The Purification and Characterization of Escherichia coli Enolase*

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SUMMARY

Enolase has been purified from aqueous extracts of Escherichia coli acetone powder by (a) heat treatment, (b) fractionation with acetone, (c) TEAE-cellulose chromatography, (d) Sephadex G-100 chromatography, and (e) crystallization. The purified, crystalline enzyme migrates as a single band in disc gel electrophoresis and is homogeneous by ultracentrifugal analysis. The molecular weight of the enzyme is approximately 90,000, as determined by sedimentation velocity and sedimentation equilibrium experiments. The subunit molecular weight estimated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate is 46,000, suggesting that the enzyme is composed of two subunits of equal size. Functionally there are many similarities between E. coli enolase and other enolases studied. Thus, the dependence on Mg2+ for activity and the inhibition by fluoride in the presence of phosphate are quantitatively very similar for all enolases. Other catalytic parameters (Km, Vmax, and pH optimum) are also similar, but minor quantitative distinction indicates that E. coli enolase is more closely related to yeast enolase than to enolases from vertebrate muscle.

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolyase; EC 4.2.1.11) has been obtained from a large number of biological sources. Most of the well characterized enolases are from vertebrate muscle, although considerable work has also been done on yeast enolase (for a recent review, see Reference 1). Based on extensive comparison of catalytic and structural properties of the enolases, it has been concluded that while the catalytic site of this enzyme has remained remarkably stable through evolution, several structural features reflect evolutionary changes and unique properties of individual enolases (1). However, one structural feature which appears to be as constant as the catalytic properties is the occurrence of two identical or very similar subunits in enolases from as widely different sources as yeast, fish, and mammals.

An obvious flaw in the conclusions from all the comparative work on enolases is the lack of data on this enzyme from prokaryotic species. Little work has been done on bacterial enolases (2, 3), and this study was therefore undertaken to purify and characterize the enzyme from one bacterial species, Escherichia coli. Preliminary work with E. coli enolase (3), suggested that E. coli would be a reasonable source of the enzyme. E. coli is also commercially available in quantities sufficient for large scale purification.

EXPERIMENTAL PROCEDURE

Activity and Protein Assays

All reagents used were reagent grade unless otherwise specified. A commercial source of ion-free water was used throughout this work. Enolase activity was measured by the ultraviolet absorption of enolpyruvate phosphate (4) by the methods described previously (5). The standard assay medium contained 1 mM D-glycerate 2-phosphate and 1 mM MgSO4 in 0.05 M Tris-HCl buffer, pH 8.1, containing 0.1 M KCl and 0.01 mM EDTA. The substrate was obtained as the water-insoluble barium salt (Sigma) and was converted to the soluble cyclohexylammonium salt (5) before use. Rates were measured with a Zeiss PMQ II spectrophotometer or with a Cary 15 recording spectrophotometer.

One unit of enolase activity (E. coli) was defined as the amount of enzyme which gave a change of 0.1 absorbance unit per min in the standard assay at 30°. Conversion to international units of activity (micromoles of substrate converted to product per min) can be made since the molar extinction coefficients for enolpyruvate phosphate is known for the conditions of the assay (6). To facilitate comparison to data for other enolases, the arbitrary unit is used in the text of this paper, but both types of units have been used in the tabulation of activity.

Protein concentration was measured either by the method of Lowry et al. (7) with lysozyme (Worthington) as the standard or by the absorbance at 280 nm. One A280 unit of protein was defined as the amount of protein which gave an absorbance of 1.0 at 280 nm when dissolved in a 1.0 ml volume. (A solution of purified E. coli enolase at 1.0 mg per ml was eventually found to have an absorbance of 0.57 at 280 nm.)

Amino Acid Analysis

E. coli enolase was hydrolyzed at 110° for 24, 48, and 72 hours in 6 N HCl containing 1:2000 (v/v) mercaptoethanol to protect

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methionine (8). Amino acid analyses were performed by the method of Moore, Spackman, and Stein (9) with a Beckman 120C amino acid analyzer. Cysteine was determined after oxidation with dimethyl sulfoxide to cysteic acid by the method of Spence and Wood (10). Tryptophan was analyzed separately by the method of Spies (11).

