Glyceraldehyde Phosphate Dehydrogenase, Phosphoglycerate Kinase, and Phosphoglyceromutase of Escherichia coli

SIMULTANEOUS PURIFICATION AND PHYSICAL PROPERTIES*

Giuseppe D'Alessio† and John Josse

From the Institute of Molecular Biology, Syntex Research, Palo Alto, California 94304

SUMMARY

A relatively simple seven-step procedure is described whereby glyceraldehyde phosphate dehydrogenase, phosphoglyceromutase, and phosphoglycerate kinase can be purified from extracts of Escherichia coli. The purified preparations (50 to 100 mg) of the first two enzymes were homogeneous when examined by polyacrylamide gel electrophoresis at pH 9 and by sedimentation techniques. The purified fraction of phosphoglycerate kinase contained small amounts of contaminating proteins. The molecular weights and sedimentation values of the purified proteins were, respectively: glyceraldehyde phosphate dehydrogenase, 144,000 and 7.5 S; phosphoglycerate kinase, 43,700 and 3.5 S; and phosphoglyceromutase, 56,300 and 4.8 S.

The amounts of these enzymes in cell extracts varied with the carbon source which the cells utilized during growth, but the three activities were invariably present in constant proportion to one another. Calculation of the number of molecules of each enzyme present in such extracts indicated a nonequimolar distribution (ratio of glyceraldehyde phosphate dehydrogenase to phosphoglycerate kinase to phosphoglyceromutase, 1:2.4:3.6).

The Embden-Meyerhof pathway of glycolysis has been of interest for many years, and several of its enzymes have been extensively studied. In particular, glyceraldehyde phosphate dehydrogenase (d-glyceraldehyde 3-phosphate:NAD+ oxidoreductase, phosphorylating, EC 1.2.1.12) has been the object of numerous investigations, and the enzymes from several sources have been well characterized (1, 2). Phosphoglycerate kinase (ATP: 3-phospho-d-glycerate 1-phosphotransferase, EC 2.7.2.3) and phosphoglyceromutase (2,3-diphospho-d-glycerate: 2-phospho-d-glycerate phosphotransferase, EC 2.7.5.3), the enzymes which catalyze the succeeding two steps in the pathway, have not yet been so thoroughly examined although important studies of kinetics and reaction mechanism have been carried out (3-5). In the last decade the enzymology of bacterial cells has been of increasing interest because so many important biological processes can be investigated to advantage in these simpler systems. The species Escherichia coli has proved to be especially useful, and a larger body of basic biological information has been accumulated on this organism than for virtually any other in nature. A relatively simple procedure is reported here whereby these three enzymes of the Embden-Meyerhof pathway can be obtained in large amounts (50 to 100 mg) from a modest quantity of E. coli cells (450 g). The molecular weight and sedimentation values of the purified proteins are also reported. The separate purification of E. coli glyceraldehyde phosphate dehydrogenase has been described by Allison and Kaplan (6), and several properties of this protein have been compared with those of glyceraldehyde phosphate dehydrogenases from other sources (6, 7). A preliminary report of the present work has also appeared (8).

EXPERIMENTAL PROCEDURE

Materials—Glyceraldehyde-3-P, glycerate-2-P, glycerate-3-P, glucose-6-P, adenine nucleotides, bovine albumin, and yeast hexokinase were products of Sigma Chemical Company. Bovine heart lactate dehydrogenase and E. coli alkaline phosphatase were purchased from Worthington Biochemical Corporation. Pyridine nucleotides, glycerate-2,3-P*, and all other commercial preparations of enzymes were obtained from Boehringer Mannheim Corporation. Snake venom phosphodiesterase was isolated according to the method of Sinsheimer and Kornberg (9). 2-Mercaptoethanol (Eastman Organic Chemicals) was redistilled before use. Norit A (Fisher Scientific Company) was activated as described by Zimmerman and Kornberg (10). Visking dialysis casing was treated for 30 min at 60° in 0.6 M NaHCO₃, 0.01 M EDTA, exhaustively washed with distilled water, and stored in 70% ethanol.

