Adenosine Triphosphate-Adenosine-5'-monophosphate Phosphotransferase from Normal Human Liver Mitochondria

ISOLATION, CHEMICAL PROPERTIES, AND IMMUNOCHEMICAL COMPARISON WITH DUCHENNE DYSTROPHIC SERUM ABERRANT ADENYLATED KINASE*

(Received for publication, May 6, 1982)

Minoru Hamada§§, Michihiro Sumida¶, Hiromichi Okuda¶, Tsutomu Watanabe¶, and Motoo Nojima¶
From the Departments of ¶Hygiene, ¶Biochemistry, and ¶Orthopedics, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime 791-02 Japan

Stephen A. Kuby§§‡‡
From the Laboratory for the Study of Hereditary and Metabolic Disorders and the Departments of Biological Chemistry and Medicine, University of Utah, Salt Lake City, Utah 84132

Adenylate kinase has been purified approximately 1360-fold to a final specific activity of 280 μmol of ATP formed min⁻¹·mg⁻¹ of protein at 30 °C from normal human liver mitochondria. The purity of the final preparation was evaluated by studies with polyacrylamide gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by sedimentation studies. The purified enzyme catalyzes transphosphorylation reactions between adenosine triphosphate and adenosine monophosphate, ATP and adenosine-5'-thiophosphate, ATP and adenosine monophosphate-3'-pyrophosphate, adenosine-5'-3-thio)triphosphate and AMP. The nearly constant ratios of these activities throughout the purification scheme suggest that all are catalyzed by the same enzyme.

The purified enzyme has a molecular weight of 25,200 by sedimentation equilibrium with the use of a partial specific volume of 0.73 ml·g⁻¹ calculated from amino acid analysis. This purified enzyme was also found to be a single polypeptide with a molecular weight of 26,500 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. From amino acid analysis, a calculated minimum molecular weight of 26,349 was obtained.

Initial velocity studies revealed a narrow specificity for adenine nucleotides. The $K_d$ values for MgATP2- and MgATP2-·S were 0.12 and 0.57 μM with $V_{max}$ values of 1.04 ±0.014 × 10³ and 7.02 × 10² μmol·min⁻¹·mg⁻¹, respectively. For the monophosphate acceptor, $K_a$ values of 0.58 and 186 μM were measured for 5'-AMP2- and AMP2-·S, respectively. The $K_d$ for MgADP2- and ADP2- were 0.53 and 0.17 μM with a $V_{max}$ of 6.40 ±0.03) × 10³ μmol·min⁻¹·mg⁻¹ of protein. The steady state kinetics, at pH 7.4, 30 °C, and essentially fixed V/2 of 0.16–0.18, of this enzyme seem to be adequately expressed by a random quasi-equilibrium type of mechanism with a rate-limiting step largely at the interconversion of the ternary complexes, as shown in rabbit muscle, calf muscle, and calf liver adenylate kinase (Hamada, M., and Kuby, S. A. (1978) Arch. Biochem. Biophys. 190, 772–792). It would appear that normal human liver mitochondrial adenylate kinase largely favors the forward reaction (ADP formation). A specific anti-liver enzyme antibody obtained from rabbit serum inhibited the purified liver mitochondrial enzyme activity, but not the purified human muscle enzyme, nor the aberrant adenylate kinase from Duchenne dystrophic serum (Hamada, M., Okuda, H., Watanabe, T., Ueda, K., Nojima, M., Kuby, S. A., Manship, M., Tyler, F. H., and Ziter, F. A. (1981) Biochim. Biophys. Acta 680, 227–237).

Adenylate kinase (ATP·AMP phosphotransferase, EC 2.7.4.3) is a ubiquitous enzyme which occurs at high activity levels in many living cells, including the human erythrocyte (1). The enzyme catalyzes an interconversion of the adenine nucleotides, thus providing a unique buffering role against rapid concentration changes of any one component of this pool. The enzyme also serves as a primary regulating agent of those reactions in which the adenine nucleotides may participate as a substrate, activator, or inhibitor. Adenylate kinases have been prepared in either crystalline or apparently homogenous forms from rabbit (2, 3), porcine (4), and rat (5) muscle, erythrocyte (6), porcine heart (7), and bovine (8), calf (3), and rat (5, 9) liver. Studies on the intracellular distribution of adenylate kinase showed it to be present in the nuclear, mitochondrial, microsomal, and supernatant fractions, although the major fractions of the total activity of liver tissues appears to be associated with the mitochondrial fraction.

Adenylate kinase from rabbit skeletal muscle was first crystallized by Noda and Kuby (2), who showed it to have a molecular weight of approximately 21,000 and to contain two free sulfhydryl groups, an observation which is consistent with the sensitivity of this enzyme to such reagents as silver and mercury.

Chiga and Plaut (10) described the partial purification of adenylate kinase from aqueous extracts of swine liver and noted marked differences from the skeletal muscle enzyme in substrate specificity as well as its insensitivity to sulfhydryl reagents. These differences in the catalytic properties of skel-
et al muscle and liver adenylate kinase suggested the possible occurrence of tissue-specific forms of this enzyme and led to a detailed examination of the molecular properties of the bovine liver mitochondrial enzyme (11).

The presence of an aberrant adenylate kinase in Duchenne dystrophic serum (12) and its similarity to the normal liver enzyme prompted this study.

This report describes the isolation of a highly purified, apparently homogeneous form of adenylate kinase from normal human liver mitochondria. Its amino acid composition and sedimentation and kinetic properties have been determined. Some specific properties of this enzyme and a comparative study with the aberrant enzyme from Duchenne dystrophic serum are also given in this report. A preliminary report on some aspects given below has been presented (38).

