A COMPARISON OF SOME OF THE PHYSICAL AND CHEMICAL PROPERTIES OF THE PHOSPHOGLYCERATE MUTASES FROM HUMAN ERYTHROCYTES

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SUMMARY: The molecular weights of both phosphoglycerate mutases from human erythrocytes have been critically examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and by exclusion chromatography. The data indicate that native monophosphoglycerate mutase (mw ∿ 56-57,200) is larger than bisphosphoglycerate mutase (mw ∿ 54-54,400) by approximately 2-3,000 g per mole. Despite differences in mass, amino acid analyses show that each enzyme is strikingly similar in composition. Thus, the values obtained for two-thirds of the residues in both proteins are either identical or nearly identical. Moderately disparate values, however, are found for lysine, threonine, glutamic acid, proline, glycine, alanine, and leucine. Of the two proteins, the monomutase is the slightly more hydrophobic, while the dimutase is the more acidic. In accord with compositional analyses, density gradient electrofocusing shows that the isoelectric points of bisphosphoglycerate and monophosphoglycerate mutase are 4.9 and 6.2, respectively. The results presented in this paper and elsewhere suggest that an extensive degree of homology exists between both red cell enzymes.

Following the physiologically important observation that 2,3-bisphospho-D-glycerate (2,3-DPG)[1] influences the complexation between oxygen and hemoglobin (1,2), an intensive investigation was initiated by several laboratories in an effort to understand the metabolic factors involved in the formation and the utilization of the above organophosphate. In this regard, two of the enzymes that have been rigorously studied are bisphosphoglycerate mutase (EC 2.7.5.4) (3-8) and monophosphoglycerate mutase (EC 2.7.5.3) (4,9-12). The first enzyme (DPGM) catalyzes the irreversible synthesis of 2,3-DPG from 1,3-bisphospho-D-glycerate (1,3-DPG) and D-glycerate-3-P (3-PGA). The second

[1] Abbreviations: 2,3-DPG, 2,3-bisphospho-D-glycerate (2,3-diphospho-D-glycerate); DPGM, bisphosphoglycerate mutase (diphosphoglycerate mutase); MPGM, monophosphoglycerate mutase; 3-PGA, D-glycerate-3-P; 2-PGA, D-glycerate-2-P.

enzyme (MPGM) requires 2,3-DPG for the interconversion of D-glycerate-3-P and D-glycerate-2-P (2-PGA).

Recently, each of the above catalysts has been purified to homogeneity from human erythrocytes (3,5,12). Consequently, several of each enzyme's physical and chemical characteristics have been described. Surprisingly, the accumulated data indicate a striking degree of similarity in the two proteins. Thus, the monomutase and the dimutase are both dimeric and both have been reported to exhibit apparently equivalent molecular weights (4,5,9). Erythrocyte DPGM has been found to generate a protein-bound phosphoryl histidine during catalysis (6). Although a similar partial reaction has not as yet been established for red cell MPGM, both the muscle (13,14) and the yeast enzymes (15) have been shown to proceed through a phosphoryl histidine intermediate. By analogy, it is almost certain that the red cell enzyme performs accordingly. Both phosphoglyceromutases possess 2,3-DPG phosphatase activity (3,12). In addition, the dimutase is capable of eliciting, to a slight degree, the principal reaction catalyzed by the monomutase (3). Contrary to the findings of Laforet et al. (10), however, it appears that homogeneous MPGM is incapable of catalyzing the transformation of 1,3-DPG to 2,3-DPG (i.e., the principal reaction conducted by DPGM) (12). Finally, the 2,3-DPG phosphatase activity of both mutases is stimulated by certain organic and inorganic anions (3,6,11, 12).

The principal thrust of this report is to elaborate on several of the physical and chemical properties of homogeneous red cell DPGM and MPGM. In doing so, we wish to clarify some of the incongruities reported in the past by several laboratories. We also wish to emphasize certain recently found distinguishing features ascribable to each enzyme.

MATERIALS AND METHODS

Materials: Outdated human erythrocytes of various blood types were obtained from The M. S. Hershey Medical Center and the Harrisburg Polyclinic Hospital blood banks. Other materials and chemicals were obtained from sources listed in previous publications (3,12,16).