Assays for Enzymatic Activity—Enzymatic assays were carried out in 1-ml incubation mixtures, following the absorbance of reduced pyridine nucleotide at 340 mp for 1 to 2 min at 23° in a Cary recording spectrophotometer. The assay for glyceraldehyde phosphate dehydrogenase was conducted according to the procedure of Allison and Kaplan (6), except that 2-mercaptoethanol (3 mm) was included in the mixture; the reaction was initiated by addition of glyceraldehyde 3-P after enzyme and other components had been previously incubated for 3 to 5 min. Phosphoglycerate kinase was assayed in the reverse direction

* This paper is contribution 73 from the Institute of Molecular Biology, Syntex Research.
† Present address, Laboratorio di Chimica Biologica, Facoltà di Scienze, Università di Napoli, Naples, Italy.
of glycolysis in an incubation mixture described by Adam (11),
substituting 20 mM Tris-chloride, pH 7.5, for triethanolamine
and including 1 mM ATP; the reaction was initiated by addition
of enzyme. Activity of phosphoglyceromutase was measured
according to the method of Czok and Eckert (12), substituting
20 mM Tris-chloride, pH 7.5, for triethanolamine and including
1 mM glyceral-3-P and 2.5 times the amount of enolase recom-
mended by those authors; the reaction was initiated by addition
of enzyme. All enzymatic activities are expressed in interna-
tional units (micromoles of substrate converted per min under
the given assay conditions), with ε = 6.22 × 105 M⁻¹ cm⁻¹ at
340 μΜ for NADH (13). When necessary, phosphoglycerate
kinase and phosphoglyceromutase were diluted for assay in 20
mM Tris-chloride, pH 8, containing 2 mM EDTA; glyceraldehyde
phosphate dehydrogenase was diluted in this buffer plus 2 mM
2-mercaptoethanol.

Sedimentation Studies—Protein solutions in Visking casing
were dialyzed at 20°C for 3 days against 1000 volumes of 0.1 mM
NaCl, 0.01 mM sodium phosphate, pH 7. The outside buffer
also contained 2 mM EDTA when samples of glyceraldehyde
phosphate dehydrogenase were dialyzed. All the sedimentation
experiments were conducted in a Spinco model E analytical ultra-
centrifuge equipped with electronic speed control. Sedimenta-
tion velocity analyses were performed with use of schlieren opti-
tes and rotor speeds of 48,000 to 57,000 rpm. Sedimentation coeffi-
cients were corrected to a standard state of water at 20°C
and plotted at [s20,w]−1 versus concentration to obtain an extrapolated
value of 0.034 Å. High speed sedimentation equilibrium dis-
mensions of molecular weights were carried out as described by
Yphantis (14). A symmetrical condensing lens mask with
0.5-mm slits was employed, and fringe photographs were taken
on spectroscopic II-G plates. With application of appropriate
baseline corrections, plates were examined both for vertical
fringe displacements as a function of radius and for radius as a
function of fringe count. Points of lowest concentration with
fringe displacements of <100 μμ were omitted in constructing
plots of log(fringe displacement) or log(fringe number) versus
(radius), and weight-average molecular weight values were
obtained from their linear least squares slopes (14). Agreement
between the two types of plots was invariably better than ±2%.
All plates were read on a Nikon microcomparator, according to
the technique of Trautman (15).

Other Methods—Protein concentration was measured ac-
 conforming to Lowry et al. (16) and by a microburet method (17).
Both techniques, when standardized with bovine albumin,
yielded similar estimates of the purified E. coli proteins described
in this paper. Polyacrylamide gel electrophoreses were carried
out as described by Davis (18). Radioactivity was measured
in a low background gas flow counter.

