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# PURIFICATION AND CHARACTERIZATION OF ALDOLASE FROM HUMAN ERYTHROCYTES

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## Summary

A procedure has been developed for the purification of human erythrocyte aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13). The process involves a specific substrate elution of the enzyme from phosphocellulose followed by a reverse ammonium sulfate fractionation. The preparation has been shown to be homogeneous by analytical ultracentrifugation, thin-layer electrophoresis, and polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The enzyme exhibits a specific activity of 16 I.U./mg protein, a $K_m$ of $7.1 \cdot 10^{-6}$ M for fructose 1,6-bisphosphate, and a substrate specificity (Fru-1,6-$P_2$/Fru-1-$P$) of 40. The native protein is a tetramer of 158 000 molecular weight possessing identical or nearly identical subunits, an isoelectric point of 8.9, a diffusion coefficient of $4.68 \cdot 10^{-7}$ cm$^2$/s, and a molecular radius of 4.56 nm. The study shows the enzyme to be a type A aldolase resembling other muscle forms in chemical and physical properties as well as amino acid composition.

## Introduction

The literature characterizing the glycolytic enzyme aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase, 4.1.2.13) from a number of tissues and species has been extensive [1—5]. Comparatively little is known, however, about the molecular properties of human erythrocyte aldolase, primarily because until now the only reported purified preparation contained contamination [6]. An isolation and purification method for the human

Abbreviations: Fru-1,6-$P_2$, fructose 1,6-bisphosphate; Fru-1-$P$, fructose 1-phosphate; GAPD, glyceraldehyde-3-phosphate dehydrogenase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Buffer A, 25 mM Tris · HCl, pH 7.3, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 M NaCl; SDS, sodium dodecyl sulfate; MTT tetrazolium, 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide.

erythrocyte enzyme is particularly important now in light of recent work by others [7,8] and ourselves [9,10] on aldolase-membrane and aldolase-actin interactions. In order to allow a precise examination of the physiological properties of human erythrocyte aldolase, we present here a method for purification as well as the catalytic and physical properties of the purified enzyme.

## Materials and Methods

### *Materials*

The following materials were obtained from Sigma Chemical Co.: fructose 1,6-bisphosphate (Fru-1,6-$P_2$), Fru-1-*P*, NADH, phosphocellulose, Trizma, $\beta$-galactosidase, cytochrome *c*, chymotrypsin, human serum albumin, rabbit muscle aldolase, GAPD, fumarase, triosephosphate isomerase, $\alpha$-glycerophosphate dehydrogenase, pyruvate kinase, dithiothreitol, Triton X-100, DTNB, MTT tetrazolium, and phenazine methosulfate. Reagents for acrylamide gel electrophoresis were from Bio-Rad Laboratories. Sephadex G-200 and Blue Dextran were from Pharmacia. Carrier ampholines were from LKB Productor. Cellulose acetate plates were from Helena Labs (Beaumont, Texas). All other chemicals were reagent grade.

### *Methods*

*Erythrocyte preparation.* The following operations were carried out at 4°C. Recent outdated whole blood was sedimented at 1460 g for 10 min in a Sorvall GSA rotor and the plasma and buffy coat carefully removed by aspiration. The erythrocytes were resuspended in four volumes of 25 mM Tris · HCl, pH 7.8, 0.1 M NaCl, and the above process repeated with particular care to remove any remaining buffy coat fragments. Two additional washes were performed with the same isotonic Tris buffer. The final centrifugation was carried out at 5000 × *g* for increased packing of the erythrocytes.

Hemolysis was then performed with five volumes of 25 mM Tris · HCl, pH 7.8, 10 mM 2-mercaptoethanol and the suspension stirred gently for 15 min. The ghosts were then sedimented at 23 000 × *g* for 45 min and the aldolase-containing supernatant solution carefully aspirated. The centrifuge brake was not used during this step in order to prevent ghosts from swirling up during the deceleration process.