**Physical Measurements**

Protein homogeneity was established by analytical disc gel electrophoresis according to the method of Davis (12) with the Tris-glycine buffer system at pH 8.3. The molecular weight of the native enzyme was determined in the Spinco model E ultracentrifuge by sedimentation-diffusion analysis with a valve-type synthetic boundary cell, or by sedimentation equilibrium according to the method of Yphantis (13) with a six-channel Yphantis cell. The schlieren optical system was used for both types of measurements and the photographic plates were measured with a Nikon 10X microcomparator. Weight average and Z-average molecular weights were calculated with the equations of Van Holde and Baldwin (14). Highly purified enolase was used for the molecular weight determinations. The buffer used was 0.1 M HCl titrated to pH 8 with Tris and contained 10⁻² M MgSO₄ and 10⁻³ M EDTA. Samples of protein were equilibrated with this buffer by passage through a Sephadex G-25 column equilibrated with buffer. The buffer used for dilutions and for solvent blanks was also passed through the same column.

Subunit molecular weight was estimated by gel electrophoresis in the presence of sodium dodecyl sulfate (15). The method of Weber and Osborn (16) was followed except that protein stock solutions of 2 mg per ml were prepared and only 25 µl of the stock solution was applied to the gels. Reference proteins were catalase (Sigma) and rabbit muscle aldolase (Sigma).

Protein concentration determinations required to establish other molecular properties were first carried out by the refractive index increment method of Bubel and Stebbilagen (17), with the ultracentrifuge with interference optics and a double sector synthetic boundary cell at a rotor speed of 7928 rpm. No significant sedimentation of the protein took place during the measurements. The system was calibrated with a known concentration of purified yeast enolase which gave a value of 4.27 fringes per mg per ml. This value was used for the calculation of E. coli enolase concentration. Based on the concentration determination, the absorbance of a 1 µg per ml solution of pure E. coli enolase was found to be 0.61 at 277 nm and 0.57 at 280 nm, and absorbance was subsequently used as a more convenient method for concentration determination.

**Purification of E. coli Enolase**

All the manipulations were carried out in a cold room at 4 °C unless other conditions are specified. The starting material, frozen E. coli D cells, was obtained from General Biochemicals (Catalogue No. 150040: grown on Kornberg medium and harvested in late log phase).

**Step 1. Acetone Powder Extraction**—The frozen cells were partially thawed at room temperature and then thoroughly mixed in a Waring blender for 30 sec. The thick cell suspension was poured slowly into 8 volumes of cold acetone (cooled to −20 °C) and stirred vigorously. If complete dispersal is a problem at this stage, more vigorous agitation, for example in a Waring blender, is indicated. The insoluble material was collected by suction filtration, resuspended in 4 volumes of cold acetone, filtered again, and washed with 2 volumes of absolute ether previously cooled to −20 °C. The filter cake was dried on a large surface area of absorbent paper to hasten solvent evaporation. The yield of air-dry acetone powder was about 20% of the wet weight of the cells. Extraction of enolase from the acetone powder was accomplished by adding the powder slowly to a cold (4 °C) solution of TME buffer (0.01 M Tris, 1 mM MgSO₄, 0.01 mM EDTA, pH adjusted to 8.0 with HCl) with vigorous stirring. The volume of buffer used was 18 ml per g of acetone powder. The extraction was carried out at 4 °C until the suspension was homogeneous. It is advantageous to extract the acetone powder overnight, in order to be able to carry out Steps 2 through 5 on the following day without interruption. The extract was centrifuged at 10,000 × g for 15 min to remove insoluble material. Re-extraction of the precipitate did not improve the yield of enolase appreciably. The yield of enolase at this stage was 5,000 to 10,000 units per g of acetone powder, depending on the batch of acetone powder and the length of the extraction period.

**Step 2. Heat Treatment**—The aqueous extract was made 1% (w/v) in MgSO₄·7H₂O by the addition of the MgSO₄·7H₂O dissolved in a minimum volume of TME buffer. The temperature of the extract was raised rapidly to 55 °C by immersion in a 70 °C water bath and maintained at 55 °C for 2 min. The extract was cooled to 10 °C in an ice bath and insoluble material was removed by centrifugation at 10,000 × g for 15 min. It was convenient to perform this step on about 500 ml of extract at one time.