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagent-grade chemicals were purchased from Sigma, Nakarai Pharmaceutical Co. (Kyoto), Boehringer Mannheim, Ya- manouchi (Tokyo), and Wako Chemical Works (Osaka). Enzymes used in the coupled enzyme tests (glucose-6-phosphate dehydrogenase, hexokinase, pyruvate kinase, and lactate dehydrogenase) and substrates (ADP, AMP, ATP, NADP⁺, NADH, phosphoenolpyruvate, and glucose) were obtained from P-L Biochemicals Inc., Sigma, and Boehringer Mannheim. ATPyS and AMPyS were purchased from Boehringer and adenosine monophosphate-3'-pyrophosphate from Sanraku-Ocean Co., Ltd. (Tokyo). Molecular weight marker proteins used were acquired from Sigma and ICN Nutritional Biochemicals. Acrylamide, Bisacrylamide, and N4-tetramethylethylenediamine were purchased from Aldrich Chemical Co. (Tokyo). High capacity phosphocellulose P-11 was obtained from Whatman; Sephadex S-300 and Sephadex G-100 were obtained from Pharmacia. Tryptophan and tyrosine were determined with the method of Goodwin and Morton (19). Cysteine was determined with 4,4'-dithiodipyridine under acidic conditions by the method of Grasetti and Murray (20); 5,5'-dithiobis-(2-nitrobenzoic acid) and p-chloromercuribenzoate were also used to determine the thiol groups (21).

**Preparation of Antisera**—Antisera to highly purified liver adenylate kinase and to crystalline porcine muscle adenylate kinase were prepared in New Zealand White rabbits. Adenylate kinase (8 mg in 0.5 ml of 0.9% saline) was emulsified with an equal volume of Freund's complete adjuvant. Two aliquots (0.1 ml) of the emulsion were injected into two footpads, and the remainder was injected intradermally at multiple sites. The rabbits received three additional intradermal injections of adenylate kinase (1 mg) in incomplete adjuvant at 10-day intervals. Seven days after the final injection, anti-enzyme antibodies titers reached maximal levels which were maintained for 3 weeks. Serum collected 7 to 20 days after the last injection was used in all the experiments described below.

**Ouchterlony Immunodiffusion**—Immunodiffusion (25) was carried out on microscope slides coated with 1 mm of 1% agarose containing 50 mM potassium phosphate, 0.5% bovine serum albumin, 1 mM MgCl₂, 0.2 mM NADH, 20 units of lactate dehydrogenase, 10 units of pyruvate kinase, plus varying and calculated amounts of Mg₃ATP and Mg₃ADP, with ATP, for the kinetic studies. Calculations employed a fixed concentration of free magnesium of 1 mM Mg²⁺ for the forward reaction. The reaction was initiated by the addition of suitably diluted adenylate kinase (dilutions in ice-cold 1 mM dithioerythritol, 1 mM EDTA, pH 7.5, with 1 mg/ml of albumin). The final ionic strength averaged approximately 0.18. Measurements of the decrease in absorbance at 340 nm were made with a Cary 219 spectrophotometer thermostatted at 30 °C. In this case, the reaction was expressed as the rate of ATP₃ disappearance where \( \frac{1}{2} \left( \Delta A_{340}/6.22 \right) \) = nmol of ATP₃ disappearance.

**RESULTS AND DISCUSSION**

All operations were carried out at 2-4 °C unless otherwise stated. Purification procedures were a modification of the method of Kuby et al. (3). The concentrations of (NH₄)₂SO₄ and the required amounts of solid (NH₄)₂SO₄ were estimated by the formulas given in Refs. 2 and 39.
Preparation of Mitochondria—Mitochondria from 2–3 kg of normal human liver were prepared by a slight modification of the method of Johnson and Lardy (26). The livers were chilled immediately after removal from the autopsy materials and were subsequently defatted and sliced into small pieces. Two hundred grams of liver were homogenized in 500 ml of cold 0.25 M sucrose for one min in a Waring Blendor run at 60% of line voltage. The homogenate was filtered through four thicknesses of cheesecloth, and the nuclei and cell debris were removed by centrifugation at 1700 \( \times g \) for 12 min in a No. 8 head of a Kubota KR 20000 centrifuge. The mitochondria present in the supernatant fraction were sedimented by acid treatment with cold 1 N acetic acid to pH 5.6, followed by a similar centrifugation at 2500 \( \times g \) for 25 min in a No. 3 rotor, and the supernatant liquid was discarded. Special care was taken to remove the fluffy layer found directly above the mitochondria. The precipitate was washed by centrifugation with the same sucrose solution.

Finally, the mitochondria were frozen at \(-20^\circ C\) (the enzyme retained full activity for many months under these conditions) and were subsequently thawed and extracted as required.

Fractionation with Zinc Acetate—To the mitochondrial extract of the fraction I supernatant, an aliquot of cold 2 N NaOH was added to adjust the pH to 6.3, and an amount of 1 M \( \text{Zn} \left( \text{Ac} \right)_2 \) (freshly prepared) was added equal to 0.008 volume of fraction I supernatant (final concentration of \( \text{Zn} \left( \text{Ac} \right)_2 \) is 7.93 mM) while maintaining the pH at 6.3 with cold 2 N NaOH. The enzyme solution was slowly stirred for 10 min at 0 \( ^\circ C \) and centrifuged at 2500 rpm for 30 min. To the supernatant liquid obtained (fraction II), a volume of 1 M \( \text{Zn} \left( \text{Ac} \right)_2 \) was added equal to 0.090 volume of fraction II (final calculated concentration of zinc acetate is 0.0684 M). The pH of the mixture was raised to 8.3 with 2 N NaOH with rapid stirring, and, during the next 30 min, stirring was continued slowly while monitoring the pH and maintaining it at pH 8.3.

The mixture was centrifuged at 2500 rpm for 30 min at 0 \( ^\circ C \). The pellet obtained was dissolved in 5/6th volume of fraction I using 0.25 M citric acid, pH 7.6, and dialyzed in pH 7.6, containing 10 mM mercaptoethanol, 0.1 M NaCl, and 0.1 mM EDTA. The enzyme solution (94.2 ml, 235.6 mg of protein) was applied to the column, then washed with 2-column volumes of the same buffer until the optical density of the washings decreased to less than 0.01. At this point, the linear gradient elution was initiated with 2 M NaCl added to the equilibrating buffer in the reservoir and 500 ml of equilibrating buffer in the mixing chamber. A flow rate of 12.5 ml/h at 3.5 \( ^\circ C \) was maintained by a 60-cm hydrostatic head, and 10-ml fractions were collected.