*Enzyme assays.* Aldolase was assayed according to the coupled enzyme procedure of Racker [11]. The reaction mixture contained 100 mM Tris · HCl, pH 8.0, 0.25 mM NADH, 1 mM Fru-1,6-$P_2$, and 10 $\mu$g each of triosephosphate isomerase and $\alpha$-glycerophosphate dehydrogenase in a final volume of 1 ml. $K_m$ values were determined for Fru-1,6-$P_2$ using substrate concentrations from $10^{-6}$ to $10^{-3}$ M and for Fru-1-*P* using $10^{-5}$—$10^{-1}$ M concentrations. NADH oxidation was monitored at 340 nm of a Beckman Model K-25 spectrophotometer.

*Protein determination.* Protein concentrations were estimated by the dye binding method of Bradford [12], using human serum albumin as the standard.

*Aldolase quantitation.* The total amount of human erythrocyte aldolase per cell was determined by combining the hemolysate activity after complete lysis with the activity from repeated salt and detergent extractions of aldolase from the ghosts. Aliquots of cells were diluted with isotonic phosphate buffer, pH

7.4, and counted in an Electrozone Celloscope (Particle Data, Inc.) using an 80 μm aperture, current of 0.5, and gain of 12.

*Ion exchange chromatography.* Cellulose phosphate was washed and equilibrated by the procedure of Peterson and Sober [13]. The cellulose was used as a batch or packed into either a 1.5 × 30 or 2.5 × 40 cm column and equilibrated with 25 mM Tris · HCl, pH 7.8, 10 mM 2-mercaptoethanol for several days prior to use.

*Ammonium sulfate fractionation.* The reverse ammonium sulfate fractionation method described by Jacoby [14] was used without modification.

*Electrophoresis.* Cellulose acetate electrophoresis was performed on cellulose acetate plates (1 × 3 inch) equilibrated in High Resolution Gelman Tris/barbitol buffer, pH 8.8. Samples of 8 μl were applied to the plates and subjected to electrophoresis for 45 min (1.5 mA/gel) in a Gelman Rapid Electrophoresis apparatus. The plates were stained for aldolase by the procedure of Penhoet et al. [15] and for protein with 0.2% Coomassie Brilliant Blue in methanol/water/acetic acid (5 : 5 : 1, v/v).

Polyacrylamide gel electrophoresis was performed on 5.6% polyacrylamide gels containing 1% SDS according to the procedure of Fairbanks et al. [16] with the following modifications. Sample solutions were made 1% in SDS, immersed in boiling water for 1 min, and dialyzed overnight prior to electrophoresis against 10 mM Tris · HCl, pH 8.0, 1 mM EDTA, 10 mM 2-mercaptoethanol and 1% SDS. Each gel was overlaid with water of the same temperature as the gel mixture. The gels were stained with Coomassie Brilliant Blue and photographed, or scanned with a Helena Laboratories Quick Scan.

*Isoelectric focusing.* Aldolase samples were electrofocused in a linear sucrose or glycerol density gradient according to the procedure of Payne et al. [17] and the LKB manual, using 2% solutions of narrow-range ampholines in a LKB Model 8100 apparatus (LKB Productor). Dithiothreitol (10 mM) was included in all sample solutions. Electrofocusing was performed at 4°C for 72 h at 500 V. Columns were eluted at a flow rate of 30 ml/h with a peristaltic pump and 1-ml fractions were collected. The pH of the fractions was immediately determined at 4°C with a Markson Flow-Thru electrode.

*Catalytic studies.* Concentrations of Fru-1,6-$P_2$ and Fru-1-*P* were determined by standard enzymatic methods [18]. $K_m$ values were evaluated using the weighted least square method of Wilkinson [19] and adapted to the IBM 360-50 Computer [20].

