Human Phosphoglycerate Kinase

I. CRYSTALLIZATION AND CHARACTERIZATION OF NORMAL ENZYME*

Akira Yoshida and Sumiko Watanabe

From the Division of Medical Genetics, Department of Medicine, University of Washington, School of Medicine, Seattle, Washington 98195

SUMMARY

Human phosphoglycerate kinase (ATP:3-phospho-d-glycerate 1-phosphotransferase, EC 2.7.2.3) was purified from red blood cells. The stepwise procedures are elimination of hemoglobin from the hemolysate by treatment with ethanol-chloroform, precipitation of the enzyme by ethanol, treatment with carboxymethyl-Sephadex, gel filtration on Sephadex G-75, treatment with diethylaminoethyl-Sephadex, and column chromatography with carboxymethyl-Sephadex. A crystalline preparation was obtained with an over-all yield of about 60%.

The sedimentation patterns on analytical ultracentrifugation and the interference pattern of sedimentation equilibrium indicated a homogeneous preparation. The sedimentation constant (s20, w) was 3.35 S at a protein concentration of 0.6%. The molecular weight was estimated as 49,600 by the sedimentation equilibrium method.

The enzyme could not be dissociated into smaller subunits by treatment with 5 M guanidine hydrochloride containing 2-mercaptoethanol, with maleic anhydride, or with sodium dodecylsulfate. The enzyme has N-acetylserine as NH₂-terminal and isoleucine as COOH-terminal.

The specific activity of the crystalline preparation was 650 to 700 units per mg at pH 7.5 and at 25°. The enzyme required Mg²⁺ or Mn²⁺ for activity.

Phosphoglycerate kinase (ATP:3-phospho-d-glycerate 1-phosphotransferase, EC 2.7.2.3) is a key enzyme for ATP generation in the glycolytic pathway. An inherited deficiency of phosphoglycerate kinase in red blood cells in man is associated with hemolytic anemia (1, 2). The recent discovery of an electrophoretic variant of phosphoglycerate kinase in New Guinea natives proved the association (3). Subsequently, several other variant of phosphoglycerate kinase in New Guinea natives proved the association (4). The recent discovery of an electrophoretic variant of phosphoglycerate kinase in New Guinea natives proved the association (5). Subsequently, several other variant of phosphoglycerate kinase in New Guinea natives proved the association (6). These findings stimulated us to study the structure of the normal and variant phosphoglycerate kinases.

MATERIALS AND METHODS

Outdated (2 to 4 weeks old) human blood, anticoagulated with acid-citrate-dextrose, ACD NIH Formula A, was used for the enzyme preparation. Phosphoglycerate kinase is stable in red blood cells during storage at 4°; there was no significant difference between enzyme activity of fresh blood and of outdated blood.

Phosphoglycerate kinase activity was routinely measured in a reaction mixture containing 0.08 M Tris-chloride, pH 7.5, 8 mM MgCl₂, 10 mM d-3-phosphoglycerate, 5 mM ATP, 0.2 mM NADH, 20 μg (about 0.7 unit) of glyceraldehyde 3-phosphate dehydrogenase, and phosphoglycerate kinase. The increase of absorbance at 340 nm was recorded on a Gilford recording spectrophotometer at 25°. One unit is defined as that amount of enzyme which catalyzes the formation of 1 pmole of 1,3-diphosphoglycerate in 1 min at 25°. Specific activity is defined as the number of enzyme units per mg of protein.

Hemoglobin was assayed by Drabkin's method (4). Protein was assayed by Lowry's method (5), with crystalline bovine serum albumin as a standard. Carbonic anhydrase activity was assayed using p-nitrophenylacetate as substrate (6). Triosephosphate isomerase (7), glyceraldehyde 3-phosphate dehydrogenase (8), glyceraldehyde 3-phosphate dehydrogenase (9), pyruvate kinase (10), and adenylate kinase (ATP production from ADP) (11) activity were assayed as previously described.

CM-Sephadex C-50 (lot 3687), DEAE-Sephadex A-50 (lot 7300), and Sephadex G-75 (lot 4118) from Pharmacia; NADH, d-3-phosphoglycerate, and ATP from Sigma; and glyceraldehyde 3-phosphate dehydrogenase (crystalline, from rabbit muscle) from Böhringer Mannheim were used.

