Phosphoglucomutase

I. PURIFICATION AND PROPERTIES OF PHOSPHOGLUCOMUTASE FROM ESCHERICHIA COLI

JAYANT G. JOSHI AND PHILIP HANDLER

From the Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina

(Received for publication, February 7, 1964)

Recognition of the general similarity of metabolic pathways at all phylogenetic levels is one of the major achievements of biochemistry. However, whereas it is clear that some reactions are common to almost all living forms, there have been, as yet, few systematic comparisons of the enzyme responsible for a single reaction as it occurs in a wide diversity of organisms. We have selected phosphoglucomutase for such a study, since presumably it must occur in all cells which can synthesize uridine diphosphate glucose from fructose. This enzyme, first discovered in an extract of rabbit muscle by Cori and Cori in 1936 (1), has since been identified in yeast (2), potato (3), and Neurospora crassa (4), among other sources. Of these, only the phosphoglucomutase from Neisseria perflava has been reported to be specific for the β form of glucose 1-phosphate (5). Both rabbit muscle and yeast phosphoglucomutase require participation of glucose 1,6-diphosphate (6, 7). The properties of the rabbit muscle enzyme have been summarized by Najjar (8).

The relatively low molecular weight, the ability to localize serine residues at the "active site" (9), and the presumed ubiquity of this enzyme were the chief factors which dictated its selection for this study. For comparative purposes, from bacterial sources, phosphoglucomutase was sought from Micrococcus lysodeikticus, Escherichia coli, and Bacillus cereus. The guanosine-cytidine contents of the deoxyribonucleic acid of these three species are 72, 52, and 34%, respectively (10). Crystalline phosphoglucomutase has been obtained from all three microorganisms. This report will describe the preparation and some properties of the enzyme from E. coli and compare these with the properties of rabbit muscle phosphoglucomutase.

EXPERIMENTAL PROCEDURE

Materials and Methods

Glucose-1-P and glucose-6-P were obtained from Sigma Chemical Company. Glucosamine 1-phosphate, galactose 1-phosphate, and N-acetylglucosamine 1-phosphate were gifts from Dr. E. A. Davidson and R. Cherniak of this department. N-Acetylcycteine was a gift from Dr. Leonard Scheffner, Mead Johnson and Company. Glucose-1,6-di-P was either prepared from human erythrocytes by the procedure of Bartlett (11) or procured from Dr. T. MacManus. After it was ascertained that fructose 1,6-diphosphate in concentrations up to 10^{-4} M does not inhibit phosphoglucomutase, an equal mixture of glucose 1,6-diphosphate and fructose 1,6-diphosphate obtained from a Dowex 1-chloride column was generally employed in enzyme assays; occasionally, however, pure glucose-1,6-di-P was employed. The purity of glucose-1,6-di-P and its concentration in the hexose diphosphate mixture were determined by assaying for total and acid-stable phosphate by the method of Fiske and SubbaRow (12), and by measuring the concentration of glucose-6-P in a neutralized acid hydrolysate with glucose-6-P dehydrogenase (13). The latter enzyme was either prepared from yeast as described by Kornberg and Horecker (13) or purchased from California Corporation for Biochemical Research. Calcium phosphate gel was prepared and aged by a standard procedure (14). E. coli 26, originally obtained from the American Type Culture Collection, was a gift from Dr. R. W. Wheat of this department. It was routinely maintained on nutrient agar slants.

Glucose 1-phosphate-32P was prepared as indicated by McCreary and Hassid (15). Radioactivity on paper strips either was detected with a Nuclear-Chicago strip scanner or the paper was cut into 1-cm strips and assayed with a Packard liquid scintillation counter.

Descending paper chromatography was conducted in one of the following solvents: Solvent 1, butanol-acetic acid water (4 : 1 : 5); Solvent 2, butanol-acetic acid-pyridine-water (30 : 6 : 20 : 24); Solvent 3, phenol-water (80:20). Electrophoresis at 50 ma was performed with the apparatus manufactured by Enseo, Inc., Salt Lake City, and conducted either at pH 6.5 (4 ml of acetic acid + 100 ml of pyridine in a total volume of 1 liter) or at pH 3.1 (278.5 ml of acetic acid + 16.1 ml of pyridine in a total volume of 1 liter). Both chromatography and electrophoresis were conducted on Whatman No. 3MM or No. 1 filter paper depending on the quantity of protein used.

Conditions for Growth of E. coli—Preliminary experiments were conducted to determine optimal conditions for obtaining a maximal yield of enzyme. Comparable cultures were grown at 37°, with aeration, in 250 ml of media containing 0.0296% MgSO_4; 7H_2O, 0.03% KH_2PO_4, 1.32% K_2HPO_4, 0.265% (NH_4)_2SO_4, and various carbon sources. Turbidity measurements were performed after 2, 7, and 9 hours, after which cells were harvested by centrifugation, washed once with 20 ml of ice-cold 0.9% NaCl, and suspended in 10 ml of 0.05 M phosphate, pH 6.8; P-glucosamine mutase activity and protein were determined by means of standard procedures. As shown in Table I, succinate did not support growth whereas, at the levels indicated, glucose, galac-
Toose, and glycerol yielded similar quantities both of cells and of soluble protein in the cell-free sonic extracts. However, the specific activities of the sonic extracts of cells grown in 1.0% glucose or 0.5% glycerol were twice as great as when galactose or lesser concentrations of glucose or glycerol were employed.

For routine purposes, therefore, cells were grown for 8 to 10 hours at 37° with continuous aeration either in batches of 10 to 12 liters in carboys, or in 45- to 50-liter batches in a biogen (American Sterilizer Company, Erie, Pennsylvania). The growth medium contained 0.0296% MgSO₄ · 7H₂O, 0.33% KH₂PO₄, 1.32% K₂HPO₄, 0.265% (NH₄)₂SO₄, and 1% glucose or 0.5% glycerol. Cells were harvested by centrifugation in the cold, washed once with 4 volumes of ice-cold 0.85% NaCl and stored at -12°. Preliminary trials showed that the enzyme is quite stable under these conditions for several weeks and for at least a year in lyophilized or acetone-powdered cells.