*Analytical ultracentrifugation.* Analytical ultracentrifugation was performed on a Spinco Model E analytical ultracentrifuge equipped with an electronic speed control and RTIC temperature control unit. Sedimentation velocity studies of purified aldolase in concentrations of 6, 3 and 1.5 mg/ml Buffer A were performed in a standard 12 mm double sector cell with an An-D rotor at 60 000 rev./min, 19.6°C, and analyzed with Schlieren optics. The sedimentation coefficient was calculated according to Schachman [21] and corrected to $s^{\circ}_{20,w}$. A partial specific volume of 0.733 $cm^3/g$ was obtained from the amino acid analysis. Sedimentation equilibrium studies were performed in a 12 mm, six-channel Yphantis cell (sapphire windows) using interference optics [22]. After dialysis against Buffer A, protein (0.5 mg/ml) sedimentation was at 16 000 rev./min for 22 h at 20°C in an An-D rotor. Subunit molecular weights

were determined by sedimentation equilibrium ultracentrifugation in 6 M guanidine hydrochloride in Buffer A at 30 000 rev./min. Photographic plate negatives of interference fringes or Schlieren boundaries were measured with a Nikon Model 6C Microcomparator equipped with digital x-y encoders.

*Gel filtration.* Sephadex G-200 was swollen in 25 mM Tris · HCl, pH 8.0, 0.1 M NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol. The gel was packed into a 2.5 × 100 cm column at room temperature and washed with the same buffer at 4°C for 10 days at 6 ml/h. Void volume, internal volume, and elution volumes were determined with Blue Dextran, tyrosine, and standard proteins, respectively. The elution volumes were correlated with molecular weights [23] or Stoke's radii [24]. Fractions of 2 ml each were collected at a flow rate of 6.0 ml/h and monitored by their absorbance at 280 nm. The following proteins were used as standards: cytochrome *c*, α-chymotrypsin, human serum albumin, GAPD, rabbit muscle aldolase, fumarase, pyruvate kinase, and β-galactosidase.

*Amino acid analysis.* Amino acid analyses were performed with a Beckman 120 C automatic amino acid analyzer after acid hydrolysis [25,26]. An internal standard of norleucine was added and the samples were sealed in reactitherm tubes (Pierce Chem. Co.) and hydrolyzed in vacuo at 110°C for 24, 48, and 72 h in 2 ml of 6 M HCl. Tryptophan was determined spectrophotometrically by the method of Edelhoch [27] and cysteine was determined with DTNB after the method of Ellman [28].

## Results

*Batch process*

Fresh hemolysate was mixed with phosphocellulose (exchange capacity = 0.94 mequiv./g) that had been equilibrated with the lysing buffer. A ratio of 1 g semi-dry phosphocellulose cake per 10 ml hemolysate was usually employed although this value was altered occasionally depending upon variations in phosphocellulose exchange capacities. The phosphocellulose/hemolysate suspension was then gently stirred for 10 min at 4°C. The suspension was then vacuum filtered and the filtrate discarded. Essentially all of the aldolase present in the hemolysate was found to bind to phosphocellulose under these conditions.

A washing process was then performed in order to remove the hemoglobin. By using the same process of resuspension, stirring, and filtration described above, the phosphocellulose was washed two times with 25 mM Tris · HCl, pH 7.8, 10 mM 2-mercaptoethanol; two times with 50 mM Tris · HCl, pH 7.8, 10 mM 2-mercaptoethanol; and two times with 50 mM Tris · HCl, pH 8.4, 10 mM 2-mercaptoethanol, thus removing approx. 98% of the hemoglobin while allowing most of the aldolase to remain bound to the cellulose ion exchanger. Between each of the above buffer changes, the cellulose was washed once with a mixture of the two transition buffers. This gradual change in ionic strength and pH was found to prevent release of aldolase that was caused by sudden changes in buffer pH or ionic strength.

A substrate elution of the aldolase was then performed by suspension of the phosphocellulose in the final wash buffer containing 2 mM Fru-1,6-$P_2$. The filtrate, which contained virtually all of the aldolase that remained bound to

the phosphocellulose, was then dialyzed against saturated ammonium sulfate at pH 7.3. The buffering of the ammonium sulfate solution was necessary because filtrate-induced pH increases approaching pH 8.0 caused aldolase denaturation during this step.

The precipitated protein was collected by centrifugation, resuspended, and dialyzed in 25 mM Tris · HCl, pH 7.3, 1 mM EDTA, 10 mM 2-mercaptoethanol.