Active fractions were pooled and concentrated by the addition of solid ammonium sulfate to 0.95 saturation. The resulting precipitate was spun down at 25,000 \( \times g \) for 20 min at 0 \( ^\circ C \), dissolved in minimum volume of the equilibrating buffer, and dialyzed overnight with stirring against three changes in 1 liter of equilibrating buffer at 3.5 \( ^\circ C \).

Agarose-Hexane ATP Affinity Chromatography—The final step of this purification procedure was carried out with the use of an agarose-hexane ATP affinity chromatography column (1.0 \( \times \) 2.5 cm, 2 ml) which was equilibrated with 0.01 M Tris-Cl buffer, pH 7.4, containing 10 mM mercaptoethanol and 0.1 mM EDTA. The concentrated enzyme solution was applied to the column, washed with 5 ml of equilibrating buffer, and eluted with a 0–1.0 M sodium chloride linear gradient, using 50 ml of equilibrating buffer in the mixing chamber. The total enzyme units were recovered in excess of 82% yield between 0.1–0.3 M NaCl. The active fractions were pooled and concentrated with saturated ammonium sulfate. The resulting precipitate was centrifuged off and dialyzed against 0.2 M Tris-Cl buffer, pH 7.6, containing 0.1 M citrate, 0.2 M NaCl, 0.01 M EDTA.

Ammonium Sulfate Fractionation—To the fraction IV solution, solid ammonium sulfate was added with stirring to give 0.40 saturation. After 30 min at 0 \( ^\circ C \), the resultant precipitate was centrifuged down at 9500 rpm for 40 min. To the supernatant, liquid solid ammonium sulfate was then added with stirring to 0.80 saturation. After 15 min, it was centrifuged at 9500 rpm for 40 min and the pellets dissolved in 5/6th volume of fraction I using 0.03 M citrate ammonia, pH 7.6 (fraction VI).

Acid Denaturation of Inert Protein—Fraction VI suspension was stirred and the pH lowered rapidly to 2.6 with 2 N HCl and kept at pH 2.6 for 5 min with stirring at 0 \( ^\circ C \) and the pH readjusted back to pH 7.6 more slowly with 2 N NaOH.

After 30 min of stirring at 0 \( ^\circ C \), the suspension was centrifuged at 9500 rpm for 45 min and the clarified supernatant liquid (fraction VII) retained. Fraction VII supernatant was concentrated with 90% saturation ammonium sulfate with gentle stirring for 20 min, and the pellets obtained were dissolved in 5/6th volume of fraction I using 0.02 M citrate ammonia, pH 7.6, and dialyzed overnight against three changes in a 50-fold volume of the suspension with 0.02 M citrate ammonia, pH 7.6 (fraction VIII).

Sephacryl S-300 Gel Filtration—Sephacryl S-300 column (2.5 \( \times \) 100 cm) was previously equilibrated with 0.01 M Tris-Cl, pH 7.6, containing 10 mM mercaptoethanol and 1 mM EDTA. The sample (874.0 mg of specific activity = 0.63 units/mg) was applied to the column and displaced with the above equilibrating buffer with use of a 68 cm height of hydrostatic head at a flow rate of 8.0 ml/h. 12-ml fractions were collected, and the active fractions were pooled and concentrated with 90% saturation ammonium sulfate. A typical gel filtration profile was shown in Fig. 1; almost 100% yield was recovered in the middle main peak having a specific activity of 1.66 \( \mu \)mol-min\(^{-1}\)-mg\(^{-1}\).

High Capacity Phosphocellulose Column Chromatography—The packed column of activated phosphocellulose (Whatman P-11) (2.6 \( \times \) 50 cm, 265.3 ml) was equilibrated with 0.1 M Tris-Cl buffer, pH 7.2, containing 10 mM mercaptoethanol, 0.1 M NaCl, and 0.1 mM EDTA. The enzyme solution (94.2 ml, 235.6 mg of protein), was applied to the column, then washed with 2-column volumes of the same buffer until the optical density of the washings decreased to less than 0.01. At this point, the linear gradient elution was initiated with 2 M NaCl added to the equilibrating buffer in the reservoir and 500 ml of equilibrating buffer in the mixing chamber. A flow rate of 12.5 ml/h at 3.5 \( ^\circ C \) was maintained by a 60-cm hydrostatic head, and 10-ml fractions were collected.

The active fractions were pooled and concentrated by the addition of solid ammonium sulfate to 0.95 saturation. The resulting precipitate was spun down at 25,000 \( \times g \) for 20 min at 0 \( ^\circ C \), dissolved in minimum volume of the equilibrating buffer, and dialyzed overnight with stirring against three changes in 1 liter of equilibrating buffer at 3.5 \( ^\circ C \).

Agarose-Hexane ATP Affinity Chromatography—The final step of this purification procedure was carried out with the use of an agarose-hexane ATP affinity chromatography column (1.0 \( \times \) 2.5 cm, 2 ml) which was equilibrated with 0.01 M Tris-Cl buffer, pH 7.4, containing 10 mM mercaptoethanol and 0.1 mM EDTA. The concentrated enzyme solution was applied to the column, washed with 5 ml of equilibrating buffer, and eluted with a 0–1.0 M sodium chloride linear gradient, using 50 ml of equilibrating buffer in the mixing chamber. The total enzyme units were recovered in excess of 82% yield between 0.1–0.3 M NaCl. The active fractions were pooled and concentrated with saturated ammonium sulfate. The resulting precipitate was centrifuged off and dialyzed against 0.2 M Tris-Cl buffer, pH 7.6, containing 0.1 M citrate, 0.2 M NaCl, 0.01 M EDTA.