EXPERIMENTS AND RESULTS

Isolation of Phosphoglycerate Kinase

All of the procedures, unless otherwise specified in the text, were carried out at 0-4°. All buffer solutions and distilled water used for the preparation contained 1 mM 2-mercaptoethanol and 1 mM EDTA (neutralized to pH 7.0).

Hemolysis of Red Cells—The blood was centrifuged at 15,000 x g for 20 min, and supernatant plasma was removed with a pipette attached to an aspirator bottle. The red blood cells were washed twice with 2 volumes of 0.9% NaCl solution and were mixed with an equal volume of citrate-glycerol solution (19.4 g of potassium citrate, 3.1 g of Na₂HPO₄, 2.8 g of Na₂HPO₄, and 400 ml of glycerol in 1000 ml). The mixture was dialyzed against 10 volumes of water. The outside water was changed after 3 hours, and dialysis was continued overnight.

The phosphoglycerate kinase activity of the hemolysate thus obtained...
was 170 units (range 135 to 210 units in 10 blood samples) per g of hemoglobin.

Hemolysates prepared by mixing the packed red cells with 2 volumes of water had 105 units phosphoglycerate kinase activity (range 86 to 117 units in the same 10 blood samples described above) per g of hemoglobin. Why the hemolysate prepared by dialysis in the presence of citrate-glycerol had 60% higher activity than the usual hemolysate is not clear. Part of the enzyme may be tightly associated with cell membranes and glycerol-citrate may facilitate the release of the enzyme. However, extraction of the red cell membrane with glycerol-citrate released only part of the expected phosphoglycerate kinase activity. Citrate alone or glycerol alone in the phosphate buffer could not increase the phosphoglycerate kinase activity in hemolysate. Citrate-glycerol did not activate the enzyme of the hemolysate prepared by osmotic shock with water.

Elimination of Hemoglobin—Ethanol-chloroform (2:1, v/v), 400 to 420 ml, cooled at -60°C, was added at one time to 1.2 liters of the ice-cold hemolysate (from 2 pints of blood, total activity 18,000 to 21,000 units) adjusted to pH 7.3 with 1 M KOH. After stirring for 20 min in an ice bath, the mixture was centrifuged in stainless steel centrifuge bottles (15,000 × g, 20 min) and the hemoglobin precipitate was discarded. Ethanol (2.5 volumes), cooled to -25°C, was added to the supernatant fluid (about 25 ml) was centrifuged, and carboxymethyl-Sephadex (3 g, dry wt), equilibrated with 0.01 M phosphate buffer, pH 7.0, was placed on a Buchner funnel and was washed with 200 ml of 0.01 M phosphate buffer at pH 7.0. The enzyme adsorbed on the carboxymethyl-Sephadex was eluted with 250 ml of 0.1 M phosphate buffer, pH 7.0, and concentrated by vacuum dialysis.

Phosphoglycerate Kinase Activity—The concentrated enzyme solution (about 25 ml) was centrifuged, and carboxymethyl-Sephadex (3 g, dry wt), equilibrated with 0.01 M phosphate buffer, pH 7.0, was added to the supernatant liquid in small portions under stirring, maintaining the pH at 6.5 with 2 N NaOH. The mixture was placed on a Buchner funnel and was washed with 200 ml of 0.01 M phosphate buffer at pH 7.0. The enzyme adsorbed on the carboxymethyl-Sephadex was eluted with 250 ml of 0.1 M phosphate buffer, pH 7.0, and concentrated by vacuum dialysis.

Carboxymethyl-Sepha dex G-75 Gel Filtration—The concentrated enzyme solution (about 25 ml) was centrifuged, and carboxymethyl-Sepha dex (2.5 × 100 cm), equilibrated with 0.01 M phosphate buffer, pH 7.5, was placed on a carboxymethyl-Sepha dex column (2.5 × 10 cm), equilibrated with 0.01 M Tris-chloride, pH 8.0. The enzyme was eluted from the column by the same buffer, while hemoglobin remained adsorbed on the ion exchanger. The eluate was adjusted to pH 7.0 with 1 M acetic acid and concentrated by vacuum dialysis to about 15 ml.