*Column chromatography*

The resuspended eluant from the batch process was then applied to a phosphocellulose column equilibrated with 25 mM Tris · HCl, pH 7.8, 10 mM 2-mercaptoethanol. A wash process similar to that used during the batch process was used to remove final hemoglobin traces. Prior to the elution step, the column was washed with one volume of 1 mM NADH in 50 mM Tris · HCl, pH 8.4, without 2-mercaptoethanol. This was effective in removing GAPD which was a persistant contaminant until this step was employed.

The aldolase was then eluted with one column volume of 50 mM Tris · HCl, pH 8.4, 10 mM 2-mercaptoethanol containing 2 mM Fru-1,6-$P_2$ (Fig. 1). A final wash with 0.5 M NaCl failed to remove additional aldolase indicating that aldolase elution was complete. The eluted aldolase was again precipitated by dialysis against saturated ammonium sulfate.

The final step involved a reverse ammonium sulfate fractionation [14] of 2% increments from 65% saturation to 45% saturation, with the bulk of the aldolase salting in at 49% saturation.

A typical purification procedure representing the washed erythrocytes from four units of whole blood is shown in Table I. With some sacrifice in yield, the process results in a 8000-fold purification and a specific activity of 16 I.U./mg protein. At this point the enzyme was stable for several months when stored as a crystalline suspension in ammonium sulfate at 4° C. Cell count, specific activ-

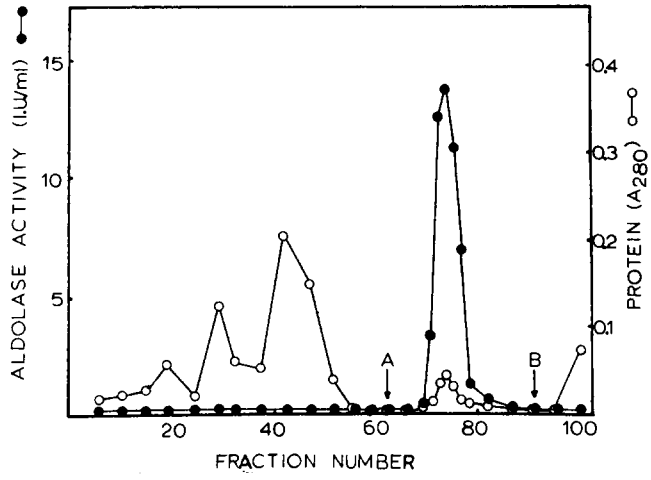


**Fig. 1. Elution of human erythrocyte aldolase from phosphocellulose. The enzyme was eluted with 2 mM Fru-1,6-$P_2$ in buffer. Arrow A indicates addition of Fru-1,6-$P_2$. Arrow B indicates addition of 0.5 M NaCl. Fractions (2 ml) were collected at a flow rate of 0.5 ml/min and assayed for aldolase activity (•) and absorbance at 280 nm (○).**

TABLE I

ISOLATION OF FRUCTOSE-BISPHOSPHATE ALDOLASE FROM HUMAN ERYTHROCYTES

| | Volume (ml) | Total units | Total protein (mg) | Units/ mg * | Yield (%) | Purification |
|---|---|---|---|---|---|---|
| Hemolysate | 2000 | 240 | 116 000 | 0.002 | (100) | (1) |
| Phosphocellulose batch | 350 | 115 | 245 | 0.42 | 48 | 210 |
| Phosphocellulose chromatography | 10 | 80 | 6 | 11.4 | 33 | 5700 |
| Reverse ammonium sulfate fractionation | 3 | 48 | 3 | 16.1 | 20 | 8000 |

* One unit of enzyme activity is that quantity of enzyme which will catalyze the reaction of 1 μmol of substrate per min at 30° C.

ity, and molecular weight parameters gave an average value of 35 000 aldolase molecules per red blood cell.

*Electrophoresis*

Thin-layer and polyacrylamide gel electrophoresis were carried out on both the hemolysate and the pure enzyme. The results of thin-layer electrophoresis of the hemolysate (Fig. 2A) and pure enzyme (Fig. 2B) demonstrated only one band when stained for enzyme activity. Polyacrylamide gel electrophoresis in SDS gave a single band when 25 μg of the pure enzyme was subjected to electrophoresis. The band was found to migrate identically with rabbit muscle aldolase when subjected to electrophoresis under identical conditions.