Ammonium Sulfate Fractionation—To the fraction IV solution, solid ammonium sulfate was added with stirring to give 0.40 saturation. After 30 min at 0 \( ^\circ C \), the resultant precipitate was centrifuged down at 9500 rpm for 40 min. To the supernatant, liquid solid ammonium sulfate was then added with stirring to 0.80 saturation. After 15 min, it was centrifuged at 9500 rpm for 40 min and the pellets dissolved in 5/6th volume of fraction I using 0.03 M citrate ammonia, pH 7.6 (fraction VI).

Acid Denaturation of Inert Protein—Fraction VI suspension was stirred and the pH lowered rapidly to 2.6 with 2 N HCl and kept at pH 2.6 for 5 min with stirring at 0 \( ^\circ C \) and the pH readjusted back to pH 7.6 more slowly with 2 N NaOH.

After 30 min of stirring at 0 \( ^\circ C \), the suspension was centrifuged at 9500 rpm for 45 min and the clarified supernatant liquid (fraction VII) retained. Fraction VII supernatant was concentrated with 90% saturation ammonium sulfate with gentle stirring for 20 min, and the pellets obtained were dissolved in 5/6th volume of fraction I using 0.02 M citrate ammonia, pH 7.6, and dialyzed overnight against three changes in a 50-fold volume of the suspension with 0.02 M citrate ammonia, pH 7.6 (fraction VIII).
extensively against the same buffer to eliminate any bound nucleotide which had been released from the gel, and concentrated with the aid of a collodion bag. A typical elution profile is shown in Fig. 2.

The purification summary is shown in Table I. The isolated enzyme was purified 1500-fold from the mitochondrial extract in 5.6% yield (for the first preparation; after the second preparation, the yield was up to 27%), and its final specific activity was 280 μmol of ATP formed min\(^{-1}\)·mg\(^{-1}\) of protein. The specific activity was similar to that of the crystalline calf liver enzyme (3); however, it was 20% less than that of the rat (5, 27) and bovine (8) liver enzymes. The purified enzyme obtained from the final step of Table I can be stored at -20 °C for periods up to several weeks and thawed with no detectable loss of activity. After 3 months, the loss of enzyme activity ranges from 5-10%. The enzyme is stable to storage at 3 °C for a month and much more stable in dilute solutions (0.2 mg·ml\(^{-1}\)), but can be stabilized by the addition of dithioerythritol (4 mM) and bovine serum albumin (1 mg·ml\(^{-1}\)). For this reason, enzyme solutions were routinely diluted in 0.05 M Tris-Cl buffer, pH 7.4, containing 1 mg·ml\(^{-1}\) of bovine serum albumin and 4 mM dithioerythritol prior to assay or storage. The enzyme activity is quite stable to dialysis in the presence of 4 mM dithioerythritol, and at low (pH 2.0) or high (pH 11.5) pH exposure at 0 °C for 1 h (data not shown).

Spectral Properties of the Normal Human Liver Adenylate Kinase—The solutions of normal, human liver adenylate kinase were colorless, and the ultraviolet absorbance spectrum of this enzyme is presented in Fig. 3. The faint shoulder in the absorption spectrum at 290 nm indicated the presence in the protein of small amounts of tryptophan and tyrosine, and this was confirmed by the spectrophotometric analysis (according to Ref. 19) of 2 tryptophan and 4 tyrosine residues in alkaline solution, and these values are close to those estimated from the amino acid analysis. The protein has an A\(_{280}/A_{260}\) ratio of 1.72, indicating the absence of significant contamination by nucleic acids or nucleotides at neutral pH. The measured absorbance coefficient of the enzyme (E\(_{1%260}\)) was found to be 0.64 at 278 nm at a protein concentration of 1.00 mg·ml\(^{-1}\) in 0.01 M Tris-Cl, pH 7.40, containing 10 mM mercaptoethanol and 0.1 mM EDTA (T/2 = 0.17) at 30 °C.

Optimal pH for the Normal Human Liver Adenylate Kinase Reaction—In the forward direction, the optimal pH was found to be approximately 7.4, as shown in Fig. 4; however, in the reverse direction, it shifted slightly to 7.9 at 30 °C (T/2 = 0.16). These data resemble those adenylate kinases obtained from other mammalian tissues.

Molecular Weight and Subunit Structure of the Normal Human Liver Adenylate Kinase—On agarose gel electrophoresis at pH 8.9, the native enzyme migrated as a single band (data not shown); and, on calibrated sodium dodecyl sulfate-polyacrylamide gels in the phosphate buffer sodium dodecyl sulfate gel system of Weber and Osborn (17) (Fig. 5), the protein also migrated as a single band in a position corresponding to a molecular weight of 26,500 compared to bovine serum albumin (68,000), γ-globulin, heavy chain (50,000), ovalbumin (45,000), chymotrypsinogen (25,700), γ-globulin light chain (23,400), apoferritin (18,500), and cytochrome c (11,700) as standards. This subunit molecular weight is very similar to that reported for the calf liver adenylate kinase (3). A molecular weight of this protein was estimated to be 25,200 by the sedimentation equilibrium method at pH 7.4 and T/2 = 0.16 (Fig. 6A) with use of a partial specific volume (δ = 0.73) of the enzyme estimated from its amino acid composition (23, 24). The protein sediments as a single component under the same conditions and yielded an s\(_{20W}\) = 2.52 S extrapolated to zero protein concentration (Fig. 6B). These data are in excellent agreement with that obtained by the method of Weber and Osborn (17), and the native normal human liver adenylate kinase is a monomer of 26,500 daltons. However, the mitochondrial enzyme seems to be easily aggregated to the dimer and possibly the tetramer (compare the rat enzyme (27)). In one preparation, an s\(_{20W}\) of 4.85 S was obtained by sedimentation velocity, with little or no concentration dependency, and, when compared to a sedimentation equilibrium run, an M\(_{0}\) of 52,800 was obtained, with evidence of still higher aggregates near the bottom of the cell.

