Red Cell Proteins. I. Two-Dimensional Mapping of Human Erythrocyte Lysate Proteins

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Human erythrocyte lysate proteins were resolved into over 250 discrete spots by two-dimensional electrophoresis using isoelectric focusing in the first dimension and electrophoresis in the presence of sodium dodecyl sulfate (SDS) in the second. The overwhelming excess of hemoglobin has made such analyses difficult in the past. However, with the ISODALT two-dimensional electrophoresis system, large numbers of red cell proteins can be mapped in the presence of hemoglobin. When hemoglobin and several other major proteins are removed by adsorption to DEAE-cellulose, additional minor components are seen, giving a total of over 275. With the use of purified preparations, the map positions of five red cell enzymes or their subunits were determined: pyruvate kinase, catalase, glucose-6-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase, and carbonic anhydrase. The mapping techniques described complement and extend those traditionally used to find human red cell protein variants.

NUMEROUS electrophoretic or kinetic variants of human nonhemoglobin red cell proteins have been described. The clinical interest in these variants stems from the association of specific variants with disease, and the theoretical interest arises from suggestions that the number of variants of a protein may vary markedly with function and that intracellular proteins may exhibit many fewer variants in general than extracellular ones. For both clinical and theoretical studies it is desirable to be able to examine large numbers of samples. Unfortunately, work in this area has been limited by the analytical techniques available.

In kinetic studies, a separate measurement or series of measurements is required for each enzyme, limiting the number of analyses that can conveniently be made. When one-dimensional electrophoresis is employed, histochemical tests for enzyme activity are used for locating enzyme positions because all of the proteins present are not resolved and are therefore not identifiable solely by position in fixed and stained electropherograms. Neither kinetic enzyme assay for kinetic variants nor one-dimensional electrophoresis is applicable to the problems of searching for variants of large numbers of different proteins, including those whose functions are unknown, in one analysis by one technique.

The two highest-resolution analytical methods presently available for proteins or protein subunits are isoelectric focusing and electrophoresis in the presence of sodium dodecyl sulfate (SDS), both in acrylamide gels. The theoretical limits of

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resolution of these two methods have not been fully explored; however, in practice they appear to lie at present between 100 and 200 entities for each and therefore are not capable of yielding separately sufficient resolution to allow a large number of proteins to be identified by position rather than by activity. Isoelectric focusing and SDS electrophoresis depend on two different and unrelated parameters: isoelectric point and molecular weight. The former is the most interesting from a genetic viewpoint, since approximately one-third of all single amino acid substitutions alter the charge of a protein sufficiently to be easily detected.[1] Note that positional variants are of interest even when their functions are unknown. For example, their linkages to known variants may be determined experimentally, and hence their chromosomal gene locations may be found.

If the techniques of isoelectric focusing and SDS electrophoresis are used two-dimensionally, theoretically the resolution should be the product of the individual resolutions of the two methods, i.e., between 10,000 and 40,000 proteins or protein subunits. A practical limit of 1000 subunits can be seen by Coomassie blue staining. This form of two-dimensional analysis was first introduced by Stegemann,[2] was perfected as a high-resolution technique by O'Farrell,[3] and was extensively modified by Anderson and Anderson[4,5] to allow large numbers of analyses to be done in parallel (so-called ISO-DALT system). Computerized data reduction systems have been and continue to be developed in this laboratory.

In this series of reports we explore the use of the ISO-DALT two-dimensional analytical system for analysis of red cell proteins. The initial problem to be solved concerns the large amount of hemoglobin present (over 95% of the total protein mass) and whether or not the minor proteins known to be present can be separated and seen in the presence of such an overwhelming amount of one protein. Additional problems considered are the reproducibility of patterns and the identification of spots associated with the major known enzymes. Subsequent reports will be concerned with specific variants, with additional enzyme identifications, and with applicable aspects of computerized data analysis.

Among the alterations in red cell enzymes previously described are deficiencies in each of seven enzymes of the Embden-Meyerhoff pathway, five enzyme deficiencies in the hexose monophosphate shunt, four nonglycolytic deficiencies, and over 80 variants of glucose-6-phosphate dehydrogenase.[6]

Whereas hemoglobin has been removed from red cell lysates by the use of chloroform-methanol extraction[7] or by selective use of ion exchangers,[8] proteins other than hemoglobin are denatured or removed by these methods. It is therefore important to develop, initially, methods for resolving as many proteins as possible without hemoglobin removal and then gain greater sensitivity for minor components by hemoglobin removal, realizing that some nonhemoglobin proteins may be lost in the process.