Table I

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Concentration</th>
<th>Specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>0.5</td>
<td>0.482</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5</td>
<td>0.680</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
<td>0.810</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.5</td>
<td>0.304</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.0</td>
<td>0.504</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.2</td>
<td>0.600</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.5</td>
<td>1.18</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.0</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* Measured in micromoles of glucose-6-P per mg of protein per 5 minutes.

Most all studies with purified enzyme preparations were carried out by direct measurement of phosphate.

Purification Procedure

Unless otherwise stated, all operations were performed at 4°.
Step 1: Typical Procedure—Frozen cells (300 g) were suspended in 7 volumes of 0.05 M phosphate, pH 6.8; 150-ml aliquots were subjected to ultrasound for 10 minutes with a Raytheon 10-kec sonic oscillator. The temperature of the sonic extract increased up to 10° during this operation. The sonic extract was centrifuged for 15 minutes at 16,000 r.p.m.; the turbid, somewhat viscous supernatant was used for the next step, and the residue was discarded. Preliminary experiments showed that sonic disruption for significantly more or less than 10 minutes in phosphate buffer resulted in somewhat lower yields, as did replacement of phosphate by an acetate or Tris-chloride buffer.

Step 2: Protamine Sulfate Precipitation—To 2150 ml of the supernatant described above were added, with continuous stirring, 322 ml (approximately 15% by volume) of 3% protamine sulfate, adjusted to pH 6.8 with 5 N NaOH. After 60 minutes, the suspension was centrifuged for 20 minutes at 8000 r.p.m., and the residue, containing almost half of the total protein and no activity, was discarded. All further operations were performed in the presence of 1 × 10⁻⁴ M cysteine.

Step 3: Ammonium Sulfate Precipitation—The 2370 ml of clear, pale yellow supernatant were brought to 0.7 saturation by slow addition of 1120 g of solid ammonium sulfate. The solution was left at -10° overnight.

Step 4: Heat Step—The precipitate from the above step was collected by centrifugation at 8000 r.p.m. for 20 minutes and dissolved in 500 ml of 0.01 M phosphate, pH 6.8, containing 0.01 M acetate. The final solution, pH 6.3, was rapidly brought to 55° with continuous stirring by placing the container in a boiling water bath, and immediately thereafter it was brought to 4° in an ice-water bath. After 60 minutes the inactive precipitate was removed by centrifugation and discarded. The pH of the supernatant was carefully adjusted to 4.9 by gradual addition of 1.7 N acetic acid. This solution was incubated at 30° for 45 minutes and the resultant precipitate was removed by centrifugation for 10 minutes at 17,000 r.p.m.

Step 5: Second Ammonium Sulfate Precipitation—Preliminary experiments showed that, at this stage, simple precipitation by ammonium sulfate at from 0.35 to 0.7 saturation effected no increase in specific activity. However, relatively high concentrations of acetate prevented coprecipitation with some of the contaminating proteins. Accordingly, the pH was readjusted to 6.3 with about 0.25 volume of saturated sodium acetate. The solution was cooled to 4° and, after 205 g of solid ammonium sulfate (0.45 saturation) had been added gradually, was left overnight at -10°.

Step 6: Calcium Phosphate Gel Treatment—The precipitate obtained by the previous procedure was collected by centrifugation, and the supernatant was discarded. The precipitate was dissolved in water, and enough 0.5 M acetate, pH 6.0, was added so that the final solution was 0.01 M with respect to acetate and contained 4.0 mg of protein per ml. Calcium phosphate gel (11 mg per ml) was gradually added (1 mg of gel for each 2 mg of protein), and the solution was stirred for 20 minutes. After centrifugation, the gel was discarded, and the supernatant was...
used for the subsequent procedure. Although best results have been obtained under these conditions, this step is somewhat empirical. Occasionally, less gel sufficed, and an excess of gel resulted in the loss of activity due to adsorption. Accordingly, the gel is added in batches routinely, and the activity in the supernatant is monitored. Almost half the total protein can be adsorbed without any loss of activity.

Step 7: Third Ammonium Sulfate Precipitation—Solid ammonium sulfate (235 g) was added to the supernatant (0.45 saturation), and after 2 hours the precipitate was centrifuged and discarded. The supernatant was brought to 0.65 ammonium sulfate saturation (114 g), and after 2 hours the active precipitate was centrifuged and the supernatant discarded. The precipitate was dissolved in 0.001 M phosphate, pH 6.8. Ultracentrifugal analysis of such a preparation showed that this fraction contained four components. The fastest moving component sedimented completely at 25,000 to 30,000 r.p.m. Hence, the solution was centrifuged in the No. 30 rotor of a Spinco preparative ultracentrifuge for 45 minutes at 25,000 r.p.m., and the residue was discarded. The clear supernatant was used for the following step.

Step 8: Second Calcium Phosphate Gel Procedure. The supernatant was diluted with 0.001 M phosphate, pH 6.8, to yield a protein concentration of 1 mg per ml. Calcium phosphate gel (11 mg per ml) was then added (4 mg of gel per mg of protein); the suspension was stirred gently for 20 minutes, and then centrifuged. In contrast to the earlier calcium phosphate gel treatment, all of the enzyme and about 75% of the total protein were adsorbed on the gel. The gel was then eluted successively with 125-ml portions of ice-cold phosphate, once at 0.001 M and three times each at 0.005 M, 0.01 M, and 0.025 M. The 0.001 M and 0.005 M eluates exhibited no activity, the 0.01 M phosphate eluate had a specific activity of 10.0 to 12.0, and the 0.025 M eluate exhibited a specific activity of 50 to 60. The latter fraction was brought to 0.45 saturation with solid ammonium sulfate and the small amount of precipitate formed after 2 hours was removed by centrifugation. The supernatant was then brought to 0.65 ammonium sulfate saturation; the resultant precipitate was collected by centrifugation and dissolved in sufficient 0.01 M phosphate to provide a protein concentration of 3 to 5 mg per ml. This solution was then dialyzed against 1 liter of the same buffer for 5 hours. A small, brownish precipitate which appeared during dialysis was removed by centrifugation at 16,000 r.p.m., yielding a clear, colorless supernatant with a specific activity of 90 to 100.