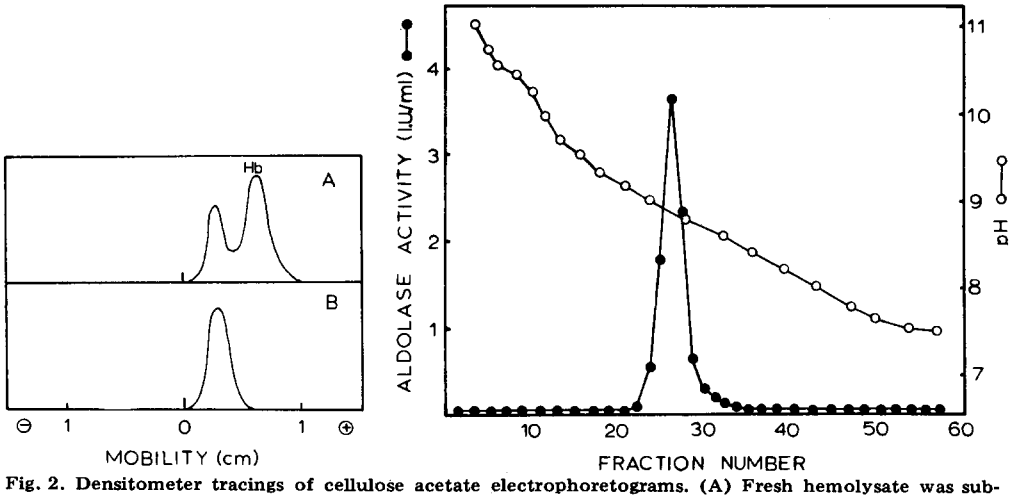


Fig. 2. Densitometer tracings of cellulose acetate electrophoretograms. (A) Fresh hemolysate was subjected to electrophoresis on cellulose acetate plates for 45 min at 1.5 mA/plate. Aldolase activity was visualized as described in Methods. (B) Purified erythrocyte aldolase was subjected to electrophoresis as described above.

Fig. 3. Isoelectric focusing of human erythrocyte aldolase. The conditions of the experiment are described under Methods.

TABLE II

COMPARISON OF CATALYTIC PROPERTIES OF ALDOLASES

| Enzyme | $K_m$ (Fru-1,6-$P_2$) (M) | $K_m$ (Fru-1-$P$) (M) | (Fru-1,6-$P_2$/Fru-1-$P$) *** |
|---|---|---|---|
| Human erythrocyte | $7.0 \cdot 10^{-6}$ | $3.0 \cdot 10^{-3}$ | 40 |
| Human heart * | $1.0 \cdot 10^{-5}$ | $2.8 \cdot 10^{-3}$ | 12 |
| Rabbit muscle ** | $3.0 \cdot 10^{-6}$ | $9.0 \cdot 10^{-3}$ | 50 |

* Data taken from Allen et al. [3].
** Data taken from Penhoet et al. [2].
*** Ratio of rates of cleavage ($V$).

*Electrofocusing*

Isoelectric focusing of hemolysate or purified enzyme showed one peak focusing at pH 8.9 (Fig. 3). SDS gels of the elution peak showed one major band and one diffuse low molecular weight minor band, the minor band coinciding with the migration of ampholines.

*Kinetics*

The catalytic properties of human erythrocyte aldolase are illustrated in Table II, along with comparisons of the human heart and rabbit muscle enzymes. The enzyme exhibits Michaelis-Menten kinetics at 30°C with $K_m$ values of $7.1 \cdot 10^{-6}$ M for Fru-1,6-$P_2$ and $3.0 \cdot 10^{-3}$ M for Fru-1-$P$. The $V$ values for the two substrates were 16.1 and 0.4 μmol/min per mg protein for Fru-1,6-$P_2$ and Fru-1-$P$ respectively. Therefore, the substrate specificity (Fru-1,6-$P_2$/Fru-1-$P$ ratio of $V$ values) of the enzyme was 40.