Enzyme Assays: DPGM and MPGM activities were assayed as previously described (3,12). One unit of enzyme activity is defined as that amount of protein which catalyzes the formation of 1.0 μmol of product per min under the conditions cited.

Molecular Weight Determination: The native molecular weights of purified DPGM and MPGM were determined at 4° by ascending exclusion chromatography on Sephadex G-100 as described by Andrews (17). The column (2.5 x 90 cm) was equilibrated with 25 mM $K\text{-}PO_4$-2 mM 2-mercaptoethanol - 5 mM EDTA (pH 7.0). After equilibration, a 4 ml sample was applied to the column. The sample contained DPGM (5 units), MPGM (25 units), and 7-10 mg of the following globular proteins: cytochrome C (12 400), α-chymotrypsinogen A (25 000), ovalbumin (43 000), and bovine serum albumin (68 000). The column was eluted at a flow rate of 15 ml/h. The resultant 2.5 ml fractions were assayed for enzyme activity and absorbance at 280 nm.

The reduced, denatured subunit molecular weight of DPGM and MPGM was determined using the sodium dodecyl sulfate polyacrylamide gel electrophoresis system of Weber et al. (18). The 11.5 x 0.6 cm gels, containing 10% total acrylamide, were electrophoresed for 12 h at 6 mA per gel. The temperature was maintained at 19°. Protein mobilities were calculated relative to that of myoglobin.

Preparation of Membrane-free Hemolysate: Erythrocyte hemolysate, equivalent to a 1:4 dilution of packed cells, was prepared by the method of Hass and Miller (16). The hemolysate was further diluted 1:1 with 5 mM $K\text{-}PO_4$-2 mM 2-mercaptoethanol (pH 7.0) and centrifuged at 60,000 g for 1 h to remove membrane and cellular debris.

Fractionation of Hemolysate with DEAE-Cellulose: Membrane-free hemolysate was fractionated by batch-wise treatment with DEAE-cellulose employing previously described procedures (3). The adsorbed protein eluate was concentrated 20-fold by ultrafiltration through an Amicon apparatus equipped with a PM-10 membrane.

Isoelectric Focusing: In preparation for isoelectric focusing, concentrated DEAE-cellulose-treated hemolysate (see above) was exhaustively dialyzed against 0.5 mM $K\text{-}PO_4$-2 mM 2-mercaptoethanol (pH 7.0). An aliquot of the dialyzed solution (1.2 ml, containing approximately 20 mg of protein) was subjected to density gradient electrofocusing (19,20), using an LKB 8100 column (110 ml capacity). The column contained a 1.5% ampholine solution (pH 3.5-10) and a 0-47% sucrose gradient. Electrofocusing was conducted at 4° and 300 V for 60 h. The absorbance at 280 nm of 0.5 ml fractions was monitored continuously with an ISCO UA-5 Absorbance Monitor. Each fraction was assayed to determine DPGM and MPGM activity, and pH.

Amino Acid Analysis: Amino acid analyses were performed by the method of Moore, Spackman, and Stein (21), using a Beckman 120C autoanalyzer as previously described (3,12).

RESULTS

Molecular Weight Determination. Previously, we and others had indicated that DPGM and MPGM possess identical molecular weights, ranging between 57,000-60,000 (3-5,9). We now wish to report that upon critical re-examination, each of the above enzymes manifests a slightly but distinctly different mass value. Thus, when DPGM, MPGM, and appropriate protein markers are subjected to as-