In this study we find over 275 spots on two-dimensional maps of red cell lysates, suggesting that the majority of red cell proteins remain to be described. The mapping of red cell soluble proteins provides a basis for understanding the structural and functional relationships of the lysate molecules and is essential for the detection of possible molecular aberrations associated with some dysfunctional conditions. A preliminary report of this work has appeared.[9]

MATERIALS AND METHODS

Fresh blood samples from healthy donors were drawn into evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) at 1.4 mg/ml as anticoagulant. Special biochemicals including reaction intermediates and related compounds were obtained from Sigma Chemical Co. 2-(*N*-morpholino)ethane sulfonic acid (MES), morpholinopropane sulfonic acid (MOPS), and cyclohexylaminoethane sulfonic acid (CHES) buffers were from Calbiochem. Agarose-NADP was from P-L Biochemicals. CNBr-activated Sepharose 4B and Sephadex G-150 were from Pharmacia, and DNAse 1 was from Worthington. CM-cellulose (CM-52) and DEAE-cellulose (DE-52) were the microgranular form and were obtained from Whatman. Chromatographic columns were constructed from disposable 20-ml syringes fitted with a filter paper bed support. Ampholines were purchased from LKB. All column procedures were monitored with an ISCO model UA-5 absorbance monitor.

Preparation of Lysate

Packed erythrocytes were washed five times with equal volumes of phosphate-buffered saline, pH 7.4. Aspiration of the buffy coat and supernatant removed 70%–90% of contaminating leukocytes and platelets and most reticulocytes. The cells were lysed by adding two volumes of glass-distilled water containing 2.5-m*M* EDTA and stirring gently for 1 hr at 4°C. Stroma and any unlysed cells were removed by centrifugation at 50,000 *g* for 20 min. Samples of lysate not to be used immediately were stored at −80°C. No changes in the protein electrophoretic patterns have thus far been observed as a result of storage.

Hemoglobin-free samples were prepared as described by Yoshida[10] by applying a lysate sample to a DEAE-cellulose column (2.0 × 7.0 cm) previously equilibrated with 5-m*M* sodium phosphate buffer, pH 6.2. The hemoglobin was eluted with two column volumes of equilibration buffer. The remaining nonhemoglobin proteins were stripped from the column with two volumes of 200-m*M* sodium phosphate buffer, pH 5.8, containing 250-m*M* sodium chloride. The samples were dialyzed overnight against phosphate-buffered saline, pH 7.4, at 4°C and subsequently concentrated by ultrafiltration (Amicon, Diaflow membrane PM 10) to the original volume.

Enzyme Purification and Analyses

To map the locations of enzymes or their subunits, each enzyme was purified from red cell lysates (*vide infra*). Enzyme activities (with the exception of catalase and hypoxanthine phosphoribosyltransferase) were measured with the aid of a computerized GeMSAEC centrifugal fast analyzer (Electro Nucleonics). Summaries of the purification procedures and measurement of activities for each enzyme follow:

Carbonic anhydrase (E.C. 4.2.1.1). Carbonic anhydrase was purified by DEAE-cellulose chromatography as described by Tanis et al.[11] following the removal of hemoglobin by chloroform-methanol extraction. Measurements of carbonic anhydrase reaction rates were calculated using a modification of the *p*-nitrophenol procedure of Tashian[12] with acetazolamide as a specific inhibitor of carbonic anhydrase activity.

Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49). Following the removal of hemoglobin by DEAE-cellulose chromatography, the affinity absorption-elution method of Yoshida[13] was used to bind the enzyme to an agarose-NADP column. Elution of the enzyme was effected with 1-m*M* NADP. Enzyme activity was calculated from the rate of NADPH formation observed at 340 nm by the procedure of Lohr and Waller.[14]

Pyruvate kinase (E.C. 2.7.1.40). Pyruvate kinase was isolated from a hemoglobin-free lysate by ammonium sulfate precipitation and CM-cellulose and DEAE-cellulose chromatography, as described by Chern et al.[8] Pyruvate kinase activity was measured by a coupled reaction with lactate dehydrogenase in which the rate of oxidation of NADH was measured at 340 nm by the procedure of Beeson and Black.[15]