Step 9: Recrystallization—The latter solution was brought to 0.45 saturation by very slow addition of saturated ammonium sulfate. After 2 hours, the precipitate was removed by centrifugation, and the supernatant was brought to 0.55 ammonium sulfate saturation. After 10 to 15 minutes, a slight turbidity appeared, and after 30 minutes, schlieren and a characteristic "silky shimmer" were observed. Further addition of ammonium sulfate to 0.65 saturation completely precipitated the enzyme as microcrystals. Recrystallization was achieved by repeating the last step. The entire procedure, including two recrystallizations, requires about 6 days. The progress of the purification procedure is summarized in Table II.

The final yield of the enzyme appears to be dependent upon the nature of the starting material. Thus, when freshly harvested cells were used for isolation of enzyme, considerable apparent stimulation of activity was observed; the total procedure yielded as much as 200% of the initially apparent activity. However, when cells which had been frozen for 4 to 6 weeks were employed, the purification pattern followed the conventional trend; the final step yielding 25 to 50% of the starting activity. The apparent stimulation of total activity observed with fresh cells was due to the removal of phosphatases capable of hydrolyzing the substrate, cofactor glucose-1,6-P, or the reaction product during the assay procedure. This was shown in the following manner. Fifteen grams of 2-week-old frozen cells were suspended in 0.05 M acetate, pH 8.0; purification was performed up to the protamine sulfate step, and the supernatant so obtained was dialyzed for 4 hours against 0.001 M cysteine. At each step samples were removed for P-glucumutase assay. After the standard incubation period, the reaction was halted by immersing the tubes in boiling water bath for 1 minute. After cooling, the suspension was centrifuged and 0.2 ml of the supernatant was assayed for free inorganic phosphate; another aliquot (0.05 ml) was used to estimate glucose-6-P by the dehydrogenase assay. As compared to the protamine sulfate-treated supernatant, the sonic extract formed 50% more inorganic phosphate, thus seemingly indicating only 50% of the total P-glucumutase units. Although the exact nature or specificity of the responsible phosphatases was not determined, it is clear that the increase in total units during purification from fresh cells was due to removal of phosphatases, rather than to "activation" of the phosphoglucomutase itself.

**Results**

**Properties of E. coli Phosphoglucomutase**—The recrystallized enzyme exhibited a specific activity of 100 at pH 7.5 and 200 at 9.0 (see below). Individual, specific assays indicated that it was devoid of phosphohexose isomerase, glucose-1-P kinase, glucose-6-phosphatase, phosphoglycerate mutase, glucose-1-P transphosphorylase, alkaline phosphatase, glucose-6-P dehydrogenase, glucose 1,6-diphosphatase, and fructose 1,6-diphosphatase activities.
Fig. 1. Ultracentrifugal pattern of E. coli phosphoglucomutase. Protein concentration was 6.3 mg per ml of 0.05 M acetate, pH 6. Exposures were taken 8, 12, 16, and 20 minutes after the rotor reached 58,780 r.p.m. at 20°C.

The crystalline preparation described above nevertheless contained 10 to 15% of a heavier component, as judged by ultracentrifugal analysis with a Spinco model E analytical ultracentrifuge and confirmed by separation and removal in a sucrose gradient by the swinging bucket technique (19). Enzyme activity was associated exclusively with the major component, which exhibited an \( s_{20,w} \) of 4.2 to 4.3 (Fig. 1), suggesting a minimum molecular weight of 62,000 to 65,000. In subsequent studies, when enzyme of maximal purity was desired, the heavy component was removed by the sucrose gradient procedure.

As compared to the conversion of glucose-1-P to glucose-6-P, \( E. \) coli P-glucosaminate catalyzed phosphate transfer between the carbon atoms 1 and 6 of N-acetylglucosamine, galactose, and glucosamine, 0.2, 0.9, and 2% as rapidly, respectively.

Phosphoglucomutase obtained from muscle (20) and yeast (7) by described procedures contains significant amounts of phosphate, presumably as serine phosphate at the active site. However, the \( E. \) coli enzyme obtained as described above is in the dephosphorylated form since (a) it is completely unable to catalyze the conversion of glucose-1-P to glucose-6-P in the absence of added glucose-1,6-di-P and (b) no phosphate was detected when 15.0 mg of purest enzyme were hydrolyzed completely in 5 N \( \text{H}_{2}\text{SO}_4 \) and phosphate was assayed by the Fiske-SubbaRow procedure.

**Stability**—Enzyme stored at 4°C in 0.05 M acetate, pH 6, 0.65 saturated with ammonium sulfate, retained full activity for 3 to 4 weeks; over 60% of the activity was lost in the following 6 weeks.

In separate trials, the influence of Mg++ plus glucose-1,6-di-P on stability during storage was tested, since the phosphorylated form of yeast P-glucosaminate has been reported to be more stable than the dephosphorylated form (7). However, neither Mg++ nor glucose-1,6-di-P improved the stability of the preparation.

**Requirements for Enzyme Activity**—Phosphoglucomutase from muscle exhibits optimal activity in the presence of Mg++, either cysteine, histidine, or imidazole, and catalytic quantities of glucose-1,6-di-P (8). \( E. \) coli P-glucosaminate also showed similar requirements. As shown in Table III, usually about 7% of maximal activity could be detected when Mg++ or cysteine was omitted from the incubation medium although as much as 12% of maximal activity was occasionally observed. Under optimal conditions, formation of glucose-6-P was proportional to enzyme concentration and was linear with time until about 70% of the glucose-1-P was converted to glucose-6-P.

**Table III**

<table>
<thead>
<tr>
<th>Conditions for phosphoglucomutase activity</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>100</td>
</tr>
<tr>
<td>Mg++</td>
<td>6.7</td>
</tr>
<tr>
<td>Cysteine</td>
<td>6.7</td>
</tr>
<tr>
<td>Glucose-1,6-di-P</td>
<td>-*</td>
</tr>
<tr>
<td>Mg++ and cysteine</td>
<td>-*</td>
</tr>
</tbody>
</table>

* Not detected.