Amino Acid Analysis—The weight of the protein in the sample to be used was estimated by the Biuret method (15). The enzyme samples were exhaustively dialyzed against 0.15 M ammonium bicarbonate and lyophilized. The samples were then hydrolyzed with twice distilled 6 N hydrochloric acid at 110 °C in sealed evacuated tubes that had been degassed by the method of Kuby et al. (3).

Fig. 2. Affinity chromatography of normal human liver adenylate kinase on ATP-hexane agarose (2-ml column bed volume). Chromatography was carried out on 347.4 units of enzyme (12.9 mg of protein). Gradient elution was identical with the aid of a collodion bag. A typical elution profile is shown in Fig. 2.

**Table I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Purification</th>
<th>Recovery of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Mitochondrial extract</td>
<td>4,500</td>
<td>29,370 mg</td>
<td>6,050.2 units</td>
<td>0.206 units/mg protein</td>
<td>1%</td>
<td>100%</td>
</tr>
<tr>
<td>II. Zn fractionation</td>
<td>590</td>
<td>2,668 mg</td>
<td>877.7 units</td>
<td>0.329 units/mg protein</td>
<td>1.59</td>
<td>14.5%</td>
</tr>
<tr>
<td>III. Acid desaturation of inert protein followed by precipitation with 90% saturation of (NH(_4))(_2)SO(_4) and dialysis</td>
<td>12.0</td>
<td>874.0 mg</td>
<td>550.6 units</td>
<td>0.630 units/mg protein</td>
<td>3.06</td>
<td>21.1%</td>
</tr>
<tr>
<td>IV. Sephacryl S-300 gel filtration</td>
<td>34.2</td>
<td>235.6 mg</td>
<td>391.5 units</td>
<td>1.66 units/mg protein</td>
<td>8.06</td>
<td>6.5%</td>
</tr>
<tr>
<td>V. Phosphocellulose chromatography followed by precipitation with saturated (NH(_4))(_2)SO(_4)</td>
<td>1.6</td>
<td>12.9 mg</td>
<td>347.4 units</td>
<td>26.93 units/mg protein</td>
<td>130.7</td>
<td>5.7%</td>
</tr>
<tr>
<td>VI. Agarose-hexane ATP affinity chromatography followed by precipitation with saturated (NH(_4))(_2)SO(_4)</td>
<td>1.5</td>
<td>1.2 mg</td>
<td>336.3 units</td>
<td>280.19 units/mg protein</td>
<td>1,360.1</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

* Initially, 3.5 kg of human liver.
* One unit = 1 μmol/min by spectrophotometric (coupled-enzyme) procedure. See Kuby et al. (3).
Adenylate Kinase from Human Liver Mitochondria

Fig. 3. Ultraviolet absorption spectra of normal human liver adenylate kinase, 2.065 mg/ml in 10 mM Tris-Cl, pH 7.4, 0.138 M NaCl, 1.0 mM EDTA, 8 mM MgCl₂ (F/2 = 0.18) at 25 °C.

Fig. 4. Effect of pH on purified normal human liver adenylate kinase activity. O, forward reaction (ATP disappearance); ●, reverse reaction (ATP formation). The following buffers (10 mM) were used: acetate (pH 5.0-6.0); potassium phosphate (pH 6.5-7.0); Tris-Cl (pH 7.4-8.5); and glycine-NaOH (pH 9.0-10.0).

the method of Crestfield et al. (28). Amino acid analyses were carried out with a Shimadzu (ISC09/S2504 (4 mm × 25 cm)) with a step gradient mobile phase at 55 °C) high pressure liquid chromatograph analyzer using postlabeling with o-phthalaldehyde (29). The amino acid composition of the normal human liver adenylate kinase protein is given in Table II and was calculated on the assumption that the protein has a molecular weight of 26,500. The protein is rich in acidic residues and sparse in aromatic and sulfur-containing amino acids. The analysis is compared to that reported for bovine liver enzyme (8) and given in Table II. Determination of tyrosine and tryptophan by the spectral method of Goodwin and Morton (19) yielded 4 Tyr and 2 Try residues/mol of protein. The method of Grassetti and Murray (20) was used for titration of sulphydryl groups under acidic conditions utilizing the reaction with the disulfide 4,4'-dithiodipyridine. By cysteic acid analyses, there are 4 half-cystine residues/subunit. There was no significant optical density change in the titration with 4,4'-dithiodipyridine. Moreover, the native enzyme is not inhibited by 5-5'-dithiobis-(2-nitrobenzoic acid) nor by p-chloromercuribenzoate, and in this respect, is in agreement with the early observations of Chiga and Plaut (10). Considerable similarity exists between the two enzymes with respect to their content of histidine, methionine, tryptophan, aspartate, and proline, and only small differences are noted in others such as arginine, tyrosine, glutamate, threonine, valine, and lysine. Substantial differences were detected in serine, glycine, alanine, leucine, isoleucine, and phenylalanine and also in the sum of the amino acid residues, for both enzymes.

The most significant difference thus far noted is the number of half-cystine residues and the presence of tryptophan in the liver enzyme compared to the rabbit muscle enzyme which

Fig. 5. Molecular weight analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified normal human liver adenylate kinase. Disruption was conducted at 40 °C for 2 h in 4 M urea, 2% sodium dodecyl sulfate, 2% mercaptoethanol, 0.1 mM EDTA, 10 mM sodium phosphate, pH 7.0 (25 °C). Standard 10% gels were used for the electrophoresis by the method of Weber and Osborn (17) with 0.1% sodium dodecyl sulfate and 0.1 M sodium phosphate, pH 7.0. Samples of 5 μg of the enzyme were analyzed electrophoretically with a constant current of 30 μA/gel at 25 °C for 4 h. Each gel had been subjected to pre-electrophoresis with 30 μl of 0.1 M mercaptoethanol in 0.1 M sodium phosphate, pH 7, prior to the electrophoresis of the standards and sample. The gels were stained with 0.25% Coomassie brilliant blue (R-250) (17) and destained by diffusion. Molecular weights selected for the polypeptide chains used as reference standards in the gel electrophoresis were summarized in Ref. 3.