Lactate dehydrogenase (E.C. 1.1.1.27). Samples of hemoglobin-free lysate were applied to a DEAE-cellulose column equilibrated with 75-m*M* sodium phosphate buffer, pH 6.4. The column was washed with equilibration buffer, and then lactate dehydrogenase was eluted with 150-m*M* sodium phosphate buffer, pH 6.8. The sample was concentrated by ultrafiltration and applied to a CM-cellulose column equilibrated with MES/KCl buffer, pH 6.5. Elution of the enzyme was accomplished by the

affinity elution procedure of Scopes[16] using MOPS/KOH buffer at pH 7.2. Lactate dehydrogenase activity was calculated from the rate of reduction of NAD, based on the method of Gay et al.[17]

Catalase (E.C. 1.11.1.6). Catalase was prepared from whole lysate by successive chromatographic procedures on DEAE-cellulose, CM-cellulose, and Sephadex G-150 according to the procedure of Aebi et al.[18] Samples containing catalase activity were rechromatographed on the CM-cellulose column. Catalase activity was assayed spectrophotometrically with perborate as substrate according to the procedure of Thomson et al.[19]

Hypoxanthine phosphoribosyltransferase (E.C. 2.4.2.8). The purification of this enzyme was carried out essentially according to the procedures of Olsen and Milman,[20] with the exception that hemoglobin-free lysate was used as the starting material. The remaining heat treatments, chromatographic procedures, and ammonium sulfate precipitations were exactly as described by those authors. The enzyme activity was determined by the hypoxanthine:phosphoribosylpyrophosphate reaction as described by Flaks.[22] The residual hypoxanthine in the reaction mixture was measured spectrophotometrically by a xanthine oxidase reaction according to the procedure of Jorgensen.[22]

Following the purification procedure for each enzyme, the sample was dialyzed overnight against distilled water, then lyophilized to dryness. Each sample was then resuspended in phosphate-buffered saline, pH 7.4, to a final concentration of 1 mg/ml.

Erythrocyte actin was also purified from whole lysate preparations to serve as a marker in the two-dimensional map. Actin was isolated by affinity chromatography according to the following procedure. One milliliter of CNBr-activated Sepharose 4B was coupled with 10 mg of DNAse 1 dissolved in 5 ml of 0.1-*M* $NaHCO_3$. Approximately 50 μl of the Sepharose were placed into an Eppendorf pipette tip fitted with a cotton plug. This microcolumn was placed into a glass culture tube 10 × 75 mm, and the apparatus was centrifuged briefly. The column was washed three times with phosphate-buffered saline, pH 7.4, by overlayering the Sepharose bed with 1-ml volumes of buffer and centrifuging to dryness. Whole lysate was then applied to the column in 1-ml aliquots and forced through the column. The bound actin was removed from the column by centrifugation of 40 μl of CHES/SDS buffer (*vide infra*) through the column. The eluate containing actin was collected in a clean culture tube and used directly as a sample for electrophoretic analysis.

Preparation of Samples for Two-Dimensional Analysis

Prior to electrofocusing, 250 μl of lysate or hemoglobin-free lysate were mixed with an equal volume of buffer containing 50-m*M* CHES/2% SDS/5% dithiothreitol (DTT)/10% glycerol, pH 9.2, in a 2-ml microfuge tube. The sample was heated for 5 min at 95°C, then rapidly cooled. Samples of purified lysate enzymes were treated identically, except that 20 μl of both sample and buffer were mixed.

Isoelectric Focusing

Two-dimensional electrophoretic separation of dissociated proteins as described by Anderson and Anderson[4] was used for mapping the samples. Some information relating to the electrophoretic technique is restated. Isoelectric focusing (first dimension) was performed on 20 samples simultaneously using the ISO apparatus. The gels contained 4% polyacrylamide with bis-acrylamide (5.6%) as cross-linker, 9-*M* urea, 2% NP-40, and 5% ampholines in the pH range of 3.5–10. The samples (20–30 μl of lysate:buffer solution or 10–15 μl of purified enzyme:buffer solution) were electrophoresed for 15 hr at 600 V, then at 700 V for 1 hr.

SDS Gel Electrophoresis

Electrophoresis on slab gels (second dimension) in the presence of SDS was performed with the DALT apparatus, which permitted 10 gels to be run simultaneously. The second-dimension gels were 10%–20% gradients of polyacrylamide with 2.7% bis-acrylamide as cross-linker. Electrophoresis was carried out at 120 mA constant current per gel. The proteins were visualized by overnight staining in 50% methanol/12% acetic acid/0.2% Coomassie brilliant blue R250 and subsequent destaining with several changes of a mixture of methanol:acetic acid ranging from 40%:6% initially to 5%:7% when destaining was complete. The protein patterns were photographed on Kodak Contrast Process Pan film using a 5500-Å narrow-band filter (Baird-Atomic) and were routinely developed in D-11 developer using nitrogen burst agitation.