*Molecular weight*

Molecular weight determinations were made from sedimentation velocity and sedimentation equilibrium ultracentrifigation. The $s^0_{20,w}$ value of $7.78 \cdot 10^{-13}$ was determined by extrapolating to zero protein concentration. High-speed equilibrium ultracentrifugation was used to determine the homogeneity and molecular weight of the enzyme. A weight-average molecular weight 158 000 was calculated from the slope ln $dy/r^2$ of fringe displace-

TABLE III

COMPARISON OF PHYSICAL PROPERTIES OF ALDOLASES

| Property | Method | Human erythrocyte | Human heart * | Rabbit muscle ** |
|---|---|---|---|---|
| $s^0_{20,w} \times 10^{13}$ | Sedimentation velocity | 7.78 | 7.79 | 7.90 |
| Molecular weight | Sedimentation velocity | 158 000 | 158 000 | 160 000 |
| | Sedimentation equilibrium | 158 000 | 158 000 | 158 000 |
| Subunit molecular weight | Sedimentation equilibrium in 6 M guanidine · HCl | 40 000 | 40 000 | 40 000 |
| | SDS electrophoresis | 40 000 | 42 000 | 40 000 |

* Data taken from Allen et al. [3].
** Data taken from Kawahara and Tanford [39].

TABLE IV

COMPARISON OF AMINO ACID COMPOSITIONS

| Residues/40 000 | Human erythrocyte | Human heart * | Rabbit muscle ** |
|---|---|---|---|
| Lys | 27 | 25 | 25 |
| His | 10 | 8 | 11 |
| Arg | 15 | 17 | 15 |
| Asp | 27 | 30 | 25 |
| Thr | 21 | 22 | 22 |
| Ser | 18 | 23 | 20 |
| Glu | 48 | 48 | 40 |
| Pro | 17 | 20 | 19 |
| Gly | 32 | 32 | 30 |
| Ala | 43 | 37 | 41 |
| Val | 21 | 20 | 21 |
| Met | 3 | 3 | 3 |
| Ile | 18 | 15 | 19 |
| Leu | 36 | 34 | 34 |
| Tyr | 12 | 12 | 12 |
| Phe | 8 | 8 | 7 |
| Trp | 4 | 4 | 3 |
| Cys | 8 | 8 | 8 |

* Data taken from Allen et al. [3].

** Data taken from Lai et al. [38].

ment. This method was also used to determine the subunit molecular weight in 6 M guanidine hydrochloride and a value of 40 000 was obtained. Both slopes were linear and, therefore, indicate a monodisperse species. Gel filtration chromatography of the purified enzyme gave a molecular weight of 160 000 and a Stoke's radius of 4.56 nm. A diffusion coefficient ($D_{20,w}$) of $4.68 \cdot 10^{-7}$ $cm^2/s$ and a frictional ratio of 1.27 were also determined. A comparison of the physical properties properties of different aldolase is seen in Table III.

*Amino acid analysis*

The amino acid composition from human erythrocyte aldolase is shown in Table IV. Analyses from human heart and rabbit muscle are included for comparison. Possible differences in residue numbers can be detected between the aldolases; however, the overall compositions are remarkably similar.

## Discussion

The present study describes a procedure for obtaining pure human erythrocyte aldolase by phosphocellulose ion exchange chromatography and reverse ammonium sulfate fractionation. The homogeneous preparation, by criteria of disc gel electrophoresis in SDS, Sephadex gel filtration, and analytical ultracentrifugation, exhibits a specific activity of 16 I.U./mg protein.

The removal of hemoglobin constituted the major problem during the purification process. The use of other ion exchangers were attempted prior to the utilization of phosphocellulose. Both CM- and DEAE-cellulose which theoretically should have allowed separation of the negatively charged hemoglobin form and the positively charged aldolase form between pH ranges from 7 and 8.5, were unsuccessful. A variety of organic solvent fractionations were

also attempted and while hemoglobin precipitation was easily affected, particularly with a 50 : 50 mixture of cold chloroform/methanol added to an equal volume of hemolysate, low aldolase yields precluded the routine use of this procedure. Phosphocellulose was therefore chosen as the ideal binding medium with its superior aldolase retention properties during the washes to remove hemoglobin. Two additional variables which could occur during the batch procedure bear comments: The cellulose was never allowed to dry during the filtration process as drying tended to cause premature aldolase release; and care was taken during the handling of the cellulose to maintain constant temperature since the pH of Tris buffer varies dramatically with temperature.