Fig. 6. Sedimentation equilibrium and sedimentation velocity measurements of normal human liver adenylate kinase. A, a molecular weight determination of normal human liver mitochondrial adenylate kinase from absorbance scanning experiments expressed in terms of a plot of (-log c, c (A) versus X² (cm²)) (square of the distance, in centimeters squared, from axis of rotation). The initial concentration of protein was 0.55 mg/ml in 0.15 M NaCl, 0.01 M Tris, 0.001 M EDTA, pH 7.4, at 4 °C. The protein was sedimented at 14,000 rpm for 7.5 h. B, sedimentation coefficients of normal human liver mitochondrial adenylate kinase as a function of protein concentration at 25.2 °C in 0.15 M NaCl, 0.01 M Tris, 0.001 M EDTA, pH 7.4, y = -0.0998 (X) + 2.5198. The protein was sedimented at 60,000 rpm for 2 h. The bar schlieren diaphragm (phase plate) angle was set at 60°.
TABLE II
Amino acid composition of normal human liver mitochondrial adenyate kinase

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Nearest integral number of residues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per 26,500 g protein</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>18</td>
</tr>
<tr>
<td>Threonine</td>
<td>12</td>
</tr>
<tr>
<td>Serine</td>
<td>20</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>22</td>
</tr>
<tr>
<td>Proline</td>
<td>13</td>
</tr>
<tr>
<td>Glycine</td>
<td>23</td>
</tr>
<tr>
<td>Alanine</td>
<td>22</td>
</tr>
<tr>
<td>Valine</td>
<td>15</td>
</tr>
<tr>
<td>Methionine</td>
<td>5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>6</td>
</tr>
<tr>
<td>Leucine</td>
<td>15</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>4</td>
</tr>
<tr>
<td>Lysine</td>
<td>12</td>
</tr>
<tr>
<td>Histidine</td>
<td>4</td>
</tr>
<tr>
<td>Arginine</td>
<td>12</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
</tr>
</tbody>
</table>

Integral number of residues × molecular weight 26,369 21,744

*Markland and Wadkins (8).
+ Calculated by the spectral method of Goodwin and Morton (19).
- Based on the determination of cysteic acid.

contains two exposed sulphhydrol groups and no tryptophan. From these and other molecular properties, it appears that the liver enzyme, compared to the muscle enzyme, may exist in a less flexible structure to allow for the necessary binding and required juxtaposition of the binding sites already partially fixed in some preferred conformation so as to facilitate phosphoryl transfer to AMP\(^{2-}\). This may be an important role in the mitochondria since \(V_{\text{max}} \ll V_{\text{max}}\) in the human liver mitochondrial enzyme (see below) and in the calf liver enzyme (14, 30).

Catalytic Properties—In Table III, a summary is given of the substrate specificities of the normal human liver mitochondrial adenyate kinase compared to the human muscle adenyate kinase. Identical substrate specificity patterns were observed for both isoenzymes; however, there are some differences in the degree of the specificity between the liver mitochondrial and the normal human muscle enzyme data. In addition to adenine nucleotide, catalytic activity was also observed in the presence of adenosine-5'-3-thiotriphosphate, adenosine-5'-thiophosphate, and adenosine monophosphate-3'-pyrophosphate. UTP, CTP, GTP, and ITP with AMP were essentially unreactive; adenosine-5'-3(thio)triphosphate plus adenosine-5'-thiophosphate proved also to be essentially unreactive, which indicates that there is little, if any, contamination by GTP-AMP transphosphorylase (31).

Adenosine-5'-O-(1-thiodiphosphate) should be a good phosphoacceptor in the presence of pyruvate kinase and phosphoenolpyruvate (Scheme 1). Also, in Scheme 1, the structures of ATPyS and adenosine monophosphate-3'-pyrophosphate are presented.

Treatment of Data and Kinetic Analyses—Calculations of MgATP\(^{2-}\) and AMP\(^{2-}\) for the forward reaction were adequately made over the range of concentrations explored by employing the set of approximate conservation equations:
Adenylate Kinase from Human Liver Mitochondria

\[
\text{ADP} = \text{MgADP}^+ + \text{ADP}^2- + \text{HADP}^2- + \text{MgHADP} + \text{NaADP}^2- \\
\text{Mg} = \text{Mg}^{2+} + \text{MgADP}^+ + \text{MgADP}^2- + \text{MgHADP} \\
\text{Na}_0 = \text{Na}^+ + \text{NaADP}^2- \\
\]

The values assigned for the chelation and dissociation constants are given in Ref. 14. The method of calculations was similar to that described in detail for the rabbit muscle ATP-creatine transphosphorylase (32) and rabbit muscle, calf muscle, and calf liver adenylate kinase (14). Calculations employed a fixed concentration of free Mg\(^{2+}\) of 1 mM for the kinetic studies on the forward reaction, where either MgATP\(^2-\) or AMP\(^2-\) were variable substrates, with each substrate varied at fixed concentrations of the complementary substrate. For the reverse reaction, where it proved impossible to maintain a fixed concentration of uncomplexed Mg\(^{2+}\), either MgADP\(^-\) or AMP\(^-\) were the variable substrates, with its paired substrate fixed.