Carboxymethyl-Sepha dex Column Chromatography—The concentrated enzyme solution was dialyzed against 0.01 M phosphate buffer, pH 7.0, and charged onto a carboxymethyl-Sepha dex column (2 × 30 cm), equilibrated with the same buffer. The enzyme was eluted with a linear gradient of buffer concentration from 0.01 M to 0.1 M (Fig. 1). The gradient was produced by adding 500 ml of 0.1 M buffer into a mixing chamber which contained 500 ml of 0.01 M buffer. The flow rate was 50 ml per hour. Phosphoglycerate kinase (Peak II) was eluted after carbonic anhydrase (Peak I), and the enzymes were completely separated by this procedure. The major phosphoglycerate kinase fractions were pooled (total activity 10,000 to 12,000 units) and concentrated by vacuum dialysis.

Besides the major phosphoglycerate kinase peak (Peak II), which represents more than 90% of total activity, two weak enzyme peaks, one at the front of the eluate and another at the carbonic anhydrase position, were observed (Fig. 1). The nature of these two phosphoglycerate kinase fractions will be discussed.

Crystallization—The concentrated enzyme solution was centrifuged and a small amount of precipitate was discarded. The supernatant liquid (about 4 ml) was placed in a dialysis bag (1 cm diameter) and dialyzed against 0.1 M phosphate buffer, pH 7.0, containing ammonium sulfate. The concentration of ammonium sulfate was increased stepwise from 30% saturation to 65% saturation over a period of 24 hours by adding saturated ammonium sulfate solution. The enzyme started to crystallize at about 35% saturation of ammonium sulfate; more than 85% of the enzyme was crystallized by the procedure. Feather-like crystals were observed (Fig. 2). The enzyme can be recrystallized by repeating the procedure.

Over-all yield of the crystalline enzyme was about 15 to 18 mg per g of hemoglobin.
Normal Human Phosphoglycerate Kinase

Vol. 247, No. 2

FIG. 2 (left). Photomicrograph of human phosphoglycerate kinase crystals. X 800.

Fig. 3 (center). Lineweaver-Burk plot of the effect of ATP concentration on phosphoglycerate kinase activity. The reaction mixture contained 50 mM Tris-chloride buffer, pH 7.5, 8 mM MgCl₂, 10 mM D-3-phosphoglycerate, 0.2 mM NADH, 0.7 unit of glyceraldehyde 3-phosphate dehydrogenase, enzyme, and various concentrations of ATP. The enzyme activity was measured at 25⁰. The concentration of MgATP was calculated from the dissociation constants (0.12 mM at 25⁰ and pH 7.5), which was estimated from values previously reported (23).

Fig. 4 (right). Effect of pH on phosphoglycerate kinase activity at 25⁰. Composition of the reaction mixture was 8 mM MgCl₂, 10 mM D-3-phosphoglycerate, 5 mM ATP, 0.2 mM NADH, 0.7 unit of glyceraldehyde 3-phosphate dehydrogenase, enzyme, and either 50 mM acetate buffer (pH 4 to 6), imidazole chloride buffer (pH 7.2 to 7.8), Tris-chloride buffer (pH 7.0 to 9.0), or sodium carbonate-bicarbonate buffer (pH 9.0 to 10.5).

Measurement of Sedimentation Constant

Ultracentrifugation experiments were carried out in a Spinco model E centrifuge. The protein was dissolved in 0.1 M phosphate buffer, pH 7.0, at a concentration of about 0.6%. Schlieren patterns of the enzyme solution showed single sedimentation boundaries. The sedimentation constant (θs,w) was calculated as 3.35 S (range 3.28 to 3.44 in five measurements) at a protein concentration of 0.6%.

Measurement of Molecular Weight and Subunit Molecular Size

The molecular weight of the enzyme and subunit molecular size were determined by sedimentation equilibrium method with the use of Rayleigh interference optics in a Spinco model E centrifuge (12).

The protein solutions used for the determination were as follow.

Solution I—The enzyme was dissolved in 0.1 M phosphate buffer, pH 7.0, and dialyzed against the same buffer. The concentration of the protein was 0.029%.

Solution II—The enzyme was dissolved in the above buffer containing 5 mM guanidine hydrochloride and 10 mM 2-mercaptoethanol, and dialyzed against the same buffer under N₂ overnight. The protein concentration was about 0.03%.

Solution III—The enzyme was dialyzed against water, lyophilized, S-carboxymethylated, and treated with maleic anhydride (13). The S-carboxymethylated amino acylated protein was dissolved in 0.1 M phosphate buffer, pH 7.0, containing 1 mM 2-mercaptoethanol and 1 mM EDTA, and dialyzed against the same buffer. The protein concentration was about 0.03%.

