Evidence for Structural Homology between Human Red Cell Phosphoglycerate Mutase and 2,3-Bisphosphoglycerate Synthase*

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Previous reports have suggested the possibility of extensive structural homology between human erythrocyte bisphosphoglycerate synthase (glycerate-1,3-P$_2$ → glycerate-2,3-P$_2$) and phosphoglycerate mutase (glycerate-3-P → glycerate-2-P). This study lends credence to that conjecture through comparative physicochemical investigations involving peptide mapping, circular dichroism, and immunological techniques. The data indicate that despite differences in function, both enzymes apparently manifest a high degree of similarity in primary, secondary, and tertiary structure. Mapping data also indicate that each protein is comprised of two apparently identical subunits.

Recently acquired evidence suggests that human red cell phosphoglycerate mutase (EC 2.7.5.3) and 2,3-bisphosphoglycerate synthase (EC 2.7.5.4) manifest extensive structural homology despite differences in primary function (1). The main role of phosphoglycerate mutase is to catalyze the reversible conversion of glycerate-3-P to glycerate-2-P in the presence of glycerate-2,3-P$_2$.

\[
\text{glycerate-3-P} \underset{\text{glycerate-2,3-P$_2$}}{\rightleftharpoons} \text{glycerate-2-P}
\]

2,3-Bisphosphoglycerate synthase, on the other hand, catalyzes the irreversible conversion of glycerate-1,3-P$_2$ to glycerate-2,3-P$_2$ in the presence of either glycerate-3-P or glycerate-2-P (2).

\[
\text{glycerate-1,3-P$_2$} + \text{glycerate-3-P} \rightarrow \text{glycerate-2,3-P$_2$} + \text{glycerate-3-P}
\]

Rose and co-workers (2) have shown that red cell bisphosphoglycerate synthase and muscle phosphoglycerate mutase both generate an enzyme-bound phosphoryl histidine intermediate during catalysis. A similar partial reaction has not yet been established for erythrocyte phosphoglycerate mutase, but by analogy with the muscle enzyme, it is almost certain that the red cell mutase functions in an analogous manner.

Through purification of the aforementioned erythrocyte enzymes it was possible to establish unequivocally that each catalyst is multifunctional (3-6). Thus, it was found that both catalysts possess intrinsic 2,3-bisphosphoglycerate phosphatase activity (glycerate-2,3-P$_2$ → P-glycerate + P$_2$). In human erythrocytes, most of the glycerate-2,3-P$_2$ phosphatase activity was shown to be associated with the bisphosphoglycerate synthase molecule, leading to the concept that the phosphoglycerate bypass as proposed by Rapoport and Luebering (7, 8) is under the control of a single enzyme (9).

Bisphosphoglycerate synthase is also capable of eliciting, to a slight degree, the principal reaction catalyzed by phosphoglycerate mutase (5). Despite the findings of Laforet et al. (10) and the recent work of Rose and Dube (2), there is no concrete evidence, however, to indicate that homogeneous red cell phosphoglycerate mutase is capable of emulating the principal reaction of bisphosphoglycerate synthase.

Despite differences in primary function, each of the above enzymes manifests certain common catalytic characteristics. In addition, both enzymes have been shown previously to possess striking degrees of similarity in several of their physical and chemical properties (1). It is the purpose of this report, therefore, to provide additional evidence in support of the concept that human erythrocyte phosphoglycerate mutase and 2,3-bisphosphoglycerate synthase are structurally homologous enzymes.

**EXPERIMENTAL PROCEDURES**

Materials — Outdated human erythrocytes, stored at 4°C in citrate/phosphate/dextrose, were obtained from the blood banks located at

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1 In previous publications, 2,3-bisphosphoglycerate synthase has been referred to as diphosphoglycerate mutase. Unlike other mutases, however, the enzyme functions principally as a catalyst for irreversible metabolite synthesis. Thus, the enzyme is indeed a synthase rather than a mutase.
The Horchey Medical Center and the Harrisburg Poly Clinic Hospital. The cells were used within 3 to 5 weeks from date of collection.

Homogeneous bisphosphoglycerate synthase and phosphoglycerate mutase were obtained by procedures described previously (5). Bovine serum albumin, rabbit muscle phosphoglycerate mutase, and bovine pancreas RNase were obtained from Miles, Boehringer, and Worthington, respectively. Human hemoglobin was a product of biophosphoglycerate synthase preparation (5).