**Fig. 2.** Effect of pH on maximal activity of \( E. \) coli phosphoglucomutase under standard conditions (see the text). The buffers used were Tris-acetate ( ), 50 \( \mu \) moles; histidine ( ), 20 \( \mu \) moles, and glycine-NaOH ( ), 50 \( \mu \) moles per 0.5 ml.
September 1964 J. G. Joshi and P. Handler 2745

Mg++
tions, activity was in the order: cysteine > thioglycolate >
N-acetylcysteine > mercaptoethanol > glutathione (reduced).
Occasionally glutathione appeared to be slightly inhibitory
(Table IV). Tolerance to high cysteine concentrations but
inhibition by glutathione at comparable concentrations has
occasionally been reported for other enzymes, e.g. in the case of
nicotinamidase from yeast (21). Neither sodium sulfite nor
borohydride could substitute for a sulfhydryl compound. In-
stead, treatment with borohydride resulted in total loss of ac-
tivity even in the presence of cysteine.

Cysteine also markedly increases the resistance of this enzyme
to heat (Fig. 5). Aliquots of enzyme were heated with and
without cysteine at the concentration used in the standard assay
in the normal buffer at pH 8.0. At varying time intervals,

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
<th>Per cent of control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>20 mM</td>
<td>100</td>
</tr>
<tr>
<td>Glutathione</td>
<td>10 mM</td>
<td>7.5</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>20 mM (100)</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>47.2</td>
</tr>
<tr>
<td>Cysteine</td>
<td>20 mM</td>
<td>100</td>
</tr>
<tr>
<td>NaGSO</td>
<td>20 mM</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td>40 mM</td>
<td>63.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>20 mM</td>
<td>100</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>20 mM</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td>40 mM</td>
<td>63.5</td>
</tr>
<tr>
<td>Thioglycolate</td>
<td>20 mM</td>
<td>64.5</td>
</tr>
<tr>
<td></td>
<td>40 mM</td>
<td>75.5</td>
</tr>
<tr>
<td>Cysteine + NaBH4</td>
<td>20 mM</td>
<td>100</td>
</tr>
<tr>
<td>NaBH4</td>
<td>5 mg</td>
<td>0</td>
</tr>
<tr>
<td>Albumin</td>
<td>200 µg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>400 µg</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Table IV**

**Effect of sulfhydryl compounds and reducing agents on phosphoglucomutase activity**

**Fig. 3.** Effect of magnesium concentration on the activity of
*E. coli* phosphoglucomutase. The enzyme was tested at pH 9.0
(●) and 7.4 (■) in Tris-acetate buffer, 50 μmoles per 0.5 ml.

**Fig. 4.** Influence of cysteine on the activity of *E. coli* phospho-
glucomutase.

Enzyme activity at pH 9.0, in the absence of 
Mg++, (Fig. 3),
was greater than that at pH 7.5 under comparable conditions.

**Metals—*E. coli* phosphoglucomutase exhibits requirement for
Mg++, and is optimally active at 1 × 10⁻³ m at both pH 7.5
and 9.0 (Fig. 3). The effects of several other metals were tested
in the presence of Mg++. Enzyme which had previously been
dialyzed against 100 volumes of 0.025 m acetate, pH 6.0, con-
taining 1 × 10⁻⁴ m EDTA, was used for this purpose. At 1 ×
10⁻³ m, Ni++, Hg++, and Zn++ were without effect, whereas
Ca++ inhibited activity 4.5%, Co++ 18.5%, Mn++ 59.7%, Cu+ 83.3%,
Fe++ 88.4%, and Al+++ more than 95%. In the absence of Mg++,
10⁻³ m Mn++ and Ni++ were 10% and 20% as effective
as Mg++ in stimulating activity; higher concentrations were
without additional effect.

**Activation by Sulfhydryl Compounds—*E. coli* P-glucomutase
was maximally active only in the presence of 2 × 10⁻² m cysteine;
higher concentrations were without additional effect (Fig. 4).
Of other sulphydryl compounds tested, at constant concentra-

**Fig. 5.** Effect of cysteine on the stability of *E. coli* phospho-
glucomutase at 45°. The enzyme was preincubated at pH 8.0
in glycine-NaOH buffer with the standard assay medium without
glucose 1-phosphate, either with (●) or without (■) cysteine.
At the times indicated, aliquots were withdrawn and assayed
under standard conditions.
samples were withdrawn and assayed by the standard procedure. Virtually no loss of activity was detected in 20 minutes at 45° in the presence of cysteine, whereas 70% of the activity was lost in its absence.

**Inhibition by Mercuribenzoate**—The specific requirement for a sulfhydryl compound in the reaction medium suggested that the enzyme may possess a sulfhydryl group essential to its catalytic activity. Accordingly, 10⁻⁴ M p-chloromercuribenzoate was added to a reaction mixture containing 10 times the normal amount of enzyme in the absence of added cysteine. After various time intervals, aliquots were removed and assayed for activity, under standard conditions but in the absence of cysteine. As shown in Table V, more than 99% of the enzyme activity was lost during the first minute and inhibition was complete after 20 minutes. Added cysteine completely protected the enzyme against mercuribenzoate inhibition. However, enzyme which had been inactivated by exposure to mercuribenzoate for 20 minutes regained only 50% of the original activity when incubated subsequently with excess cysteine for 20 minutes.

Titration of the enzyme with mercuribenzoate by the procedure of Boyer (22) revealed the presence of two to three sulfhydryl groups per mole, the first of which reacted with the reagent in less than 1 minute, in accord with the inhibition pattern noted above. When enzyme was oxidized with performic acid and the cysteic acid content estimated by the procedure of Moore (23), the data revealed the presence of 2 moles of cysteine per mole of protein. The reason for the slightly excessive value obtained by mercuribenzoate titration is not clear.