RESULTS

Enzymatic Activities in Cell Extracts

Earlier work by Vogell et al. (19) and by Pette, Luh, and
Büchner (20) has established that the enzymes of the Embden-
Meyerhof pathway from triose phosphate isomerase to enolase
are members of a constant proportion group. That is, in cell
extracts from different species, ranging from yeast cells to insects
to mammals, these activities are present in relatively constant proportion
to one another. For example, in each extract examined the ratio
of glyceraldehyde phosphate dehydrogenase to phosphoglycerate
kinase to phosphoglyceromutase was in the range of 1:0.6-
1:2:0.6-1.2 when these activities were expressed in terms of
micromoles of substrate utilized per min per g of wet tissue
(20). We have preliminarily investigated this situation in a
common bacterial species (E. coli) grown on different carbon
sources. Typical results are shown in Table I. Although there
seems to be relatively more phosphoglycerate kinase and phos-
phoglyceromutase activity in E. coli extracts (ratio of glyceralde-
hyde phosphate dehydrogenase to phosphoglycerate kinase to
phosphoglyceromutase, 1:1.7:5.4) than in those studied by
Pette et al. (20), the three activities are nevertheless in approxi-
mately constant proportion to one another no matter which of
the three carbon sources the cells utilized. (The methods of
enzyme assay used here differ somewhat from those employed
by Pette et al. (20), but these differences do not appear to account
for the disparity in the glyceraldehyde phosphate dehydrogenase
to phosphoglycerate kinase to phosphoglyceromutase ratio.)
In other experiments the ratio of activities of these three enzymes
did not change significantly during the cycle of growth on a
given carbon source. Cells grown to stationary phase in a
glucose-containing medium were chosen as a source of these
enzymes for purposes of purification.

Purification of Enzymes

All operations were conducted at 0 to 5°C unless otherwise
indicated; centrifugations were performed at 15,000 × g for 30
min. Tris-EDTA buffer refers to 20 mM Tris-chloride, pH 8,
containing 2 mM EDTA. Procedures are summarized in Table
II. In all subsequent studies the most purified fractions were
employed.

Growth of Bacteria and Preparation of Extract—E. coli (Hfr
Hayes, strain K12-3000, from the collection of J. Monod, Pasteur
Institute) were grown to stationary phase with vigorous aeration
in a glucose-yeast extract medium described earlier (21).
Growth and harvesting of cells were carried out by Grain Proc-

### Table I

| Medium carbon source | Specific activities | Proportion of GDH: PK: PGM |
|----------------------|---------------------|---------------------------|
|                      | GDH | PK | PGM |
| Glucose (0.5%)       | 0.4 | 0.7 | 1.7 | 1:1.7:4.2 |
| Glycerol (0.5%)      | 0.4 | 0.6 | 2.2 | 1:1.5:5.5 |
| Succinate (1.0%)     | 0.2 | 0.4 | 1.5 | 1:2.0:6.5 |

* GDH, glyceraldehyde phosphate dehydrogenase; PK, phosphoglycerate kinase; PGM, phosphoglyceromutase.
Fraction step Protein

I
II
III
IV-DH
IV-K-M
V-DH
V-K
V-M

Abbreviations are defined in Table I.

See Fig. 1.

See Fig. 2.

The cell paste could be stored at $-10^\circ$ for over 3 years without loss of relevant enzymatic activities. Cells (400 g) were disrupted by high speed stirring with glass beads (Superbrite, average diameter 200 μ, Minnesota Mining and Manufacturing Company; 1.3 kg) in 450 ml of Tris-EDTA buffer. The procedure was carried out in a 1-gallon stainless steel blending container with an outer jacket through which polyethylene glycol at $-5^\circ$ was circulated; the temperature of the extract never exceeded $10^\circ$. The mixture was stirred at slow speed to form a thick suspension and then blended for 20 min at 75% of maximum speed. Tris-EDTA buffer (1.35 liters) was added, and the mixture was stirred at slow speed for an additional 10 min. After the beads had settled for 10 min, the liquid was decanted, and the beads were washed by slow speed stirring with 1 liter of Tris-EDTA buffer. The combined extract and wash were centrifuged, and the supernatant liquid was decanted (Fraction I, 2.4 liters).

Streptomycin Precipitation of Inactive Materials—Streptomycin sulfate (Charles Pfizer and Company; 31 g in 625 ml of Tris-EDTA buffer) was added with stirring to Fraction I. The suspension was slowly stirred an additional 15 min and then centrifuged; the supernatant fluid was collected (Fraction II, 3 liters).

Ammonium Sulfate Fractionation—Solid ammonium sulfate (Nutritional Biochemicals Corporation, enzyme grade; 1.2 kg) was stirred into the 3 liters of Fraction II. The precipitate was removed by centrifugation, and 0.7 kg of ammonium sulfate was added with stirring to the supernatant. After this suspension had stood overnight (15 to 18 hours), it was centrifuged, and the precipitate was collected in 120 ml of Tris-EDTA buffer. The solution of concentrated protein was clarified by a final centrifugation (Fraction III, 0.9 liters).