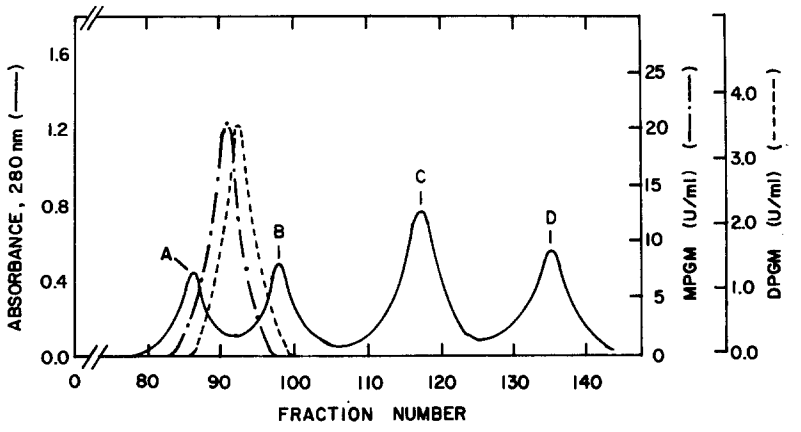

Figure 1. Determination of the native molecular weights of diphosphoglycerate and monophosphoglycerate mutase by ascending exclusion chromatography. DPGM and MPGM were chromatographed in the presence of the following globular proteins: bovine serum albumin (A), ovalbumin (B), α-chymotrypsinogen A (C), and cytochrome C (D). Fractions were analyzed for 280 nm absorbance (———), MPGM (—·—), and DPGM activity (- - - -). Detailed procedures are outlined under Materials and Methods.

cending exclusion chromatography on Sephadex G-100, the results depicted in Figure 1 are obtained. Analysis of these results gives native molecular weights of 54,000 and 56,000 for the dimutase and the monomutase, respectively. Despite the small difference in the above masses, repeated experiments under the conditions cited have consistently produced virtually identical results.

Further corroboration of the above finding has been obtained through polyacrylamide gel electrophoresis experiments conducted in the presence of sodium dodecylsulfate, using relatively long (11.5 cm) gel columns. Under these conditions, a distinct separation of DPGM and MPGM subunits is obtained (Figure 2), yielding respective molecular masses of 27,200 and 28,600 daltons. Consequently, there appears to be little doubt that native MPGM is the larger of the two dimeric enzymes by about 2,000-3,000 g/mole.

Amino Acid Composition. In light of the molecular weight difference between DPGM and MPGM, the previously reported amino acid composition for the dimutase (3) has been revised slightly to accomodate a macromolecular mass of

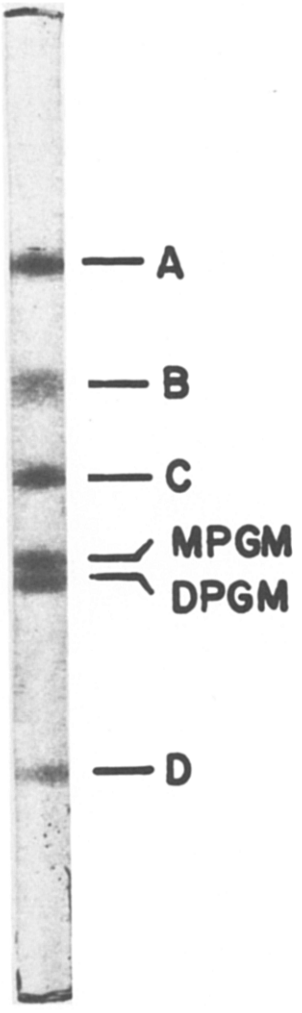

Figure 2. Determination of DPGM and MPGM subunit molecular weights by polyacrylamide gel electrophoresis. During electrophoresis, the following standard proteins were used: bovine serum albumin (A), ovalbumin (B), glyceraldehyde-3-P dehydrogenase (C), and myoglobin (D). Electrophoresis and data analysis were conducted as described under Materials and Methods. Appropriate control gels were run in order to distinguish MPGM from DPGM.

54,000 rather than 57,000 daltons. When this is done and the results are compared with those obtained for MPGM (mw = 56,000), the amino acid compositions of the two proteins are strikingly similar (Table I). Thus, moderately disparate values are found for lysine, threonine, glutamic acid, proline, glycine, alanine, and leucine, while those for the remaining residues are either identi-

TABLE I

COMPARISON OF HUMAN ERYTHROCYTE DIPHOSPHOGLYCERATE AND MONOPHOSPHOGLYCERATE MUTASE AMINO ACID COMPOSITION[a]

Amino Acid Residue	DPGM Amino Acid Residues per 54,000 MW	MPGM Amino Acid Residues per 56,000 MW
Lysine	25	33
Histidine	14	13
Arginine	32	32
Aspartic acid	46	46
Threonine	10	19
Serine	25	21
Glutamic acid	69	62
Proline	21	27
Glycine	26	33
Alanine	26	47
Half cystine[b]	5	5
Valine	27	25
Methionine	5	8
Isoleucine	27	27
Leucine	56	47
Tyrosine	14	13
Phenylalanine	9	13
Tryptophan[c]	9	9
TOTALS	446	480
CALCULATED MW	53,937	56,306

[a]Amino acid analyses were performed as described in Materials and Methods. The above values have been rounded off to the nearest whole number.