In contrast, rabbit muscle phosphoglucomutase offers two sulfhydryl groups immediately reactive with mercuribenzoate, whereas 70% of the activity was lost during the first minute and inhibition was complete after 20 minutes. Added cysteine completely protected the enzyme against mercuribenzoate inhibition. However, enzyme which had been inactivated by exposure to mercuribenzoate for 20 minutes regained only 50% of the original activity when incubated subsequently with excess cysteine for 20 minutes.

**Kinetics**—E. coli P-glucomutase was assayed at varying substrate concentrations, and at two concentrations of glucose-1,6-di-P, by the modification of the method of Fiske and SubbaRow suggested by Bartlett to measure acid labile phosphate. From a Lineweaver-Burk plot (30) of the data so obtained, Kᵢₘ for glucose-1-P was found to be approximately 5.35 x 10⁻⁴ and 6.0 x 10⁻⁵ M at glucose-1,6-di-P concentrations of 1.3 x 10⁻⁴ and 8.35 x 10⁻⁹ M, respectively. Concentrations of substrate above 8 x 10⁻⁹ M were inhibitory; no activity was detectable at 4 x 10⁻⁹ M. Variation in the concentration of glucose-1,6-di-P did not alleviate this inhibition. The inhibition of E. coli P-glucomutase by urea proved to be formally competitive in contrast to that of several other Mg⁺⁺-dependent enzymes (31). At a glucose-1,6-di-P concentration of 1.3 x 10⁻⁴ M, Kᵢₘ for urea was 0.8 M.

**Lack of Stimulation of E. coli Phosphoglucomutase by Histidine or Imidazole**—It has been observed repeatedly (26, 32) that muscle P-glucomutase activity is stimulated about 5-fold by preincubation with either histidine or imidazole and Mg⁺⁺ at appropriate concentrations. Further, this phenomenon was observed only when the phosphorylated form of the enzyme was employed. In contrast, preincubation with 0.04 M histidine and 0.001 M Mg⁺⁺ did not enhance the activity of the E. coli enzyme. Since E. coli P-glucomutase as isolated is in the nonphosphorylated form, in separate experiments it was preincubated with glucose-1,6-di-P for 10 minutes at 4°, then with imidazole (0.04 M) and Mg⁺⁺ (0.001 M) for another 10 or 20 minutes at 30°, and activity was measured under standard conditions. Again, no stimulation of activity was observed. Essentially identical results were obtained whether the incubations were conducted at pH 7.4 or 9.0. In a preliminary attempt to ascertain the mechanism of the imidazole stimulation of muscle P-glucomutase, this enzyme was preincubated with imidazole and Mg⁺⁺ in standard fashion for 20 minutes and then titrated with mercuribenzoate. No increase in reactivity with this reagent was detected.

**Failure of Inhibition by Diisopropyl Phosphofluoridate**—A series of hydrolytic enzymes with serine phosphate at the active center have been shown to be inhibited by DFP (33); the status of muscle phosphoglucomutase in this regard is somewhat uncertain (33-35). Preincubation of E. coli P-glucomutase with DFP at concentrations from 10⁻⁴ to 10⁻³ M for periods as long as 160 minutes at pH 7.5, 8, or 8.5 in glycine-NaOH or Tris-acetate buffer, with or without glucose-1,6-di-P at 0° or 30°, failed to occasion any detectable inhibition. The ambiguous
previous experience with muscle P-glucosumutase probably reflects the presence of a powerful enzyme inhibitor in some commercial samples of DFP (36).

**Effect of Anions on Enzyme Activity**—Phosphoglucomutase activity is known to be affected by the species and concentration of anions in the assay mixture. Cori, Colowick, and Cori (37) observed that catalysis of the formation of glucose-6-P from glucose-1-P by extracts of rabbit muscle is inhibited by various salts. A more detailed study of the effects of salts on catalysis of the phosphoribomutase reaction by rabbit muscle P-glucosumutase has been reported by Klenow (38). In the present study, the effects of various salts on the action of *E. coli* P-glucosumutase at 30° and by nitrate at 25.1° and 31° were examined. The data presented in Fig. 6 show that this enzyme is quite sensitive to anion concentration. When the ions here considered are arranged according to their inhibitory effect, the order bears a striking resemblance to the Hofmeister "lyotropic series." Similar results have recently been reported for the acetoacetic decarboxylase (39) from *Clostridium acetobutylicum*. The latter enzyme, however, is resistant to divalent anions such as sulfate, carbonate, and phosphate, whereas *E. coli* P-glucosumutase was inhibited by sulfate, as well as by borate and citrate. About 50% of the inhibition by citrate could be overcome by increasing the Mg++ concentration to 2.4 × 10^{-3} M in the assay mixture; higher concentrations of Mg++ were inhibitory. Thus, the citrate anion is inhibitory per se and also as a chelating agent. Inhibition by borate has also been observed with the P-glucosumutase of rabbit muscle (40) and of pea seedlings (41).

The influence of anions on muscle P-glucosumutase proved to be markedly dependent upon the presence of imidazole or histidine. In the absence of the latter, anions do not significantly alter the catalytic activity of the enzyme. When histidine was present in the assay medium, anions appeared to enhance activity whereas, as shown in Fig. 7, after the enzyme had been preincubated with histidine, an inhibition pattern resembling that found with the *E. coli* enzyme was apparent. It is noteworthy that both the stimulatory and inhibitory patterns follow the order of the Hofmeister series of anions. This phenomenon is now being studied in detail.

**Amino Acid Composition**—Total amino acid composition was determined on two independent acid hydrolysates of *E. coli* P-glucosumutase which had been freed of any contaminant protein by the sucrose gradient-swinging bucket technique described above. The procedure was essentially that of Moore and Stein (42) and employed a Spinco automatic amino acid analyzer. The value for cysteine was obtained after performic acid oxidation. Table VI summarizes these data, which are expressed as residues per mole of protein, molecular weight 62,000. For comparison, the table also presents the amino acid composition of rabbit muscle P-glucosumutase recalculated from the original data (43), which were based on a molecular weight of 67,000, although this value has since been shown to be quite close to 62,000 (44). The low values for serine, aspartic acid, and cystine (cysteine) in the *E. coli* enzyme are particularly noteworthy.