DEAE-Sephadex Chromatography of Glyceraldehyde Phosphate Dehydrogenase—Fraction IV-DH (590 ml) was applied to the top of a column (4 cm $\times$ 30 cm) of DEAE-Sephadex A-50 which had been equilibrated with 50 mM Tris-chloride, pH 8, 2 mM EDTA. (The higher ionic strength buffer was necessary to avoid swelling of the gel during loading of the column.) Active fractions were combined and dialyzed overnight against 10 to 20 volumes of a solution of phosphoglycerate kinase, which eluted together in later fractions (Fig. 1). Fractions containing enzymatic activity were combined in two different pools (Fractions IV-DH and IV-K-M, respectively) and treated separately.

**TABLE II**

**Purification of enzymes**

| Fraction | Step                | Protein | GDH<sup>a</sup> Specific activity (units/mg protein) | Total activity (units) | PGK<sup>a</sup> Specific activity (units/mg protein) | Total activity (units) | PGM<sup>a</sup> Specific activity (units/mg protein) | Total activity (units) |
|----------|---------------------|---------|-------------------------------------------------|-----------------------|-------------------------------------------------|-----------------------|-------------------------------------------------|-----------------------|
| I        | Extract             | 15      | 0.4                                             | 14,500                | 0.7                                             | 25,000                | 1.7                                             | 60,000                |
| II       | Streptomycin        | 7       | 0.8                                             | 17,000                | 1.1                                             | 24,000                | 2.4                                             | 50,000                |
| III      | Ammonium sulfate    | 60      | 2.2                                             | 16,000                | 3.3                                             | 34,000                | 5.6                                             | 40,000                |
| IV-DH    | Sephadex G-150<sup>b</sup> | 1.6   | 8.5                                             | 8,000                 | 7.9                                             | 13,000                | 12                                              | 20,000                |
| V-DH     | DEAE-Sephadex       | 13-27   | 30                                              | 4,000                 | 98                                              | 7,000                 | 124                                             | 13,000                |
| VI-DH    | Sephadex G-200      | 0-13    | 40                                              | 2,500                 |                                                  |                       |                                                  |                       |
| V-K      | DEAE-cellulose<sup>c</sup> | 11    |                                                  |                       |                                                  |                       |                                                  |                       |
| V-M      |                      | 25      |                                                  |                       |                                                  |                       |                                                  |                       |

<sup>a</sup> Abbreviations are defined in Table I.

<sup>b</sup> See Fig. 1.

<sup>c</sup> See Fig. 2.

![Fig. 1. Sephadex G-150 gel filtration of Fraction III, containing *Escherichia coli* glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, and phosphoglyceromutase. Fractions IV-DH and IV-K-M were obtained from eluates pooled as shown by the arrows. Details are given in the text.](http://www.jbc.org/content/425/4/2415/F1.large.jpg)
FIG. 2. Chromatography on DEAE-cellulose of Fraction IV-K-M, containing Escherichia coli phosphoglycerate kinase and phosphoglyceromutase. Fractions V-K and V-M were obtained from eluates pooled as shown by the arrows. Details are given in the text.

FIG. 3. Polyacrylamide gel electrophoreses of Escherichia coli enzyme fractions. Electrophoreses of 50 to 100 μg of protein were carried out in gels at pH 9 according to the method of Davis (18), and the protein bands were stained with Amido black. Extract (EXTR, Fraction I), glyceraldehyde phosphate dehydrogenase Fraction VI-DH (GDH), phosphoglycerate kinase Fraction V-K (PGK), and phosphoglyceromutase Fraction V-M (PGM) were examined.

neutral saturated ammonium sulfate (prepared by addition of 10 ml of 28% ammonium hydroxide per liter of ammonium sulfate solution saturated at 2°). After the suspension of precipitated protein had been removed from the dialysis sac, it was centrifuged and the precipitate was dissolved in 5 to 10 ml of Tris-EDTA buffer. Alternatively, the pooled eluates could be concentrated by pervaporation in a Schleicher and Schuell collodion bag filtration apparatus. Any turbidity in the concentrated protein solutions was removed by centrifugation (Fraction V-DH).