**Step 3. Acetone Fractionation**—Acetone, 43 ml/100 ml of solution from Step 2 was cooled to −20 °C and added to the stirred protein solution in a fine stream over a period of 2 to 3 min. The temperature of the solution was kept below 10 °C at all times. In the initial stages a gelatinous precipitate formed, which subsequently coalesced as more acetone was added. The precipitate in the 30% acetone solution was removed by centrifugation at 10,000 × g for 15 min and discarded. An additional 57 ml of acetone per 100 ml of original heat supernatant was cooled to 10 °C in an ice bath and insoluble material was removed by centrifugation at 10,000 × g for 15 min. It was convenient to perform this step on about 50 ml of extract at one time.

**Step 4. TEAE-cellulose Titration**—This step was incorporated to remove a large amount of material which adsorbs strongly to TEAE prior to the column step. The procedure prevented overloading the column and greatly improved resolution. TEAE-cellulose (Brown Company, coarse grade, 0.81 meq per g) was washed with 1 N NaOH and 1 N HCl to remove colored impurities and was equilibrated with TME buffer. Increments of TEAE-cellulose were added to the fraction from Step 3 to adsorb protein and nucleic acid impurities. The 280-nm absorbance and enolase activity in the supernatant fluid were measured after each addition. The titration was stopped when

*The abbreviation used is TEAE-cellulose, triethylaminoethyl cellulose.*
the enolase activity began to decrease, i.e., after the addition of
about 6 mg of moist TEAE-cellulose per A\textsubscript{280} unit present in the
original solution. TME buffer was added when necessary to
keep the cellulose in a thick slurry. The TEAE-cellulose was
removed by filtration under gentle suction and washed once
afterwards. The TEAE-cellulose was immediately applied to a TEAE-cellulose column.

**Step 4. TEAE-cellulose Chromatography**—The protein solution from Step 4 was applied to a TEAE-cellulose column, 4 x 50
cm, and washed with 300 ml of TME buffer prior to elution.
A 0 to 0.25 M NaCl gradient (in TME buffer) was used to elute
the column. Total elution volume was 2 liters. The first 500
ml of effluent was discarded, after which 3-min fractions were
collected at a flow rate of 7.5 ml per min. Enolase eluted at a
conductivity of 5 to 10 mmho. Enolase reproducibly eluted
between resolution and speed. Flow rates were fast enough to
allow for completion of the column step in 5 hours, with a 50% yield of activity. Resolution
was not optimal since the protein often occupied 50 to 70%
of the total column capacity and there was some skewing of the
enolase peak.

Steps 6 and 7. Gel Filtration on Sephadex G-100—A sample of
150 mg of protein from the TEAE-cellulose column was dis-
solved in 10 ml of TME buffer and applied to a Sephadex G-100
column, 3 x 85 cm, and eluted with 0.05 M EDTA at a flow
rate of about 12 ml per hour (downward flow). Fractions were
collected every 20 min and those fractions with 800 enolase
units per A\textsubscript{280} unit of protein were pooled. Protein was pre-
cipitated with 80% saturated ammonium sulfate (0.53 g per
ml). This step was repeated on another G-100 column as be-
fore. By proper selection of elution fractions, nearly pure
enolase was obtained at this stage.

**Step 5. Crystallization**—A concentrated enolase solution (10
to 20 mg per ml) was made about 70% saturated in ammonium
sulfate by addition of 0.44 g of solid (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} per ml of solu-
tion. The pH was adjusted to 6 with dilute acetic acid if
necessary. Increments of a saturated solution of ammonium
sulfate at pH 6 were added until turbidity was evident. Crystal-
ization occurred within a few hours. At the early stages of
crystallization amorphous precipitates were sometimes obtained,
especially when contaminating proteins were present. In these
cases, the precipitates were removed by centrifugation and fur-
ther additions of ammonium sulfate eventually resulted in
crystals. The crystallization step was used both for purification
and for storing the enzyme. Suspensions of the crystalline
enzyme in ammonium sulfate solution at pH 6 have been stored at 4° for more than a year without any activity loss. The only
critical factor observed for the crystallization was the pH,
which had to be 6 or lower. The crystalline enzyme can be
dissolved simply by titrating the ammonium sulfate suspension
of crystals from pH 6 to 8.

