A Revised Preparation of Yeast (Saccharomyces cerevisiae)
Pyruvate Kinase*

(Received for publication, April 9, 1975)

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A revised preparation of pyruvate kinase from Saccharomyces cerevisiae is reported. By purifying this cold labile enzyme at room temperature, an improved recovery and specific activity was obtained. More than 350 mg of pure enzyme with a specific activity of 350 to 400 units/mg at 30°C were obtained from a pound of fresh yeast. The last step of the preparation, passage of the enzyme over Sephadex G-100, was required to remove a contaminating protease. The molecular parameters of the new preparation are: molecular weight, 209,000; four subunits of identical size; \( E_{280} \text{nm} \) 0.51; pH 6.6; and pH optimum, 6.28. Kinetic parameters are: \( K_m \) for P-enolpyruvate and ADP, 0.09 and 0.16 mM in the presence of saturating Fru-1,6-P_{2}, and 1.8 and 0.34 mM in the absence of Fru-1,6-P_{2}; \( K_a \) for Fru-1,6-P_{2}, 0.014 mM. No free NH-terminal amino acid could be detected. Amino acid composition was determined and compared with other pyruvate kinase preparations.

**ENZYME PROPERTIES**

Homogeneity—The purified enzyme was homogeneous by two independent criteria; (a) a single protein band either for dissociated subunits (Fig. 1B) or for native enzyme (Fig. 2B) was obtained by analytical polyacrylamide gel electrophoresis, (b) its plot of log net fring versus square of radius of equilibrium sedimentation was linear (Fig. 3B). The enzyme sedimented as a single peak during velocity sedimentation (data not shown).

Stability—The preparation exhibits the same cold lability (1) and susceptibility to inactivation by Fru-1,6-P_{2} as that reported for the original preparation. The purified enzyme, at 3.8 mg ml^{-1}, was stable at room temperature in 0.1 M sodium phosphate, pH 6.5, in the presence of 25% glycerol, 5 mM EDTA, and 5 mM mercaptoethanol for a minimum period of 31 days (Table 1IV). The enzyme may be stored at 0°C as a suspension in 90% saturated ammonium sulfate.

Extinction Coefficient—The extinction coefficient determined by dry weight gave an average value of 0.51 ml mg^{-1} cm^{-1} at 280 nm. The value obtained by the method of

† Direct proof for a protease contamination at this stage of purification is not possible. The suspected protease contamination is not sufficiently large to see on polyacrylamide gel or by analytical ultracentrifugation; furthermore, no protease activity could be detected in an azoalbumin assay described by Tomarelli (5) or in a fluorescine-coupled assay (6).
### Summary of yeast pyruvate kinase purification

Assay conditions are described under "Experimental Procedure." The yields are given for 1 pound of yeast.

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Toluolysis (37°)</td>
<td>475</td>
<td>12,400</td>
<td>280,000</td>
<td>22.6</td>
<td>100</td>
</tr>
<tr>
<td>II. (NH4)2SO4 fraction (40-55%)</td>
<td>102</td>
<td>2,500</td>
<td>197,000</td>
<td>79</td>
<td>70</td>
</tr>
<tr>
<td>III. Dialysate</td>
<td>111</td>
<td>3,010</td>
<td>223,000</td>
<td>74</td>
<td>80</td>
</tr>
<tr>
<td>IV. DEAE-cellulose phosphate, pH 7.5</td>
<td>390</td>
<td>1,680</td>
<td>160,000</td>
<td>95</td>
<td>70</td>
</tr>
<tr>
<td>V. Cellulose phosphate, pH 6.5</td>
<td>246</td>
<td>390</td>
<td>135,000</td>
<td>346</td>
<td>48</td>
</tr>
<tr>
<td>VI. (NH4)2SO4 precipitation</td>
<td>14.6</td>
<td>350</td>
<td>126,000</td>
<td>360</td>
<td>45</td>
</tr>
<tr>
<td>VII. Sephadex G-100</td>
<td>178</td>
<td>396</td>
<td>123,000</td>
<td>345</td>
<td>44</td>
</tr>
</tbody>
</table>

Scopes (7) using the ratio of $A_{360}/A_{280}$ was 0.515 ml mg$^{-1}$ cm$^{-1}$. The extinction coefficient of pyruvate kinase from other sources is compared in Table V.

**pH Optimum and Isoelectric Point**—A pH profile of pyruvate kinase in the presence of saturating Fru-1,6-P$_x$ shows a bell-shaped curve with an optimum at pH 6.28 (data not given) in agreement with earlier data (8). Fig. 6S shows a pI = 6.6.

**Molecular Weight**—High speed sedimentation equilibrium according to the method of Yphantis (9) gave an average molecular weight of 209,000 ± 6,000 from three determinations (Fig. 3S). A molecular weight of 56,500 to 58,500 was obtained for the subunits by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 4S). The molecular weight determined by electrophoresis of native enzyme on polyacrylamide gels, as described by Hedrick and Smith (10) was 220,000 ± 6,500 (Fig. 5S). An identical value was obtained for enzyme in crude extract. Purified pyruvate kinase mixed with crude extract gave a single activity band on polyacrylamide gels of varying acrylamide concentration.

**Amino Acid Composition and NH$_2$-terminal**—The preparation has a typical amino acid composition (Table III). No free NH$_2$-terminal amino group was detected.

**Kinetic Parameters**—The kinetic parameters for this preparation, except for the specific activity, are not significantly different from those reported for the original preparation (1).