Sephadex G-200 Gel Filtration of Glyceralddehyde Phosphate Dehydrogenase—Fraction V-DH (5 to 10 ml) was slowly applied to the bottom of a bed of Sephadex G-200 gel which was mounted in a column (5.1 cm² x 95 cm; Pharmacia Fine Chemicals) and had been equilibrated with Tris-EDTA buffer. The same buffer was then pumped upward through the column at a rate of 10 ml per hour. The enzyme was eluted as a sharp peak closely followed by a small, broad peak of inert protein. Fractions constituting the first half to two-thirds of the enzyme peak (those which yielded a single band in polyacrylamide gel electrophoresis) were pooled and concentrated as described in the preceding section. Neutral saturated ammonium sulfate containing 2 mM EDTA (0.18 volume) was added to the concentrated protein solution (to make it 0.8 M in ammonium sulfate), and the purified enzyme was stored at 2° in this medium (Fraction VI-DH, 5 to 10 ml). As shown in the following paper, the enzyme can be crystallized at this point, but no further purification is evident.

DEAE-Cellulose Chromatography of Phosphoglycerate Kinase and Phosphoglyceromutase—A column (3.8 cm² x 25 cm) of DEAE-cellulose (Whatman DE-52, microgranular) was equilibrated with Tris-EDTA buffer, and Fraction IV-K-M (935 ml) was applied to the top of the bed. After the column had been washed with 5 bed volumes of Tris-EDTA buffer, a linear salt gradient (0 to 0.15 M NaCl in a total volume of 1 liter of Tris-EDTA buffer) was applied. The average flow rate was 30 ml per hour, and fractions of 5.3 ml were collected. The phosphoglyceromutase and phosphoglycerate kinase activities eluted separately at concentrations of 0.08 M and 0.11 M NaCl, respectively, although there was a consistent and unexplained trailing of the phosphoglyceromutase peak into the phosphoglycerate
The complete reaction mixture (0.3 ml) contained 62 mM Tris-chloride, pH 7.5, 3.3 mM MgCl₂, 1 mM 2-mercaptoethanol, 0.1 mM NAD+, 0.7 mM ATP, 0.1 mM glyceraldehyde-2-P, 5 mM glyceraldehyde-3-P₃, 6.5 ml, and Fraction V-M containing phosphoglyceromutase, and phosphoglyceromutase assay. No activity was detected if glyceraldehyde-3-P was omitted; in the phosphoglycerate kinase assay there was no activity unless ATP and glyceraldehyde-3-P were both included; and in the phosphoglyceromutase assay there was 0.04 mM Pi, (10⁶ cpm per pmole), 0.6 µg of glyceraldehyde phosphate dehydrogenase (Fraction VI-DH), and 0.2 µg each of phosphoglycerate kinase (Fraction V-K) and phosphoglyceromutase (Fraction V-M). After incubation for 15 min at 37°C, the mixture was heated at 100° for 2 min and then chilled to 0°. Ice-cold 2 N HCl (0.1 ml) and Norit A (20 mg in 0.2 ml of water) were added. Nucleotide adsorption to the charcoal was complete within 5 min at 0°, and the Norit was collected and exhaustively washed with cold water on a Millipore microfiber glass prefiltter (AP 20). The Norit was dried under an infrared lamp and assayed for radioactivity. The millimicromoles of ATP-γ-P formed were calculated from the given specific radioactivity of ATP-γ-P and therefore yields low estimates.

\[ ^{32}P-\text{ATP-γ-P exchange catalyzed by E. coli glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, and phosphoglyceromutase} \]

The complete reaction mixture (0.3 ml) contained 62 mM Tris-chloride, pH 7.5, 3.3 mM MgCl₂, 1 mM 2-mercaptoethanol, 0.1 mM NAD+, 0.7 mM ATP, 0.1 mM glyceraldehyde-2-P, 5 mM glyceraldehyde-3-P₃, 0.7 mM ATP, (10⁶ cpm per pmole), 0.6 µg of glyceraldehyde phosphate dehydrogenase (Fraction VI-DH), and 0.2 µg each of phosphoglycerate kinase (Fraction V-K) and phosphoglyceromutase (Fraction V-M). After incubation for 15 min at 37°C, the mixture was heated at 100° for 2 min and then chilled to 0°. Ice-cold 2 N HCl (0.1 ml) and Norit A (20 mg in 0.2 ml of water) were added. Nucleotide adsorption to the charcoal was complete within 5 min at 0°, and the Norit was collected and exhaustively washed with cold water on a Millipore microfiber glass prefiltter (AP 20). The Norit was dried under an infrared lamp and assayed for radioactivity. The millimicromoles of ATP-γ-P formed were calculated from the given specific radioactivity of ATP-γ-P and therefore yields low estimates.