Table 1. Human Erythrocyte Enzyme Activities of Whole Lysate and Lysate After Removal of Hemoglobin by DEAE-Cellulose Chromatography

Enzyme*	Lysate (1:2)	Lysate† (Hb-free)	Yield (%)
Catalase	Slope = −8.0	Slope = −3.4	42.5
MDH	13,000 μmoles/min/liter	3,000 μmoles/min/liter	23.1
LDH	6,400 μmoles/min/liter	2,200 μmoles/min/liter	34.4
GOT	400 μmoles/min/liter	90 μmoles/min/liter	22.5
TIM	60,000 μmoles/min/liter	16,250 μmoles/min/liter	27.1
PK	2,350 μmoles/min/liter	1,306 μmoles/min/liter	55.5
G-6-P DH	750 μmoles/min/liter	548 μmoles/min/liter	73.0
PGI	4,800 μmoles/min/liter	0 μmoles/min/liter	0
CA	1,380 μmoles/min/liter	0 μmoles/min/liter	0
6-PG-DH	750 μmoles/min/liter	0 μmoles/min/liter	0
Aldolase	395 μmoles/min/liter	0 μmoles/min/liter	0
AK	4,150 μmoles/min/liter	38 μmoles/min/liter	0.9
GPT	125 μmoles/min/liter	19 μmoles/min/liter	15.2
HPRT	357.3 μmoles/min/liter	357.3 μmoles/min/liter	100.0

*Abbreviations: MDH = malate dehydrogenase; LDH = lactate dehydrogenase; GOT = glutamate-oxaloacetate transaminase; TIM = triosephosphate isomerase; PK = pyruvate kinase; G-6-P DH = glucose-6-phosphate dehydrogenase; PGI = phosphoglucose isomerase; CA = carbonic anhydrase; 6-PG-DH = 6-phosphogluconate dehydrogenase; AK = adenylate kinase; GPT = glutamate-pyruvate transaminase; HPRT = hypoxanthine phosphoribosyltransferase.

†Samples of hemoglobin-free lysate concentrated to the original volume.

Identification of Enzymes in Lysate Maps

Spots in the lysate maps that correspond to purified proteins were determined by co-electrophoresis of purified samples with lysate and by the use of an optical comparator that enabled us to superimpose gels optically and determine the localization of the purified proteins.

RESULTS

Enzyme Analysis of Whole Lysate and Hemoglobin-Free Lysate

The activities of 14 erythrocyte enzymes were determined on pooled fresh lysate and lysate from which the hemoglobin was removed by DEAE-cellulose chromatography using a centrifugal fast analyzer.[23] These activities are presented in Table 1. Activity values are expressed as micromoles per minute per liter (international units) for a 1:2 dilution of lysate or hemoglobin-free lysate concentrated to the original volume. Of the 14 enzymes tested, acceptable recovery was possible for 9 enzymes, but the chromatographic procedures resulted in total loss of activity for 5 other enzymes.

Table 2. Summary of Purifications of Six Human Erythrocyte Enzymes

Enzyme	Lysate* (1:2)	Final Product	Overall Purification
Catalase	Slope/mg = 0.053	Slope/mg = 2.72	51.3
Carbonic anhydrase	0.009 μmoles/min/mg	1.75 μmoles/min/mg	194.4
G-6-P DH	0.005 μmoles/min/mg	39.66 μmoles/min/mg	7932.0
HPRT	0.0024 μmoles/min/mg	23.33 μmoles/min/mg	9720.8
LDH	0.043 μmoles/min/mg	15.69 μmoles/min/mg	364.9
Pyruvate kinase	0.016 μmoles/min/mg	79.38 μmoles/min/mg	4961.3

*Total protein of lysate based on a hemoglobin concentration of 300 mg/ml of packed cells.

Purification of Six Enzymes From Erythrocyte Lysate

In order to determine the locations of some enzyme components of the lysate, six enzymes were purified by conventional procedures, and their activities are listed in Table 2. Most of the enzymes were recovered with high activities relative to the starting sample. In each case the increase achieved by purification was sufficient to identify the enzyme in a two-dimensional electrophoretic pattern.