As will be seen, under these conditions of a reasonable fixed ionic strength at 30 °C and pH 7.4, the steady state kinetics conformed to first degree velocity expressions (33) for two substrate reactions. For the quasi-equilibrium random mechanism similar to the one proposed for calf brain ATP-creatine transphosphorylase (34) and where independent binding was not invoked, the following intrinsic constants may be given as shown in Scheme 2, where \(MA = \text{MgATP}^2-, B = \text{AMP}^2-, MC = \text{MgADP}^2-\), and \(C = \text{ADP}^2-\). For the limiting cases, where the concentrations of products may be set to zero initially, then for the forward and reverse initial velocities, respectively,

\[
\text{vi}_f = \text{V}_{\text{max}} \left( \frac{1 + K_A}{(MA)} \right) \left( \frac{1 + K_B}{(B)} \right) \left( \frac{1 + K_{MC}}{(C)} \right)^{-1} \left( \frac{K_{MC} \cdot K_{MC}}{(MA)(B)} \right) \]

\[
\text{vi}_r = \text{V}_{\text{max}} \left( \frac{1 + K_A}{(MC)} \right) \left( \frac{1 + K_B}{(C)} \right) \left( \frac{1 + K_{MA}}{(MA)} \right)^{-1} \left( \frac{K_{MA} \cdot K_{MA}}{(MC)(C)} \right) \]

where \(vi_f\) and \(vi_r\) denote initial velocities for forward and reverse reactions. For the variable MgATP\(^2-\) (MA) and fixed AMP\(^2-\) (B), e.g. a primary plot of \(1/vi_f\) versus \(1/(MA)\) would yield

\[
\frac{1}{vi_f} = \frac{1}{\text{V}_{\text{max}}} \frac{1}{(1 + K_B)} \frac{1}{(1 + K_{MA})} \frac{1}{V_{\text{max}}} \frac{1}{(1 + K_{MC})} \frac{1}{(1 + K_{MA})(1 + K_B)} \]

and, from the appropriate secondary plots of slopes and ordinate intercepts and relation (1), values \(K_A = K_B = K_M = K_C\) may be estimated for the forward reaction. Thus,

\[
\text{Slope} \left( \frac{1}{vi_f} \text{versus} \frac{1}{MA} \right) = \frac{K_A + K_B + K_{MA}}{\text{V}_{\text{max}}} \]

\[
\text{y intercept} = \frac{1}{\text{V}_{\text{max}}} + \frac{K_B}{(1 + K_B)} \frac{1}{\text{V}_{\text{max}}} \]

Also, for variable AMP\(^2-\) and fixed MgATP\(^2-\), again all four constants (and \(V_{\text{max}}^2\)) may be estimated for the forward direction. Within the experimental errors and calculation uncertainties, the values for the kinetic parameters proved to be the same, and average values will be presented together with some of their ranges of uncertainty. Similarly, appropriate

\[
\frac{1}{vi_r} = \frac{1}{\text{V}_{\text{max}}} \frac{1}{(1 + K_B)} \frac{1}{(1 + K_{MC})} \frac{1}{V_{\text{max}}} \frac{1}{(1 + K_{MA})} \frac{1}{(1 + K_B)(1 + K_{MC})} \\
\text{Slope} \left( \frac{1}{vi_r} \text{versus} \frac{1}{MC} \right) = \frac{K_A + K_B + K_{MA}}{\text{V}_{\text{max}}} \]

\[
\text{y intercept} = \frac{1}{\text{V}_{\text{max}}} + \frac{K_B}{(1 + K_B)} \frac{1}{\text{V}_{\text{max}}} \]

Kinetic analyses of the enzyme-catalyzed bimolecular reversible reaction included initial rate measurements as a function of substrate concentration. In Fig. 7 (A and B) are shown double reciprocal plots of reaction velocity versus variable MgATP\(^2-\) at constant AMP\(^2-\) concentrations and variable AMP\(^2-\) at constant MgATP\(^2-\) concentrations. The plots resulted, in each case, in a series of straight lines intersecting at a common point. Secondary plots of the data (see insets) allow calculation of \(V_{\text{max}}\) and dissociation constants from the binary and ternary enzyme-substrate complexes. \(K_i\) (i.e. \(K_{ii}\) values from these data are \(1.19 \times 10^{-3}\) and \(5.60 \times 10^{-3}\) for MgATP\(^2-\) and AMP\(^2-\).

Initial rate measurements carried out in the direction of MgATP\(^2-\) formation with ADP\(^2-\) or MgADP\(^2-\) as the variable substrate are shown in Fig. 7 (C and D). Values for \(K_{A\text{DPP}}\) and \(K_{A\text{DPADP}}\) were found to be \(1.68 \times 10^{-5}\) and \(5.25 \times 10^{-5}\) M, respectively. These data suggested a quasi-equilibrium random mechanism with a rate-limiting step at the interconversion of the ternary complexes, as reported for rabbit muscle, calf muscle, and calf liver adenylate kinase (14). But these data alone will not exclude the possibility of a random mechanism in which product release is rate-limiting. Moreover, the data presented in Fig. 7 are consistent with the conclusion of Rhoads and Lowenstein (37), viz. that any mechanism involving covalent enzyme intermediates is unlikely in view of the sets of intersecting double reciprocal plots. \(K_{A\text{DPP}}\) and \(K_{A\text{DPADP}}\) values (i.e. \(K_{A\text{DPP}} = 2.4 \times 10^{-4}\) M and \(K_{A\text{DPADP}} = 5.6 \times 10^{-5}\) M) (see Table IV) are similar to those for the calf enzyme (14). \(V_{\text{max}}/E_i\) and \(V_{\text{max}}/E_i\) values are also of similar magnitude for the calf liver adenylate kinase (14), with \(V_{\text{max}}\) being smaller than \(V_{\text{max}}\) for the liver enzymes. This observation might be of