Enzyme Assays - Bisphosphoglycerate synthase was assayed essentially by the method described previously (5) except that the final concentrations of the ingredients in the reaction mixture were modified as follows: 25 mM glycerol (pH 7.8), 1.5 mM dichloro-



resultant supernatant solution was lyophilized. The lyophilized tryptic hydrolysate was dissolved in 1.0 ml of 0.2 M NH₄HCO₃ (pH 8.5) and subsequently digested with trypsin. Digestion was initiated by the addition of an aliquot of trypsin solution (5 mg/ml), resulting in a protein to peptide ratio of 50:1 (w/w). The digestion mixture was incubated for 24 h at 37°C. After 24 h, any undigested particles were removed by centrifugation and the resultant supernatant solution was lyophilized.

Column peptide mapping. Employing a Beckman 120C autoanalyzer, was performed essentially by the method of Hill and DeLalio (12). The lyophilized tryptic hydrolysate was dissolved in 1.0 ml of 0.2 M pyridine/acetate (pH 3.1) and an appropriate aliquot was applied under pressure to a jacketed column (0.9 x 38 cm) containing Beckman type PA-28 resin. The column was thermostatted at 55.5°C. The data, therefore, confirm the previously stated conjecture (2-6) that each enzyme is comprised of two apparently identical subunits. It is noteworthy that both elution profiles (A and B in Fig.

Circular Dichroism - Spectra were obtained with a Cary 60 spectro
topolarmeter equipped with a model 6001 (CD) accessory. Measurements were conducted over a 1-mm path length at 21°C. Prior to analysis, protein samples were dialyzed at 700 x g for 12-18 h against 10 mM KPO₄, 2 mM mercaptoethanol (pH 7.0). Final protein concentrations were adjusted to 0.3 mg/ml, spectrophotometrically. Absorbance indices (1 mg/ml at 280 nm) used for bisphosphoglycerate synthase and phosphoglycerate mutase were 1.65 (5) and 1.56, respectively. Mean residue ellipticity, [θ], was calculated from the observed ellipticity, θ, by the method of the following equation:

\[ [\theta] = \frac{\theta_{222} \times (\text{mean residue wt})}{10 \times (\text{path length in cm}) \times (\text{mg/ml of protein})} \] (1)

The mean residue weight of each protein was calculated from its amino acid composition. The fractions (fₒ, etc.) of a, b, and random structures were calculated by the method of Reed et al. (13) from the relationships:

\[ f_a + f_b + f_r = 1 \] (3)

Values for [θ]₀, [θ]ₐ, and [θ]ₛ were those reported by Chen et al. (14). The experimental data were analyzed with a PDP-12 computer (Digital Equipment Corp.). Bisphosphoglycerate synthase and phosphoglycerate mutase were found to manifest no change in either total or specific activity during CD measurements, indicating no apparent alteration in macromolecular structure as the result of ultraviolet irradiation.

Immunology - Antiserum against bisphosphoglycerate synthase was produced in female New Zealand White rabbits, weighing approximately 3.5 kg. One milliliter of the purified enzyme antigen (3 mg in 20 mM KPO₄, 6 mM EDTA, 2 mM β-mercaptoethanol, pH 7.0) was emulsified by brief sonication with an equal volume of complete Freund's adjuvant (Difco Laboratories). The antigen-adjuvant emulsion was administered to animals, using the following regimen. A dorsal intradermal injection, employing multiple sites, was given initially and again 2 weeks later. At the beginning of the 4th week, a third and final dorsal injection was given subcutaneously. Empirical precipitin tests conducted in capillary tubes indicated that anti-bisphosphoglycerate synthase had reached its maximum titer within 7 to 8 days after the final injection. The rabbits were then exanguinated by cardiac puncture and blood serum was obtained by standard procedures (15). The serum's γ-globulin component was concentrated by repeated precipitation with ammonium sulfate (15). The final precipitate was dissolved in 0.05 M KPO₄, 0.145 M NaCl (pH 7.5) and was exhaustively dialyzed against the same buffer. The IgG-rich fraction was centrifuged to remove insoluble material and was stored at -20°C.