**RESULTS**

**Purification of E. coli Enolase**—The results of a typical puri-
ification from 500 g of cells, wet weight, are summarized in Table

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Typical purification of E. coli enolase from 83 g of acetone powder</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Enolase units</th>
<th>Protein (g)</th>
<th>Enolase units/\textsubscript{mg}protein</th>
<th>Enolase units/\textsubscript{A\textsubscript{280} unit}</th>
<th>% Yield</th>
<th>\textsubscript{A280}/\textsubscript{A260}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Extraction</td>
<td>1200</td>
<td>372,000</td>
<td>12.6</td>
<td>30 (4.4)</td>
<td>4.8</td>
<td>100</td>
</tr>
<tr>
<td>(2)</td>
<td>Heat treatment</td>
<td>1180</td>
<td>390,000</td>
<td>8.0</td>
<td>49 (7.2)</td>
<td>6.4</td>
<td>105</td>
</tr>
<tr>
<td>(3)</td>
<td>Acetone fractionation</td>
<td>100</td>
<td>440,000</td>
<td>3.7</td>
<td>120 (17.5)</td>
<td>54</td>
<td>119</td>
</tr>
<tr>
<td>(4)</td>
<td>TEAE cellulose titration</td>
<td>210</td>
<td>400,000</td>
<td>2.6</td>
<td>154 (22)</td>
<td>110</td>
<td>108</td>
</tr>
<tr>
<td>(5)</td>
<td>TEAE cellulose chromatography</td>
<td>275</td>
<td>205,000</td>
<td>0.47</td>
<td>435 (64)</td>
<td>405</td>
<td>55b</td>
</tr>
<tr>
<td>(6)</td>
<td>Sephadex G-100 chromatography I</td>
<td>30</td>
<td>120,000</td>
<td>0.194\textsuperscript{c}</td>
<td>620\textsuperscript{c} (91)</td>
<td>1090</td>
<td>32\textsuperscript{b}</td>
</tr>
<tr>
<td>(7)</td>
<td>Sephadex G-100 chromatography II</td>
<td>30</td>
<td>100,000</td>
<td>0.095\textsuperscript{c}</td>
<td>1020\textsuperscript{c} (149)</td>
<td>1780</td>
<td>29</td>
</tr>
<tr>
<td>(8)</td>
<td>Crystallization\textsuperscript{d}</td>
<td>3</td>
<td>80,000</td>
<td>0.074\textsuperscript{c}</td>
<td>1080\textsuperscript{c} (158)</td>
<td>1900</td>
<td>22</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Units defined in the text are reported in this table. The values in parenthesis in this column are the international
units (molecules of product formed per min).

\textsuperscript{b}Yield could be improved by retreatment of side fractions.

\textsuperscript{c}Protein estimates based on the finding that a 1 mg/ml solution had a 280 nm absorbance of 0.57.

\textsuperscript{d}Crystalline enzyme was 95% pure by disc gel electrophoresis (maximum specific activity for electrophoretically pure enzyme
was 1150 enolase units/mg protein).
**Table II**

**Sedimentation-diffusion properties of E. coli enolase**

Diffusion was measured at 6166 rpm to about half-height. The rotor speed was then increased to 59,780 rpm for the sedimentation measurements. All measurements were made at 20°. The numbers given are the mean values ± standard error of several independent microcomparator measurements of the photographic plates.

<table>
<thead>
<tr>
<th>Protein conc.</th>
<th>$S_{app}$</th>
<th>$D_{app} \times 10^{7}$ sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/ml</td>
<td>(Svedbergs)</td>
<td></td>
</tr>
<tr>
<td>10.2</td>
<td>4.81 ± 0.13</td>
<td>5.65 ± 0.21</td>
</tr>
<tr>
<td>6.90</td>
<td>4.92 ± 0.52</td>
<td>5.78 ± 0.59</td>
</tr>
<tr>
<td>3.45</td>
<td>5.21 ± 0.15</td>
<td>5.85 ± 0.16</td>
</tr>
<tr>
<td>a</td>
<td>5.40 ± 0.30$^b$</td>
<td>5.95 ± 0.30$^b$</td>
</tr>
</tbody>
</table>

$^a$Extrapolation to zero protein concentration.

$^b$Includes extrapolation error.

**Table III**

**Molecular weight of E. coli enolase**

The numbers are the mean values ± standard error of multiple measurements of the schlieren photographs (four measurements for the hinge point molecular weight; two for the weight average molecular weight). Z-average molecular weight was obtained with a single set of measurements.