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The complete reaction mixture (0.3 ml) contained 62 mM Tris-chloride, pH 7.5, 3.3 mM MgCl₂, 1 mM 2-mercaptoethanol, 0.1 mM NAD+, 0.7 mM ATP, 0.1 mM glyceraldehyde-2-P, 5 mM glyceraldehyde-3-P₃, 0.7 mM ATP, (10⁶ cpm per pmole), 0.6 µg of glyceraldehyde phosphate dehydrogenase (Fraction VI-DH), and 0.2 µg each of phosphoglycerate kinase (Fraction V-K) and phosphoglyceromutase (Fraction V-M). After incubation for 15 min at 37°C, the mixture was heated at 100° for 2 min and then chilled to 0°. Ice-cold 2 N HCl (0.1 ml) and Norit A (20 mg in 0.2 ml of water) were added. Nucleotide adsorption to the charcoal was complete within 5 min at 0°, and the Norit was collected and exhaustively washed with cold water on a Millipore microfiber glass prefiltter (AP 20). The Norit was dried under an infrared lamp and assayed for radioactivity. The millimicromoles of ATP-γ-P formed were calculated from the given specific radioactivity of ATP-γ-P and therefore yields low estimates.

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was complete dependence upon glycerate-3-P. Nevertheless, it was desirable to confirm independently the catalytic functions of these proteins. Stoichiometry studies of the individual reaction steps are difficult because of instability of some of the intermediates, notably glycerate-1,3-P₂, and because of tediousness in quantitative isolation of such intermediates. Penefsky et al. have shown that ³²P can be exchanged into the γ position of ATP by means of the concerted action of glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase (22). In a modification of this procedure, with the use of purified E. coli enzymes, such an exchange can be demonstrated and shown to be dependent upon glycerate-2-P, provided phosphoglyceromutase is also present in the reaction mixture (Table III and Fig. 4). For equations, see Scheme 1.

**Molecular Weights and Sedimentation Coefficients of Purified E. coli Enzymes**—The high speed technique of sedimentation equilibrium was employed to determine molecular weights of the purified proteins. Logarithmic plots of the interference fringe data eschericus (radius)² yielded strictly linear relationships with all samples of both glyceraldehyde phosphate dehydrogenase and phosphoglyceromutase, as expected from the electrophoretic homogeneity of these proteins (Fig. 3). However, with the highest concentrations of phosphoglycerate kinase there was slightly increasing slope of the logarithmic plot toward the base of the cell, a finding indicative of heterogeneity (14). Molecular weights obtained from the slopes of the logarithmic plots are listed in Table IV. The values given for phosphoglycerate kinase were obtained from linear portions of the plots and are estimates of the smallest protein species present. In view of gel electrophoresis which show that the major protein component in the phosphoglycerate kinase preparation is of smaller size than contaminating minor components (Fig. 3), we conclude that the molecular weight listed in Table IV is a valid size estimate for the main component in Fraction V-K and assume that this is E. coli phosphoglycerate kinase. Sedimentation coefficients of the purified enzymes have also been measured and are recorded in Table IV. The contaminants present in purified phosphoglycerate kinase (Fig. 3) were not apparent in the schlieren patterns of sedimenting protein (Fig. 5).

**DISCUSSION**

We hope that the information given here will contribute toward continuing elucidation of biochemical function as it is carried out in E. coli. For example, with use of various carbon sources it should be possible to devise genetic techniques for isolation of mutant cells with altered glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, or phosphoglyceromutase. Such mutants would be of considerable interest in studies of

**TABLE IV**

| Protein         | Molecular weight | Analytical S.D. | Analytical Range | n₁/₀  |
|-----------------|------------------|-----------------|------------------|-------|
| GDH             | 144,000          | 3,900           | 140,500–148,000  | 7.1   |
| PGK             | 43,700           | 1,100           | 42,200–45,000    | 3.5   |
| PGM             | 56,300           | 1,200           | 55,400–57,000    | 4.8   |

* Abbreviations are defined in Table I.