Once the aldolase-containing batch eluant was applied to the phosphocellulose column, the enzyme bound very tightly and the remaining hemoglobin could be easily removed by continued washing. At this point GAPD was the most troublesome contaminant concomitant with substrate elution. An NADH wash in the buffer without 2-mercaptoethanol was found to be successful in eluting all the GAPD prior to the aldolase elution. The reverse fractionation process was successful only when the protein sample was greater than 70% pure; otherwise a wide overlap in protein solubility profiles occurred.

Results from thin-layer electrophoresis of the hemolysate show a single band when stained for enzyme activity. This agrees with the results of Weber et al. [29] that show a single activity band for rabbit erythrocytes. Thin-layer electrophoresis of the pure enzyme also shows a single band when stained for either protein or enzyme activity, again suggesting a single form of the native enzyme.

Isoelectric focusing, another excellent method for detecting isozymal enzyme forms, suggests a single native form of aldolase in the human erythrocyte. When a sample of hemolysate was focused in a pH gradient from 3.5 to 10, a single activity profile was found. When the pure enzyme was focused within a pH gradient from 7 to 9, a single activity peak was again found at pH 8.9; this compares closely to the pH of 8.5 reported for rabbit muscle aldolase by Susor et al. [30]. Notwithstanding the report by Weber et al. [29] that aldolase inhibition by antiserum antialdolase C in rabbit erythrocytes illustrates the presence of the type C isozyme, the present results suggest the presence of only a single form in the human red cell.

We conclude that the predominant if not exclusive form of aldolase found in human erythrocytes is type A, found also in vertebrate muscle [1] and heart tissue [3]. This is not entirely unexpected since the embryonic origin of blood, the mesoderm, is the same for muscle and heart. Interestingly, the liver (type B aldolase) and brain (type C aldolase) arise from yet other germ layers, the endoderm and ectoderm, respectively [31].

An important variable in determining the amount of aldolase present in the hemolysate is the ionic strength of the lysing buffer. The concentration of 25 mM Tris was chosen because aldolase retention by the ghosts was negligible. As the ionic strength is decreased, particularly with a Tris concentration between 5 and 10 mM, aldolase retention by the ghosts approaches 20% of the total cell aldolase content. This is why the assessment of aldolase content in red cells from individuals possessing an erythrocyte disorder must be performed with some caution. For example, reports for ref. 32 and against ref. 33 aldolase

deficiency in red cells from individuals having hereditary spherocytosis could easily be based upon differences in aldolase release from membrane stroma, especially since the two afore-mentioned reports used different methods for erythrocyte and hemolysate preparation. Our quantitation of aldolase per red cell and a specific activity value for the enzyme should help avoid difficulties in determining aldolase content, as aldolase could simply be reported as some percent of the total cell quantity thus avoiding volume and hemoglobin concentration variables.

The association of human erythrocyte aldolase with ghosts has been reported [34—37] but the nature of this binding has not been characterized. The binding studies that have been reported [7,8] have used only the rabbit muscle enzyme. When the general physical and kinetic properties of the human erythrocyte enzyme are compared with the rabbit muscle enzyme, they appear similar enough to use the rabbit muscle form for binding determinations. However, results from the amino acid analysis suggest that at least the glutamic acid/glutamine content may vary between the two enzymes and could, for example, account for the difference in isoelectric points between the rabbit muscle and red cell enzyme. If these amino acids are located in critical binding domains on the aldolase molecule, the use of the rabbit muscle enzyme for studies with human erythrocyte ghosts may not represent a natural binding phenomenon. While binding studies using the rabbit muscle enzyme represent an important model system for observing enzyme-membrane interactions and possibly the effect of this interaction on conformation and catalytic activity of the enzyme the possible physiological role of this specificity of association in vivo remains conjectural.

Therefore, in our current studies on the physiological behavior of glycolytic enzymes, human erythrocyte aldolase is being used for studies with ghosts because sufficient quantities can now be obtained.

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