**Fingerprint Analysis**—Before undertaking an amino acid sequence analysis, comparison of muscle and *E. coli* P-glucosumutase by the fingerprint procedure appeared advisable. The two enzymes were assayed simultaneously and by identical procedures. About 5.0 mg of each enzyme in 1.0 ml of water were dialyzed twice against 25 ml of 8 M urea for 12 hours. The urea was removed by dialysis for 2 days against 6 liters of water, with the water changed every 12 hours. This procedure precipitated all the protein within the dialysis tubing. The denatured enzymes were suspended in water and enough 5% ammonium carbonate was added to bring the concentration to

---

**Fig. 6.** Effect of nitrate on the activity of *E. coli* phosphoglucomutase at 31° and 25.1°.

**Fig. 7.** Effect of some anions on the activity of rabbit muscle phosphoglucomutase. Reaction mixtures 1, 2, and 3 contained chloride; 4, 5, and 6 contained bromide; and 7, 8, and 9 contained iodide. In Systems 1, 4, and 7, the enzyme was not preincubated with histidine and Mg++, but was assayed in their presence. In Systems 2, 5, and 8, the enzyme was assayed in Tris-acetate buffer containing cysteine. Curves 3, 6, and 9 represent the activity of enzyme which had been preincubated with 0.04 M histidine and 0.001 M Mg++ for 40 minutes at 4° and then assayed in their presence.
from the E. coli enzyme can be matched with those from the
As will be seen, approximately one-half the peptides derived
tral peptides was difficult because of trailing and intermixing.
results are shown in Fig. 8, which indicates the ninhydrin-
used for paper chromatography and electrophoresis. Typical
lyophilized, and the residues, dissolved in 0.5 ml of water, were
muscle. Unshaded areas indicate ninhydrin-positive peptides;
stippled areas indicate histidine-containing peptides.

<table>
<thead>
<tr>
<th>Residue</th>
<th>E. coli</th>
<th>Muscle*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>37</td>
<td>59</td>
</tr>
<tr>
<td>Threonine</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>Serine</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>69</td>
<td>43</td>
</tr>
<tr>
<td>Proline</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Glycine</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>Alanine</td>
<td>65</td>
<td>49</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Valine</td>
<td>42</td>
<td>39</td>
</tr>
<tr>
<td>Methionine</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Leucine</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Lysine</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>Histidine</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Arginine</td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>

* Original values (43) recalculated for a molecular weight of 62,000 (44).
† Estimated as cysie acid (23).

0.05% and the pH to 8.0. A freshly prepared solution of
crystalline trypsin (5 µg per mg of enzyme) and 1 drop of toluene
were added, and the tubes were incubated at 30°. After 12
hours an equal amount of trypsin was added and incubation was
continued for another 24 hours. The solutions were then
lyophilized, and the residues, dissolved in 0.5 ml of water, were
used for paper chromatography and electrophoresis. Typical
results are shown in Fig. 8, which indicates the ninhydrin-
reactive and histidine-containing peptides.

Under these conditions, about 45 to 50 independent peptides
were apparent in the E. coli enzyme. Clean separation of neu-
tral peptides was difficult because of trailing and intermixing.
As will be seen, approximately one-half the peptides derived
from the E. coli enzyme can be matched with those from the
muscle enzyme. This difference is even more striking when the
histidine-containing peptides are compared.

The sum of lysine + arginine in the E. coli enzyme is 61 per
mole, whereas only two-thirds this number of tryptic peptides
were observed. Nevertheless, this circumstance suggests that,
if the enzyme consists of more than one polypeptide chain, these
are nonidentical. An attempt to establish this by estimation
of the NH₄-terminal groups was unsuccessful. With the procedure
of Fraenkel-Conrat, Harris, and Levy (45), 5 mg of each
P-glucoumutase were treated with 20 µl of liquid dinitrofluoro-
benzene for 8 hours at room temperature. However, after
hydrolysis no α-N-dinitrophenylamino acid was detected in the
ether layer. Attempts are being made to deaggregate these
enzymes by various reagents and conditions as revealed by
ultracentrifugal analysis.

Phosphorylation of Phosphoglucomutase—Studies of muscle
P-glucoumutase have shown that the enzyme reacts with sub-
strate, presumably glucose-1,6-di-P, with phosphorylation of
seryl residues on the enzyme (8, 9). Two different seryl residues
were found to so participate, and it has been found that both lie
within a single strand of the enzyme, separated by only 4 resi-
dues (46). However, in no case does the total amount of phos-
phate accepted by the enzyme exceed 1 phosphate per mole (8),
suggesting that only 1 of the 2 seryl residues may be phosphoryl-
ated on any one enzyme molecule. As a preliminary to a similar
study of E. coli P-glucoumutase, these observations were first
confirmed with the muscle enzyme and identical conditions then
employed with the bacterial enzyme.

Phosphorylation was generally accomplished by the procedure
of Milstein (34) with the use of phosphorus phosphate for prepara-
tion of glucose-1-32P. About 20 g of potato were homogenized
with an equal volume of water in a Waring Blender at top speed
for 45 seconds, and the homogenate was filtered through cheese-
cloth. After centrifugation at 18,000 × g for 10 minutes at 4°,
the supernatant was used as the source of phosphorylase. Five
milliliters of a 2.5% solution of soluble starch, 1.5 ml of phos-
phorylase solution, and 0.1 ml of 1 M phosphate containing 2 to
5 mc of Hi-32PO₄ were incubated in a tube (1 cm × 20 cm) for 8
hours at room temperature. The contents were transferred to a
tube (8 cm × 20 cm), and into this solution was introduced a
minimal dialysis sac containing 5 mg of P-glucoumutase plus
phosphate-1,6-di-P, Mg++ and cysteine at the concentrations
used in the standard assay. The system was equilibrated over-
night, after which the dialysis sac was removed and the contents
dialized with constant stirring, twice against 2 liters of 0.05 M
acetate, pH 6.0, for 4 hours, and overnight against 6 liters of this
buffer. The entire cycle of dialyses was then repeated against
distilled water.