Centrifugation was carried out at 21,740 rpm for 20 hours. Plots of logarithum of fringe displacement with respect to radius from 2 pints of outdated blood. An example of yield and activity of the enzyme at various steps of purification is shown in Table I.

Yield and activity of phosphoglycerate kinase in purification from 1 liter of human blood

<table>
<thead>
<tr>
<th>Steps of purification</th>
<th>Total volume</th>
<th>Total protein</th>
<th>Phosphoglycerate kinase activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysate............</td>
<td>1,200 ml</td>
<td>135 x 10⁶ mg</td>
<td>20,500 units/ mg protein 0.15</td>
<td>100</td>
</tr>
<tr>
<td>Supernatant of ethanol-chloroform treatment...</td>
<td>1,100 ml</td>
<td>2.2 x 10⁶ mg</td>
<td>18,000 units/ mg protein 8.2</td>
<td>88</td>
</tr>
<tr>
<td>Precipitate with ethanol, dissolved in buffer...</td>
<td>70 ml</td>
<td>1.7 x 10⁵ mg</td>
<td>17,500 units/ mg protein 10.3</td>
<td>85</td>
</tr>
<tr>
<td>Eluate from CM-Sephadex, concentrated by vacuum dialysis...</td>
<td>20 ml</td>
<td>300 mg</td>
<td>17,000 units/ mg protein 50.5</td>
<td>83</td>
</tr>
<tr>
<td>Sephindex G-75 gel fraction, concentrated by vacuum dialysis...</td>
<td>40 ml</td>
<td>120 mg</td>
<td>14,000 units/ mg protein 117</td>
<td>68</td>
</tr>
<tr>
<td>Eluate from DEAE-Sephadex, concentrated by vacuum dialysis...</td>
<td>20 ml</td>
<td>400 mg</td>
<td>14,000 units/ mg protein 68</td>
<td>68</td>
</tr>
<tr>
<td>CM-Sephadex column effluent, concentrated by vacuum dialysis...</td>
<td>5 ml</td>
<td>21 mg</td>
<td>13,000 units/ mg protein 600</td>
<td>63</td>
</tr>
<tr>
<td>Crystallization...</td>
<td>18 ml</td>
<td>12 mg</td>
<td>12,000 units/ mg protein 670</td>
<td>59</td>
</tr>
</tbody>
</table>
TABLE II

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid residues</th>
<th>Number of amino acid residues per molecule*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>11.82</td>
<td>50</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.79</td>
<td>19</td>
</tr>
<tr>
<td>Serine</td>
<td>4.73</td>
<td>27</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9.82</td>
<td>38</td>
</tr>
<tr>
<td>Proline</td>
<td>4.03</td>
<td>21</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.18</td>
<td>45</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.45</td>
<td>45</td>
</tr>
<tr>
<td>Half cystine</td>
<td>2.29</td>
<td>11</td>
</tr>
<tr>
<td>Valine</td>
<td>8.94</td>
<td>45</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.70</td>
<td>14</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.53</td>
<td>20</td>
</tr>
<tr>
<td>Leucine</td>
<td>10.07</td>
<td>44</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.44</td>
<td>4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.90</td>
<td>17</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.26</td>
<td>44</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.67</td>
<td>6</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.74</td>
<td>12</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.67</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

* Closest integral number of amino acid residues per enzyme molecule of molecular weight of 49,600.

These results indicate that human phosphoglycerate kinase does not dissociate into smaller subunits under conditions which commonly cause dissociation.

Amino Acid Composition

The crystalline enzyme was dialyzed against water and lyophilized. The protein was hydrolyzed for 20 hours, 44 hours, and 68 hours at 110°C in hydrochloric acid of constant boiling point in an evacuated, sealed tube. For the determination of cysteine content, a sample of protein oxidized with performic acid (17) was subjected to amino acid analysis. Tryptophan was estimated by measuring the absorbance at 294.4 and 290 nm in 0.1 M NaOH solution, correcting for spurious absorption as described by Goodwin and Morton (14). The amino acid composition of human phosphoglycerate kinase is presented in Table II. The number of amino acid residues per molecule is also given in the table. Hexosamine was not detected in the amino acid analysis.