[b]Average of three determinations as cysteic acid after 24 h hydrolysis (22).

[c]Determined by the procedure of Goodwin and Morton (23).

cal or nearly so. Of the two proteins, MPGM is the slightly more hydrophobic, while DPGM is the more acidic. The latter property stems from the fact that DPGM contains 8 less lysine and 7 more glutamic acid residues than MPGM. The number of other charged amino acids in both molecules is, for all practical purposes, identical.

Isoelectric Point Determination. In accord with the amino acid analyses, density gradient isoelectric focusing shows that the isoelectric points of red cell DPGM and MPGM are 4.9 and 6.2, respectively (Figure 3). These pI's are

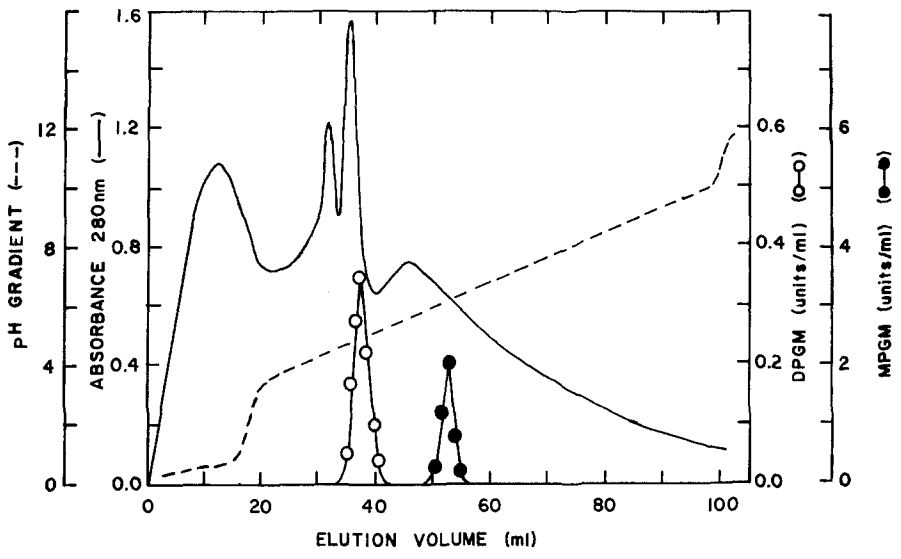

Figure 3. Isoelectric point determination of DPGM and MPGM by density gradient electrofocusing of DEAE-treated erythrocyte hemolysate. Resultant fractions were analyzed for 280 nm absorbance (———), pH (- - - -), DPGM (O——O), and MPGM activity (●——●). Experimental details are presented under Materials and Methods.

in agreement with the approximate values reported previously by Rose and Whalen (6), and Sasaki et al., (5). At this juncture, it is worthwhile noting that the difference in coulombic properties of the two phosphoglycerate mutases has allowed us to separate DPGM from MPGM by ion exchange chromatography (16) and polyacrylamide gel electrophoresis[2].

DISCUSSION

In order to acquire a better understanding of the regulation of 2,3-DPG levels within the human erythrocyte, we have purified both red cell phosphoglycerate mutases to homogeneity. This currently places us and others in position to compare many of the physical, chemical, and catalytic properties of the two enzymes.

As already pointed out in the text, many of the characteristics of both mutases are strikingly similar. This suggests that extensive structural homo-

[2] L. F. Hass et al., unpublished data.

logy may exist between both proteins. The experiments required either to prove or disprove the above hypothesis, however, have not yet been performed. Nevertheless, the molecular weight and compositional data presented in this paper favor the homology concept. If it can be ultimately demonstrated that the concept is true, the question concerning the genetic origin of each enzyme is immediately brought into focus. Thus, one might legitimately ask whether each mutase arises from a different structural gene or whether both enzymes arise from a common gene with a subsequent transformation of one enzyme into the other. An investigation designed to answer the latter question is currently underway.

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