In various studies, enzyme so phosphorylated was hydrolyzed
by various procedures. Tryptic digestion was conducted after
denaturation with urea as in the fingerprint studies. Acid
digestion of protein in amounts up to 5 mg was conducted in
2.0 ml of 6 N HCl at 100° for 45 minutes. Larger quantities of
protein (up to 60 mg) were allowed to stand for 36 to 48 hours
at room temperature in concentrated HCl.

When smaller quantities of protein had been used, the hydro-
lysatc was lyophilized, dissolved in 0.5 M acetic acid, streaked
on Whatman No. 3MM paper, and subjected to electrophoresis at
pH 6.5 for 90 minutes. Radioactive spots were eluted with 0.5
M acetic acid, lyophilized, and independently chromatographed
on paper with Solvent 1. When desired, the entire procedure of
Electrophoresis and chromatography were repeated, and radioactive areas were eluted, lyophilized, and saved for subsequent analyses.

When larger quantities of protein had been used, the lyophilized hydrolysate was dissolved in water and fractionated on a Dowex 50-H+ (200 to 400 mesh) column (1 cm × 16 cm). The column was eluted in succession with 20 ml of water and 1 M ammonium formate; 2-ml fractions were collected and 5-μl aliquots were spotted on paper and assayed for radioactivity. In various runs, 40 to 70% of the total radioactivity appeared in the water eluate and the remainder in the ammonium formate eluate. The radioactive fractions were lyophilized and purified further by paper chromatography and electrophoresis as described, and used for amino acid and sequence analysis.

Muscle P-glucosamutase-2p so prepared was denatured with urea and subjected to tryptic hydrolysis for varying times; the digests were then used for paper chromatography on Whatman No. 3MM paper with Solvent 1. As the course of tryptic digestion of the fully labeled enzyme was followed, two labeled peptides were observed to increase. With the chromatographic system employed, after 18 hours the solvent front had run off the paper while the two labeled peptides migrated 2.5 and 5 inches, respectively. The latter, which was present invariably in about twice the amount of the former, was later shown to contain the amino acid sequence identified by Milstein and Sanger (34). No attempt was made to examine the more slowly migrating material.

In order to establish the stoichiometry of phosphorylation of E. coli P-glucosamutase, the conditions described above were employed except that the enzyme was dialyzed against 32 μmoles of glucose-1-2p, 1.3 to 3.5 × 10⁵ c.p.m. per μmole. After removal of all excess glucose-1-2p by dialysis as indicated, the enzyme solution was centrifuged and aliquots (5 or 10 μl) of the supernatant were counted. The data so obtained were not consistent, varying from 0.4 to 1.05 moles of 32P per mole of enzyme. It has been assumed from these observations that the fully phosphorylated enzyme bears 1 phosphate per mole.

When E. coli P-glucosamutase was phosphorylated with glucose-1-32P generated by potato phosphorylase as described above, and the course of tryptic digestion was followed as indicated for muscle P-glucosamutase, only one 32P-containing peptide was apparent; the Kₚ was that of the major of the two 32P-peptides from muscle P-glucosamutase.

**Peptide at Active Site of E. coli Phosphoglucomutase—**Electrophoresis of partial acid hydrolysates of E. coli P-glucosamutase-32p, at pH 6.5, yielded four radioactive spots. Three of these were identified as P₁, serine phosphate, and glucose monophosphate, respectively, by their chromatographic behavior in Solvents 1, 2, and 3, and by electrophoretic behavior at pH 6.5 upon comparison with authentic samples. In each case, the radioactive material matched perfectly the behavior of the authentic marker.

The unidentified fourth radioactive material was eluted from the paper with 0.5 M acetic acid, lyophilized, and hydrolyzed with 1 ml of 6 N HCl for 45 minutes at 100°. After removal of the HCl by lyophilization, the residue was dissolved in water and subjected to electrophoresis on paper at pH 6.5. Seven radioactive peaks were then located with a strip scanner. Portions of the paper containing maximal radioactivity from each peak were then eluted, and each portion was purified by a second cycle of electrophoresis and chromatography. The radioactive materials on the chromatograms were eluted, lyophilized, and hydrolyzed in sealed tubes for 24 hours at 110°. The amino acids in each tube were identified by their chromatographic behavior in Solvents 1, 2, and 3. Table VII summarizes the composition of each peptide and presents the serine phosphate peptides found in muscle P-glucosamutase by Harshman and Najjar (46) and by Milstein and Sanger (34). It is evident that the partial amino acid sequence here identified in the 32P-containing peptide is identical with that found in muscle P-glucosamutase by Milstein and Sanger, viz. threonine-alanine-serine phosphate-histidine-aspartic acid (NH₄⁺).

**Immunoechemical Comparison—**Before it was recognized that the amino acid composition and fingerprint pattern of rabbit muscle and E. coli P-glucosamutase differ so significantly, an immunoechemical comparison was undertaken as follows. E. coli and rabbit muscle P-glucosamutase were stored at 0° and suspended in 0.65 saturated ammonium sulfate at a concentration of 4 mg per ml. As desired, 0.5 ml was centrifuged at 18,000 × g for 10 minutes at 4°, the supernatant was discarded, and the precipitate was dissolved in 0.5 ml of 0.85% NaCl. After mixing with an equal volume of Freund's adjuvant in a syringe, the mixture was administered intramuscularly to a rabbit. One rabbit received E. coli P-glucosamutase, two muscle P-glucosamutase, and a fourth received 1.0 ml of adjuvant-0.85% NaCl mixture only. This procedure was followed twice weekly. Each animal was bled before injection and serum was prepared by conventional procedures. Qualitative precipitin reaction was observed by the agar diffusion technique, with the use of undiluted serum. Ouchterlony agar diffusion plates were prepared as described by O'Connor (47). Precipitation was observed after 72 to 96 hours at 4°.