NH2-terminal and COOH-terminal Residues

The NH2-terminal amino acid of the enzyme was determined by Sanger's dinitrophenyl method (18) and by Edman's phenylisothiocyanate method (19). No free NH2-terminal amino acid was detected by either method, indicating that the NH2-terminal of the enzyme is blocked. After digestion of the S-carboxymethylated enzyme (20) by Streptomyces griseus protease and subsequently by carboxypeptidase A and carboxypeptidase B, N-acetylated was obtained from the digest. The recovery of N-acetyserine was more than 0.82 mole per 50,000 g of enzyme protein. Details of the method for identification of blocked NH2-terminal residues of proteins will be reported in a separate paper.

An attempt was made to determine the COOH-terminal amino acid of S-carboxymethylated enzyme by the hydrazinolysis method (21). Only isoleucine appeared as a free amino acid after hydrazinolysis at 100°C for 6.5 hours. Without correction for recovery, 1 mole of terminal isoleucine was obtained per 51,000 g of enzyme protein.

After incubation of lyophilized phosphoglycerate kinase or S-carboxymethylated phosphoglycerate kinase with carboxypeptidase A and B, only a small amount (1 mole per 130,000 g of protein) of isoleucine appeared after digestion at 38°C for 3.5 hours.

Enzymatic Properties

Enzyme Activity—The specific activity of crystalline human phosphoglycerate kinase measured in the reaction mixture containing 8 mM Tris-chloride at pH 7.5, 8 mM MgCl2, and 10 mM d-3-phosphoglycerate was 600 to 700 units per mg at 25°C. The specific activity was about 1000 at 35°C.

The crystalline enzyme has no measurable activity of carbonic anhydrase, triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, glycerolaldehyde 3-phosphate dehydrogenase, pyruvate kinase, or adenylate kinase.

Michaelis Constant—The rate of the enzyme reaction as a function of the concentration of d-3-phosphoglycerate indicated the usual Michaelis-Menten relationship. The Michaelis constant (Km) for d-3-phosphoglycerate was estimated as 1.1 mM under the standard assay conditions.

On the other hand, the rate of the enzyme reaction as a function of the concentration of ATP (or MgATP) did not fit the
usual Michaelis-Menten relationship, as shown in a Lineweaver-Burk plot (22) (Fig. 3). At higher concentrations of ATP, the $K_m$ was estimated as about 1 mm while at lower concentrations it was estimated as 0.37 mm.

**pH Optima**—The effect of pH was examined using 0.05 m buffer of sodium acetate (pH 4 to 6), imidazole chloride (pH 7.2 to 7.8), Tris-chloride (pH 7.0 to 9.0), and sodium carbonate-bicarbonate (pH 8.0 to 10.5) in the reaction mixture. A truncate pH activity profile, with maximal activity at pH 7.2 to 9.0, was observed (Fig. 4). Phosphate buffer inhibited the reaction.

**Stability and Other Properties**—The enzyme in the crude hemolysate is stable at neutral pH (pH 8.5 to 8.0) at 4°C for at least a week, but is more rapidly inactivated at acidic pH (lower than pH 6.0) and at alkaline pH (higher than pH 8.5). The purified crystalline enzyme dissolved at a low concentration in a buffer (Tris-chloride or imidazole chloride) of neutral pH is rapidly inactivated (more than 10% inactivation per 24 hours at 4°C). When bovine serum albumin was added to the enzyme solution at a final concentration of 10 mg per ml, the enzyme activity remained unchanged for at least a week at 4°C. Mg$^{2+}$ or Mn$^{2+}$ at a concentration of 5 mm to 10 mm is essential to the enzyme reaction. Higher Mg$^{2+}$ or Mn$^{2+}$ concentration (>50 mm) slightly inhibited the enzyme reaction.