<table>
<thead>
<tr>
<th>Protein conc.</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hinge point $x 10^{-3}$</td>
</tr>
<tr>
<td>10.2</td>
<td>77.1 ± 1.7</td>
</tr>
<tr>
<td>6.8</td>
<td>81.5 ± 1.1</td>
</tr>
<tr>
<td>3.4</td>
<td>96.0 ± 2.1</td>
</tr>
<tr>
<td>a</td>
<td>92.0 ± 4.0$^b$</td>
</tr>
</tbody>
</table>

$^a$Extrapolation (of 1/$M_{app}$) to zero concentration.

$^b$Includes extrapolation error.

1. The purity of the enzyme at different stages of purification was estimated by specific activity and by analytical dis gel electrophoresis. Based on the latter criterion, it was estimated that the crystalline enzyme generally contained at most 5% of a contaminating component. This contaminant could eventually be removed by repetition of Steps 5 through 8 to give an electrophoretically pure enzyme with a specific activity of 1150 units per mg.

**Molecular Properties**—All the molecular properties were determined with the highest purity enzyme (1150 units per mg). The molecular weight of E. coli enolase was determined by sedimentation-diffusion measurements and by sedimentation equilibrium. The sedimentation-diffusion results are summarized in Table II. Single and symmetrical schlieren peaks were obtained in all cases, indicating homogeneity. The molecular weight calculated from this data was 82,000 ± 8,200. The observed error in the measurement of $S_{app}$ and $D_{app}$ is about ±5%, because of microcomparator and extrapolation uncertainty. The molecular weight calculated from $S$ and $D$ consequently could have a 10% error.

Molecular weight data from the sedimentation equilibrium measurements are summarized in Table III. The hinge point calculation is the most reliable estimate of the molecular weight (92,000 ± 4,000), but a weight average and a Z-average molecular weight calculated from the same schlieren pattern agree with the hinge point molecular weight within the precision of the measurements (about 5%). A plot of $(1/x) \cdot (dc/dx)$ versus $c$ was linear across 70% of the Yphantis cell at all protein concentrations. This supports the conclusion that the purified enzyme was homogeneous with respect to molecular weight.

The subunit molecular weight of E. coli enolase was investigated by acrylamide gel electrophoresis in sodium dodecyl sulfate. When the method was applied to E. coli enolase, with bovine catalase (subunit molecular weight 57,000) and rabbit muscle aldolase (subunit molecular weight 40,000) as reference proteins a subunit molecular weight of 46,000 ± 1,000 was calculated as the average of three duplicate gels. The data is consistent with two subunits of equal size for E. coli enolase, and thus agrees with the general subunit structure of other enolases.

The amino acid analysis of E. coli enolase is presented in Table IV. A comparison of the amino acid composition of E. coli enolase with those of other enolases (1) reveals little meaningful information, except that both the yeast and E. coli enzymes have low cysteine content compared to the vertebrate muscle enolases.

**Catalytic Properties**—The catalytic properties of E. coli enolase were initially determined with an enzyme sample with a specific activity of 850 units per mg (about 75% pure). However, subsequent determinations of $K_m$ and $V_{max}$ with the pure enzyme did not reveal any quantitative or qualitative differences. The characteristic features of all enolases, the Mg$^{2+}$ activation and fluoride inhibition (in the presence of phosphate) were studied, together with the usual catalytic parameters, $K_m$, $V_{max}$, and pH optimum. Whenever necessary, the results were corrected for the effect of pH, Mg$^{2+}$, and K$^+$ on the extinction coefficient of enolpyruvate phosphate (6).

The $K_m$ of $1 \times 10^{-4}$ M is somewhat higher than that observed for enolases from vertebrate sources (0.4 to 0.6 $\times 10^{-4}$ M) but identical with that for yeast enolase. The Mg$^{2+}$ optimum ($2 \times 10^{-3}$ M) and the fluoride inhibition index ($0.5 \times 10^{-12}$ M) are both within the range of those observed for other enolases (1, 18). The pH optimum of 8.1 is significantly higher than that for vertebrate enolases (pH 7) and only somewhat above that of yeast and plant enolases (pH 7.6 to 8.0). The specific activity of E. coli enolase (180 units per mg) is twice as high as that of the vertebrate enolase (about 90 units per mg). Yeast enolase with 130 units per mg is intermediate between the vertebrate enolases and E. coli enolase in specific activity.