For glyceraldehyde phosphate dehydrogenase partial specific volume (θ = 0.734 cc per g) was calculated from the amino acid composition of the protein given in the following paper (28, 29).

Three molecular weight analyses were carried out at 18,140 rpm for 24 hours. The θ₀,₀ values reported by Allison and Kaplan (3) were included along with data of the present study in determining the θ₀,₀ value.

For phosphoglycerate kinase partial specific volume was assumed to be θ = 0.75 cc per g (30). Six molecular weight analyses at two different rotor speeds were carried out (40,410 rpm for 26 hours and 30,170 rpm for 25 hours).

For phosphoglyceromutase partial specific volume was assumed to be θ = 0.74 cc per g (31). Three molecular weight analyses were carried out at 25,210 rpm for 22 hours.

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**Scheme 1.** 2-PGA, 3-PGA, and 1,3-diPGA represent glycerate-2-P, glycerate-3-P, and glycerate-1,3-P₂, respectively; and [3-PGA*GDH.NAD] represents acyl-enzyme complex (27).

**FIG. 5.** Schlieren boundaries observed during sedimentation of E. coli phosphoglycerate kinase. A valve-type synthetic boundary cell (32) was employed in this experiment in which solvent (0.1 M NaCl, 0.01 M sodium phosphate, pH 7) was layered on a solution of purified phosphoglycerate kinase (8.5 mg of Fraction V-K per ml). Photographs were taken at 4, 12, and 20 min after attaining a rotor speed of 52,350 rpm. The angle of the schlieren diaphragm was 70°, and temperature was 8°.
bacterial physiology and metabolic control, and the altered proteins would provide useful analogues for investigation of enzyme structure and mechanism. Such projects at the present time would be overwhelmingly difficult in complex multicellular organisms but are quite feasible with a bacterial system such as E. coli.

One relatively simple analysis that can be made with the data at hand relates to an interesting hypothesis proposed by Mier and Cotton about the constant proportion group of Embden-Meyerhof pathway enzymes (triose phosphate isomerase to enolase) (19, 20, 33). These authors noted that the data compiled in earlier studies of Pette et al. (20), taken together with recorded molecular weight and specific activity values of purified enzymes, could be interpreted in terms of an operon hypothesis. They calculated that approximately equimolar amounts of each of the five enzymes were present in the various cell extracts studied by Pette et al., and proposed that, if these proteins were all members of the same operon (34), a single long strand of polycistronic messenger RNA might be transcribed from the entire operon and subsequently give rise to equimolar amounts of each of the proteins coded by the operon. A corresponding calculation with the presently available E. coli enzyme data (Tables I, II, and IV) shows that the ratio of molecules of glyceraldehyde phosphate dehydrogenase to phosphoglycerate kinase to phosphoglyceromutase is 1:2:4:3:6. (This calculation is independent of the method of assay of the respective enzymes so long as the same technique is employed with both the extract and the purified protein.) Therefore, in this bacterial species such an operon hypothesis does not seem to hold unless there are additional extrachromosomal genes for one or more of these three proteins in E. coli strain K12-3000 or disproportionate amounts of protein are synthesized during the translation process. Location of the genes for these three proteins on the E. coli chromosome map (35) would be of much interest.

The measured size of E. coli glyceraldehyde phosphate dehydrogenase is very similar to the reported molecular weights of analogous proteins isolated from rabbit muscle (36, 37), pig muscle (38), and yeast (39). The sedimentation coefficient, in agreement with previous measurements by Allison and Kaplan (6), is indistinguishable from those of corresponding proteins from a wide variety of sources (6). There is only one recorded value of phosphoglycerate kinase molecular weight with which to compare that of the E. coli enzyme; the size of the yeast protein is reported as 34,000 daltons (30). Finally, the molecular weight of E. coli phosphoglyceromutase is near the values reported for the enzymes from rabbit skeletal muscle (64,000 (31), 57,000 (32)) and from chicken breast muscle (66,000 (33)) but is about half that found for the yeast enzyme (112,000 (31)).

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