### Table IV

<table>
<thead>
<tr>
<th>Defined kinetic parameters</th>
<th>Defined equilibrium</th>
<th>Defined intrinsic dissociation constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_1)</td>
<td>(E \cdot MA = E + MA)</td>
<td>(K_{MA\text{ATPP}})</td>
<td>(1.1 \times 10^{-5})</td>
</tr>
<tr>
<td>(K_2)</td>
<td>(E \cdot B = E + B)</td>
<td>(K_{AM\text{P}})</td>
<td>(5.6 \times 10^{-5})</td>
</tr>
<tr>
<td>(K_3)</td>
<td>(E \cdot MA \cdot B = E \cdot MA + B)</td>
<td>(K_{AMBP})</td>
<td>(2.3 \times 10^{-4})</td>
</tr>
<tr>
<td>(K_4)</td>
<td>(E \cdot MA \cdot B = E \cdot B + MA)</td>
<td>(K_{AMBP})</td>
<td>(5.0 \times 10^{-4})</td>
</tr>
<tr>
<td>(K_5)</td>
<td>(E \cdot C + E = C)</td>
<td>(K_{ADP})</td>
<td>(1.6 \times 10^{-5})</td>
</tr>
<tr>
<td>(K_6)</td>
<td>(E \cdot MC = E + MC)</td>
<td>(K_{ADP})</td>
<td>(5.2 \times 10^{-5})</td>
</tr>
<tr>
<td>(K_7)</td>
<td>(E \cdot MC \cdot C = E \cdot C + MC)</td>
<td>(K_{ADP})</td>
<td>(3.1 \times 10^{-6})</td>
</tr>
<tr>
<td>(K_8)</td>
<td>(E \cdot MC \cdot C = E \cdot MC + C)</td>
<td>(K_{ADP})</td>
<td>(1.0 \times 10^{-6})</td>
</tr>
<tr>
<td>(K_9)</td>
<td>(E \cdot MC \cdot C = E \cdot MA \cdot B)</td>
<td>(K_{ADP})</td>
<td>(1.0 \times 10^{-6})</td>
</tr>
<tr>
<td>(V_{\text{max}}/E_i)</td>
<td>(E \cdot MA \cdot B = E \cdot MC \cdot C)</td>
<td>(K_{ADP})</td>
<td>(1.0 \times 10^{-6})</td>
</tr>
<tr>
<td>(V_{\text{max}}/E_i)</td>
<td>(E \cdot MA \cdot B = E \cdot MC \cdot C)</td>
<td>(K_{ADP})</td>
<td>(1.0 \times 10^{-6})</td>
</tr>
</tbody>
</table>

*\(K_i\), dissociation constant of the particular substrate from the binary complex. \(K_o\), dissociation constant of the particular substrate from the ternary complex.

\(\text{Calculated on the basis of molecular weight of the enzyme, } 26,400.\)
Adenylate Kinase from Human Liver Mitochondria

FIG. 7. Kinetics at pH 7.4, r = 0.16-0.18, and 30 °C of the forward reaction, MgATP$^2-$ + AMP$^2-$ → MgADP$^2-$ + ADP$^3-$, and of the reverse reaction, MgADP$^2-$ + ADP$^3-$ → MgATP$^2-$ + AMP$^2-$, catalyzed by ATP-AMP transphosphorylase from normal human liver. A, primary plots: (1/u) versus 1/MgATP$^2-$ at several fixed values of AMP$^2-$, Mg$^{2+}$ fixed at 1.0 mM. Secondary plots: slopes (●—●) and ordinate intercepts (○—○) from linear extrapolated primary plots versus 1/AMP$^2-$, Mg$^{2+}$ fixed at 1.0 mM. Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/B)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$. Some physiological importance if the mitochondrial adenylate kinase plays any regulatory role in oxidative phosphorylation, for it may indicate that this enzyme is already partially fixed in some preferred configuration so as to facilitate phosphoryl group transfer to AMP$^2-$, which might be its important role in the liver mitochondria.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$. Some physiological importance if the mitochondrial adenylate kinase plays any regulatory role in oxidative phosphorylation, for it may indicate that this enzyme is already partially fixed in some preferred configuration so as to facilitate phosphoryl group transfer to AMP$^2-$, which might be its important role in the liver mitochondria.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$. Some physiological importance if the mitochondrial adenylate kinase plays any regulatory role in oxidative phosphorylation, for it may indicate that this enzyme is already partially fixed in some preferred configuration so as to facilitate phosphoryl group transfer to AMP$^2-$, which might be its important role in the liver mitochondria.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$. Some physiological importance if the mitochondrial adenylate kinase plays any regulatory role in oxidative phosphorylation, for it may indicate that this enzyme is already partially fixed in some preferred configuration so as to facilitate phosphoryl group transfer to AMP$^2-$, which might be its important role in the liver mitochondria.

Preparation and Properties of Antibodies Directed against Human Liver Adenylate Kinase—Injection of purified normal human liver adenylate kinase into rabbits led to the production of antibodies directed against this enzyme. As shown in Fig. 8, serum from injected animals could inactivate the liver enzyme to at least 65%, whereas preimmune serum was inactive, and there was no inactivation of the enzyme from normal muscle, the erythrocyte enzyme, and the serum enzymes from normal and Duchenne dystrophies. Furthermore, double immunodiffusion studies indicated that the antibody was capable of forming precipitin bands with the liver enzyme (Fig. 9). Also, sodium dodecyl sulfate-acrylamide gel electrophoresis of immunoprecipitates indicated that the antibody prepared from immune serum precipitated the liver adenylate kinase from linear extrapolated primary plots versus 1/MgATP$^2-$, Secondary plots fitted to the expressions, slope = $(K_s/V_{max}) + (K_v/K_s)(1/M)$; y intercept = $(1/V_{max}) + (K_s/V_{max})(1/M)$. Some physiological importance if the mitochondrial adenylate kinase plays any regulatory role in oxidative phosphorylation, for it may indicate that this enzyme is already partially fixed in some preferred configuration so as to facilitate phosphoryl group transfer to AMP$^2-$, which might be its important role in the liver mitochondria.
We also gratefully acknowledge the assistance in preparation of the manuscript by K. Miyoshi of this laboratory.

Acknowledgments—We wish to express our appreciation to Drs. R. Fukunish, R. Tabei, and H. Mori of the Department of Pathology, Ehime University School of Medicine, for kindly supplying the autopsied material and helpful suggestions, to K. Oka for his dedicated technical assistance during the progress of this research, and to T. Takaku and K. Kameda of the Central Laboratory Analysis Division.

We also gratefully acknowledge the assistance in preparation of the manuscript by K. Miyoshi of this laboratory.

REFERENCES
25. Ouchterlony, O. (1962) Prog. Allergy 6, 30-154