Immunodiffusion plates were prepared from 1.0% agar in phosphate-buffered saline, pH 7.5. Gel diffusion tests were conducted by incubating the aforementioned plates (containing appropriate amounts of antiserum and test antigens) in a humid chamber for 24 h at room temperature. Results were recorded directly onto photographic paper with a Bessier enlarger.

RESULTS

Peptide Mapping - Prior to mapping tryptic digests of bis-



Illustrated in Fig. 2 are the peptide maps obtained from tryptic digests of S-carboxamidomethylated bisphosphoglycerate synthase and phosphoglycerate mutase. The elution patterns of the synthase (Fig. 2A) and the mutase (Fig. 2B) both manifest approximately 30 peaks instead of the respective theoretical maxima of 58 and 66 based on amino acid analyses (1). The data, therefore, confirm the previously stated conjecture (2-6) that each enzyme is comprised of two apparently identical subunits.
FIG. 1. Maps of trypic peptides of bovine serum albumin (A) and human hemoglobin (B). Each chromatogram represents an analysis performed on 3.3 to 4.0 mg of S-carboxamidomethylated protein. The large peak emerging at approximately 5 h is due to ammonia. The techniques employed are described in detail under "Experimental Procedures."

FIG. 2. Peptide maps of trypic digests of S-carboxamidomethylated derivatives of human erythrocyte bisphosphoglycerate synthase and phosphoglycerate mutase. The various elution profiles are represented as follows: (A) bisphosphoglycerate synthase, (B) phosphoglycerate mutase, and (C) a mixture of peptides from both enzymes. In all cases, peptides equivalent to 1.0 mg of synthase and 0.8 mg of mutase were used. Apparently homologous peptides common to both enzymes are indicated by arrows. The large peak eluting at approximately 5 h is due to ammonia. The techniques employed are identical with those mentioned in Fig. 1.

Circular Dichroism—The far ultraviolet CD spectra for bisphosphoglycerate synthase and phosphoglycerate mutase are shown in Fig. 3. Except for slight differences in the minima above 220 nm and the extents of ellipticity, both spectra are essentially equivalent. The occurrence of minima at 208 and 223 to 225 nm indicates the presence of a significant amount of $\alpha$ helix in each protein (17). This is corroborated by the calculations from CD data (Table I) of $\alpha$ helix ($\alpha$), $\beta$-pleated sheet ($\beta$), and random structure ($\rho$).

The most significant aspect of this study, however, is the apparently close agreement in secondary structure between bisphosphoglycerate synthase and phosphoglycerate mutase as indicated by the data in Table I. That the values of $\alpha$, $\beta$, and $\rho$ for the above enzymes are indeed credible is supported by the additional data presented for bovine serum albumin and ribonuclease A. Thus, our fractional values for RNase obtained by CD are in excellent accord with those obtained from x-ray analyses by Kartha et al. (18). Despite slight disparities, CD calculations for bovine serum albumin made by us and by Reed et al. (13) are also in good agreement. This is particularly true in light of the report by the latter investi-
**DISCUSSION**

The data presented here and elsewhere (1) give evidence relating to extensive structural homology between two functionally distinct enzymes. Moreover, both of these enzymes have been obtained from the same species. This work, therefore, is unlike other numerous investigations which have concerned themselves with the characterization of functionally identical proteins from different species.

Despite considerable foundation in reality, all of the evidence in favor of structural homology between human red cell bisphosphoglycerate synthase and phosphoglycerate mutase is, nevertheless, of an indirect nature. Thus, additional experimentation will be required to lend more substance to our hypothesis. Areas for further investigation that immediately suggest themselves are sequence analysis and x-ray crystallography. With regard to the latter, we must point out that McPherson (23) has already crystallized red cell bisphosphoglycerate synthase from solutions of polyethylene glycol.

If our hypothesis concerning bisphosphoglycerate synthase and phosphoglycerate mutase is indeed correct, it would be of definite interest to determine the nature of those structural features which are responsible for the elicitation of one catalytic activity versus another. It would also be of interest to obtain clues leading to an explanation for the degrees of overlapping polyfunctionality exhibited by each enzyme.

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Phosphoglycerate Mutase and Bisphosphoglycerate Synthase Homology

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