Initial velocity versus concentration of P-enolpyruvate under conditions specified under "Experimental Procedure" is sigmoidal with a Hill coefficient, $n_H = 2.3$ and $K_m = 1.8$ mM. The sigmoidal curve becomes hyperbolic in the presence of Fru-1,6-P$_x$. The $K_m$ for P-enolpyruvate under the same condition, but with 1 mM Fru-1,6-P$_x$ is 0.09 mM. The $K_m$ for ADP is 0.34 mM without Fru-1,6-P$_x$ and 0.16 mM with Fru-1,6-P$_x$. $K_m$ for Fru-1,6-P$_x$ is 0.014 mM at 1 mM P-enolpyruvate.

### REFERENCES

Purification and Properties of Yeast Pyruvate Kinase

Supplementary Material

To A Purified Preparation of Yeast (Saccharomyces cerevisiae) Pyruvate Kinase

Supplementary Text

Purification and Properties of Yeast Pyruvate Kinase

Supplementary Text

Fig. 1 A S 1.2 -!

Supplementary Text

Supplementary Text

Fig. 2 S

Supplementary Text

Fig. 3 S

Supplementary Text

Fig. 4 S

Supplementary Text

Fig. 5 S

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Fig. 6 S

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Fig. 7 S

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Fig. 8 S

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Fig. 9 S

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Fig. 10 S

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Fig. 11 S

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Fig. 12 S

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Fig. 13 S

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Fig. 16 S

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Fig. 17 S

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Fig. 18 S

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Fig. 19 S

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Fig. 20 S

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Fig. 21 S

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Fig. 22 S

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Fig. 23 S

Supplementary Text
Purification and Properties of Yeast Pyruvate Kinase

Fig. 3

Molecular weight determination of native yeast pyruvate kinase by gel electrophoresis according to Andrews and Smith (1978). Electrophoresis was performed at 80 V for 4 hr in a 10% acrylamide gel. The molecular weights of the standards were calculated using the following values: 1. horse heart myoglobin, 2. ovalbumin, 3. bovine serum albumin, 4. horse spleen tyrosinase, 5. chymotrypsinogen A, 6. carbonic anhydrase A, 7. aldolase, 8. lactate dehydrogenase, and 9. pyruvate kinase. The gel was stained with Coomassie Blue R-250 and scanned with a densitometer. The molecular weight of the purified enzyme was calculated from the migration distance and the molecular weight of the standards.

Fig. 4

Separation of subunits molecular weight determination of yeast pyruvate kinase by gel electrophoresis according to Andrews and Smith (1978). Electrophoresis was performed at 80 V for 4 hr in a 10% acrylamide gel. The molecular weights of the standards were calculated using the following values: 1. horse heart myoglobin, 2. ovalbumin, 3. bovine serum albumin, 4. horse spleen tyrosinase, 5. chymotrypsinogen A, 6. carbonic anhydrase A, 7. aldolase, 8. lactate dehydrogenase, and 9. pyruvate kinase. The gel was stained with Coomassie Blue R-250 and scanned with a densitometer. The molecular weight of the purified enzyme was calculated from the migration distance and the molecular weight of the standards.

Table I

**Table I**

<table>
<thead>
<tr>
<th>Pyruvate Kinase Activity at Various Stages During Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Cell extract</td>
</tr>
<tr>
<td>G20-200</td>
</tr>
<tr>
<td>G20-200</td>
</tr>
<tr>
<td>G20-200</td>
</tr>
</tbody>
</table>

Fig. 5

**Stability of Pyruvate Kinase Activity at Various Stages During Purification**

The activity of the purified enzyme was measured at different stages of the purification process. The enzyme was stored at 4°C in 10% glycerol and 50 mM Tris-HCl, pH 7.5. The activity was assayed at 30°C in a standard assay mixture containing 100 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 5 mM ATP, and 1 mM NADH. The reaction was started by the addition of NAD⁺, and the increase in absorbance at 340 nm was monitored.

In conclusion, the purification of yeast pyruvate kinase was successful, and the enzyme retained its full activity throughout the purification process. Further studies are needed to investigate the mechanism of action of this enzyme and its role in cellular metabolism.
## Purification and Properties of Yeast Pyruvate Kinase

### Table V

<table>
<thead>
<tr>
<th>Property</th>
<th>Reference</th>
<th>Precipitate</th>
<th>80% Alcohol</th>
<th>95% Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (kDa)</td>
<td>ref. (196)</td>
<td>100,000</td>
<td>100,000</td>
<td>100,000</td>
</tr>
<tr>
<td>Western blot analysis</td>
<td>ref. (196)</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>ref. (196)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Specific activity (U/mg)</td>
<td>ref. (196)</td>
<td>200,000</td>
<td>200,000</td>
<td>200,000</td>
</tr>
<tr>
<td>Glucose uptake (μmol/g)</td>
<td>ref. (196)</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

### Table VI

| Comparison of Purified Yeast Pyruvate Kinase by Various Purification Techniques |
|-------------------------------|------------------------|-------------|-------------|-------------|
| Method                        | Reference              | Precipitate | Alcohol     | Alcohol     |
| Methanol essentially removes  | ref. (196)             | Present     | Present     | Present     |
| Cell debris                   | ref. (196)             | Present     | Present     | Present     |
| Macromolecular complexes      | ref. (196)             | Present     | Present     | Present     |
| Nucleic acids                 | ref. (196)             | Present     | Present     | Present     |
| Protein fractions             | ref. (196)             | Present     | Present     | Present     |
| Lipids                        | ref. (196)             | Present     | Present     | Present     |
| Phosphate                     | ref. (196)             | Present     | Present     | Present     |

### References

A revised preparation of yeast (Saccharomyces cerevisiae) pyruvate kinase.
S L Yun, A E Aust and C H Suelter


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