
</tr>
<tr>
<td>VII.</td>
<td>ATPase affinity chromatography, followed by precipitation with saturated (NH₄)₂SO₄</td>
<td>5.2</td>
<td>75.4</td>
<td>5,300</td>
<td>30</td>
<td>438</td>
</tr>
<tr>
<td>VIII.</td>
<td>Sephacryl S-200 filtration, followed by precipitation with saturated (NH₄)₂SO₄</td>
<td>1.85</td>
<td>22.6</td>
<td>3,700</td>
<td>166</td>
<td>1,023</td>
</tr>
<tr>
<td>IX.</td>
<td>Crystallization</td>
<td>1.65</td>
<td>15.4</td>
<td>2,500</td>
<td>185</td>
<td>1,160</td>
</tr>
<tr>
<td>X.</td>
<td>Crystals</td>
<td>1.65</td>
<td>15.4</td>
<td>2,500</td>
<td>185</td>
<td>1,160</td>
</tr>
</tbody>
</table>

*One unit = 1 molecules by spectrophotometric (coupled-enzyme) procedure. See Kubo et al., Arch. Biochem. Biophys., (1951) 182, 34-52.
FIGURE 1B

B. Crystals of ATP-MgPpyrophosphorylase (adenylate kinase) from human liver (prep. 1).
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