Administration of rabbit muscle P-glucosamutase to rabbits failed to elicit formation of precipitating antibodies. E. coli P-glucosamutase, however, readily induced specific antibody formation, and in the Ouchterlony plates the amount of precipitate was proportional to the amount of E. coli P-glucosamutase in the well. There was absolutely no visible cross-reaction with rabbit muscle P-glucosamutase.

For quantitative precipitin reactions a γ-globulin fraction

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Threonine</th>
<th>Alanine</th>
<th>Serine</th>
<th>Histidine</th>
<th>Aspartic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
in 0.1 N NaOH; the alkaline-carbonate solution was employed in collection and storage of enzyme and its extreme sensitivity to mercuribenzoate, contrasted with the inhibition of the muscle enzyme by preincubation with cysteine and the relative insensitivity of the latter enzyme to sulfhydryl reagents; and (c) the striking increase in activity of the muscle enzyme effected by preincubation with imidazole and the insensitivity of the bacterial enzyme to this procedure. Indeed, in view of these striking differences, it is all the more remarkable that these enzymes differ in specific activity (turnover number or \( V_{max} \)) only by a factor of 4. Although these findings may reflect the conditions necessary for maintaining these two proteins in catalytically active conformation.

As shown in Fig. 9, all \( E. \text{coli} \) P-glucosomutase was precipitated as an enzyme-antibody complex; 1 \( \mu \)g of enzyme precipitated about 16 \( \mu \)g of antibody protein. Increasing the amount of antibody above this amount decreased the amount of precipitate.

**DISCUSSION**

Table VIII attempts to summarize a comparison of the phosphoglucomutases from rabbit muscle and \( E. \text{coli} \). From the evolutionary standpoint, the similarities are perhaps more significant than the differences here noted. The essentially identical molecular weights, amino acid sequence at the active site, and the requirements for glucose 1,6-diphosphate and Mg\(^{2+}\) indicate both a similarity in the catalytic mechanisms and homology of the gene or genes responsible for biosynthesis of these enzymes.

The present data do not indicate the number of polypeptide chains per enzyme molecule since P-glucosomutase from both sources may be added to the growing list of proteins which lack N\(_{\text{H}}\)-terminal groups detectable by dinitrophenylation. If these enzymes are multistranded, the number of peptides liberated by tryptic hydrolysis strongly suggests that the peptide chains are not identical. Although the similar molecular weights and identical active site peptides argue for the genetic homology of these enzymes, as well as of the enzyme from yeast (49), this concept is difficult to reconcile with the gross differences in behavior and extreme difference in cysteine (cysteine) content. The argument for homology would be strengthened by demonstration of transitional forms. In any case, the amino acid compositions and tryptic fingerprint patterns of these enzymes indicate that with the exception of the sequence at the active site, there are numerous differences in the amino acid sequences of their polypeptide chains. It is therefore not surprising that they fail to cross-react with specific antibodies.

The fact that the \( E. \text{coli} \) enzyme, as isolated, is in the dephosphorylated form may not necessarily indicate the steady state of this enzyme in vivo, since under certain conditions a phosphate-free P-glucosomutase can be isolated from muscle (50). Although the present data, as well as studies to be reported subsequently, indicate that the phosphorylated enzyme formed by reaction with substrate involves similar reactions with substrate in both instances, there are differences in the conditions essential to catalysis which are noteworthy. Most striking of these are (a) the optimal pH for activity, which is 1.6 units greater with the \( E. \text{coli} \) than for the muscle enzyme; (b) the absolute dependence on the presence of a sulfhydryl compound in the medium of the \( E. \text{coli} \) enzyme and its extreme sensitivity to mercuribenzoate, contrasted with the inhibition of the muscle enzyme by preincubation with cysteine and the relative insensitivity of the latter enzyme to sulfhydryl reagents; and (c) the striking increase in activity of the muscle enzyme effected by preincubation with imidazole and the insensitivity of the bacterial enzyme to this procedure. Indeed, in view of these striking differences, it is all the more remarkable that these enzymes differ in specific activity (turnover number or \( V_{max} \)) only by a factor of 4. Although these findings may reflect the conditions necessary for maintaining these two proteins in catalytically active conformation.
tions, it seems equally likely that they may reflect a fundamental difference in the detailed catalytic mechanism, e.g. participation of $\text{S}^\text{\textmd{}}$ as a proton acceptor in the E. coli enzyme at pH 9 and of imidazole in a similar capacity for the muscle enzyme at pH 7.4. Details of a study of the mechanisms of these two enzymes will be reported subsequently.

**SUMMARY**

A procedure is described for preparation of crystalline phosphoglucomutase from *Escherichia coli*, and a comparison with the rabbit muscle enzyme is reported. Both enzymes require glucose 1,6-diphosphate and Mg$^{++}$ for activity, have molecular weights of about 62,000, and have no detectable NH$_2$-terminal $\alpha$-amino groups. Marked differences were observed in their amino acid compositions and tryptic fingerprint patterns. Although the muscle enzyme has 2 seryl residues which are phosphorylated by substrate, the *E. coli* enzyme has only 1 active serine; the pentapeptide in which this is located is identical with that of 1 of the 2 active serine of the muscle enzyme. Although the muscle enzyme exhibits an optimal pH of 7.4 and marked stimulation on preincubation with imidazole, and is inhibited by preincubation with cysteine while relatively insensitive to mercuribenzoate, the *E. coli* enzyme shows optimal activity at pH 9.0, is unaffected by preincubation with imidazole or histidine, requires cysteine in the medium, and is extremely sensitive to mercuribenzoate. The significance of these findings with respect to catalytic mechanisms and evolution is discussed.

**Acknowledgments**—We gratefully acknowledge our indebtedness to the following: Mr. Roscoe Price for growing quantities of *E. coli* 26; Dr. W. R. Guild for cooperation in the ultracentrifugal analyses; Dr. W. L. Byrne for assisting in the swinging bucket analyses; Dr. W. Buckley for helping with the immunological study; Mrs. Sue Neece for assisting in the preparation of the muscle enzyme; and Dr. R. L. Hill, Mr. James Naylor, and Mrs. Constance Harris for conducting the amino acid analyses.

**REFERENCES**