**DISCUSSION**

Phosphoglycerate kinase activity (3-phosphoglycerate $\rightarrow$ 1,3-diphosphoglycerate) in normal human red blood cells has been reported as 22.6 ± 2.9 units (2) and 18.8 to 27 (1) units per 10$^6$ erythrocytes at $37^\circ$, i.e. about 80 units per g of hemoglobin in a hemolysate. These earlier values are significantly lower than the value obtained in this work, i.e. 105 units at $25^\circ$ (or 150 units at $35^\circ$) g of hemoglobin in the hemolysate prepared by osmotic shock or 170 units at $25^\circ$ (or 260 units at $35^\circ$) g of hemoglobin in the hemolysate prepared by dialysis in the presence of glycerol citrate. The difference between the value of previous workers and the present value is probably due to the methods of enzyme activity measurement. The previous workers estimated the enzyme activity in hemolysates by the rate of ADP production, which was measured indirectly with pyruvate kinase. Since the Michaelis constant of pyruvate kinase for ADP production is high (0.3 mm) (24), the true rate of ADP production cannot be measured under the assay conditions used. In the present work, the rate of 1,3-diphosphoglycerate production was measured in the presence of excess NAD-glyceraldehyde 3-phosphate dehydrogenase as commonly applied to the determination of yeast and animal phosphoglycerate kinase activity (25, 26). Since the Michaelis constant of NAD-glyceraldehyde 3-phosphate dehydrogenase for 1,3-diphosphoglycerate is low ($K_m \approx 0.8$ mm) (9), a true rate of 1,3-diphosphoglycerate production can be measured by this method. Although triosephosphate isomerase activity is high, glyceral 3-phosphate dehydrogenase activity is very low in hemolysates. Therefore, the phosphoglycerate kinase assay method used did not lead to overestimation of NADPH production.

From the specific activity of the human crystalline phosphoglycerate kinase (680 to 700) and from enzyme activity in the hemolysate (about 20,000 from 1 liter of blood), one can estimate that 1 liter of normal human blood contains about 30 mg of phosphoglycerate kinase. By the purification method described in this paper, about 15 to 18 mg of crystalline enzyme can be obtained from 1 liter of blood after about 5000-fold purification.

Phosphoglycerate kinase has been crystallized from yeast (27) and from rabbit and pig muscle (28). It has also been reported that the enzyme was crystallized from human red blood cells (28). However, the specific enzyme activity of the reported human phosphoglycerate kinase was only 5 to 7% of that of the enzyme preparation obtained in the present work. It is likely that the previous preparation contained mostly carbonic anhydrase, which exists in large quantity in red blood cells, and can be crystallized by the method described by these authors (28). Specificity (absence of carbonic anhydrase activity), homogeneity, and molecular size of their enzyme preparation were not reported.

Although early measurements of the molecular weight of phosphoglycerate kinase from yeast (29), pig, and rabbit (26) were in the range of 34,000 to 38,000, more recent measurements have shown that phosphoglycerate kinase from rabbit muscle and yeast has a molecular weight of 45,000 to 47,000 (30, 31). These values are close to the molecular weight of the human enzyme (mol wt 49,000) estimated by the sedimentation equilibrium method in this work. There also is a structural similarity in human, rabbit, and pig phosphoglycerate kinase. All three enzymes contain 1 tyrosine and 1 tryptophan residue per 12,000 to 12,500 g of protein (26). The specific activity of each of these three enzymes under similar assay conditions is 800 to 1,000 units per mg at 35°C.

Human phosphoglycerate kinase could not be dissociated into smaller subunits by treatments which have caused dissociation of various other proteins. The human enzyme seems to consist of a single chain polypeptide with N-acetylene as NH$_2$-terminal and isoleucine as COOH-terminal residues.

The elution profile of the enzyme from CM-Sephadex column chromatography (Fig. 1) showed two minor (altogether less than 5% of total activity) and one major enzyme peaks. Starch gel electrophoresis of the crude hemolysate frequently, although not always, showed two minor and one major enzyme bands (see accompanying paper (32)). Beutler (33) reported that only a major band of phosphoglycerate kinase was observed in starch gel containing ATP. The two minor components and one major component found by starch gel electrophoresis appear to correspond in mobility to the two minor and one major enzyme peaks of the CM-Sephadex elution profile. However, the separated enzyme peaks each showed the same electrophoretic pattern on starch gel as that of the unfractionated enzyme sample or the crude hemolysate.

Therefore, the two minor enzyme peaks from CM-Sephadex column chromatography are presumably artifacts due to the association of the enzyme with more acidic protein impurities which eluted at lower phosphate buffer concentrations.

**REFERENCES**

Human Phosphoglycerate Kinase: I. CRYSTALLIZATION AND CHARACTERIZATION OF NORMAL ENZYME
Akira Yoshida and Sumiko Watanabe


Access the most updated version of this article at http://www.jbc.org/content/247/2/440

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/247/2/440.full.html#ref-list-1