When E. coli enolase was treated with EDTA in attempts to remove all residual activity prior to the Mg$^{2+}$ activation studies,
TABLE IV

Amino acid composition of E. coli enolase

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>moles amino acid per analysisa</th>
<th>Extrapolated or average moles</th>
<th>Moles per 90,000 grams protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr</td>
<td>48 hr</td>
<td>72 hr</td>
</tr>
<tr>
<td>asp</td>
<td>0.208</td>
<td>0.211</td>
<td>0.210</td>
</tr>
<tr>
<td>thr</td>
<td>0.097</td>
<td>0.095</td>
<td>0.089</td>
</tr>
<tr>
<td>ser</td>
<td>0.096</td>
<td>0.086</td>
<td>0.080</td>
</tr>
<tr>
<td>glu</td>
<td>0.226</td>
<td>0.224</td>
<td>0.225</td>
</tr>
<tr>
<td>pro</td>
<td>0.053</td>
<td>0.056</td>
<td>0.055</td>
</tr>
<tr>
<td>gly</td>
<td>0.222</td>
<td>0.221</td>
<td>0.221</td>
</tr>
<tr>
<td>ala</td>
<td>0.286</td>
<td>0.285</td>
<td>0.285</td>
</tr>
<tr>
<td>cysb</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>val</td>
<td>0.113</td>
<td>0.118</td>
<td>0.120</td>
</tr>
<tr>
<td>met</td>
<td>0.052</td>
<td>0.051</td>
<td>0.049</td>
</tr>
<tr>
<td>ilu</td>
<td>0.136</td>
<td>0.136</td>
<td>0.160</td>
</tr>
<tr>
<td>leu</td>
<td>0.146</td>
<td>0.141</td>
<td>0.141</td>
</tr>
<tr>
<td>tyr</td>
<td>0.052</td>
<td>0.052</td>
<td>0.051</td>
</tr>
<tr>
<td>phe</td>
<td>0.057</td>
<td>0.058</td>
<td>0.058</td>
</tr>
<tr>
<td>lys</td>
<td>0.185</td>
<td>0.182</td>
<td>0.188</td>
</tr>
<tr>
<td>his</td>
<td>0.034</td>
<td>0.036</td>
<td>0.036</td>
</tr>
<tr>
<td>arg</td>
<td>0.051</td>
<td>0.051</td>
<td>0.053</td>
</tr>
<tr>
<td>trp</td>
<td>--</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a normalized to give best overall fit with asp, glu, gly, and ala.

b determined as cysteic acid in DMSO oxidized sample (10).

c by the method of Spies (11).

It was found that the enzyme was unstable in the absence of Mg++, and that substantial and irreversible loss of activity was observed after very short exposure of the enzyme to EDTA. Other enolases do not show this denaturation in the absence of Mg++, although removal of Mg++ has been found to greatly facilitate dissociation of both yeast and rabbit muscle enolase into inactive monomers (19-21).

DISCUSSION

The purpose of the work on E. coli enolase was to compare a procaryotic enolase to previously studied enolases from eucaryotic sources. The results indicate that E. coli enolase is very similar to the other enolases in both physical and catalytic properties. There is some suggestion from the data that E. coli enolase more closely resembles yeast enolase than the vertebrate enolases (primarily in pH optimum, Km, and specific activity).

The finding of a single electrophoretic form of E. coli enolase is consistent with the findings of Pfleiderer et al. (22) who detected only one band of enolase activity (determined by an activity stain) by starch gel electrophoresis of E. coli enolase extracts. Many other enolases have been shown to be single molecular forms, although multiple molecular forms have been observed in yeast (22-24) and some fish species (25-27).

Based on the available evidence, enolases, regardless of source, are characterized by the following properties: (a) a molecular weight of 80,000 to 100,000; (b) two subunits of similar or identical size; (c) two active sites per active dimer, based on the binding stoichiometry of substrate analogues to enolases (1, 28); (d) an absolute dependence on divalent cations (Mg++, Mn++, or Zn++) for activity; and (e) specific inhibition by fluoride ion in the presence of phosphate. The observed differences in catalytic properties are minor.

REFERENCES

The Purification and Characterization of *Escherichia coli* Enolase

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