Two-Dimensional Separation of Lysate Proteins

A typical two-dimensional map of lysate proteins and protein subunits is shown in Fig. 1. A total of 250 spots representing either monomeric proteins or protein

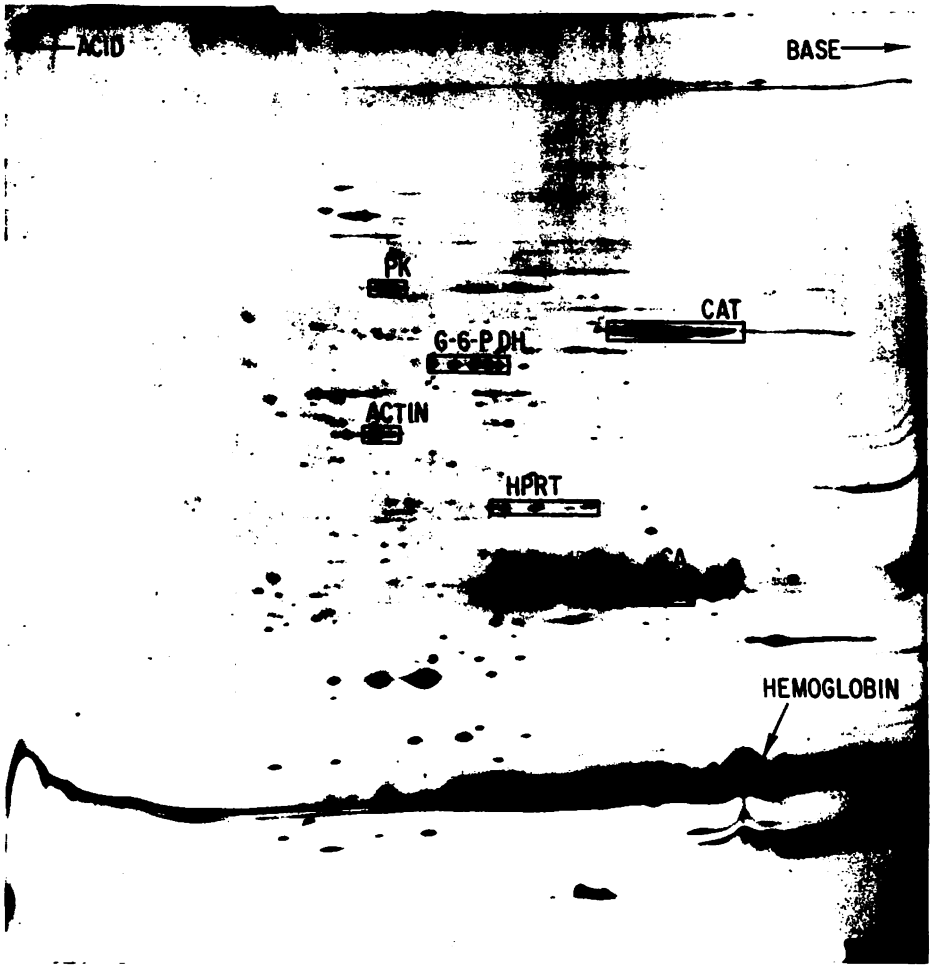


Fig. 1. Two-dimensional separation of human erythrocyte lysate proteins. The sample was 12.5 μl of fresh lysate denatured in 12.5 μl of buffer containing 2% SDS/5% DTT. Isoelectric focusing was from right to left and molecular weight separation from top to bottom. The map locations of known erythrocyte enzymes are labeled. CAT = catalase; PK = pyruvate kinase; G-6-P DH = glucose-6-phosphate dehydrogenase; HPRT = hypoxanthine phosphoribosyltransferase; CA = carbonic anhydrase. Hemoglobin and actin are also labeled. Molecular weight values were determined by SDS electrophoresis of known proteins.

subunits ranging in molecular weight from 10,000 to 100,000 d can be visualized following electrophoresis of 25 μl of the lysate:buffer preparation containing approximately 1.9 mg of total protein. Thus, even in the presence of hemoglobin, other erythrocyte lysate proteins can be readily resolved by this procedure.

The localization of enzymes or enzyme subunits identified by electrophoresis of purified samples is also shown. Of the enzymes identified, only lactate dehydrogenase is of such small quantity as to make visualization difficult. Several of the proteins appear as rows of small spots along the horizontal axis, indicating charge heterogeneity, which may be due to deamidation or the addition of charged groups such as sialic acid residues. Over 900 two-dimensional gels of lysates have been run to date. Based on this experience and the highly reproducible patterns obtained, we conclude that the charge heterogeneity is characteristic of the proteins and is not due to artifacts introduced by sample preparation or electrophoretic procedures.

The diagrammatic representation of a typical pattern of spots is shown in Fig. 2. The diagram gives a better notion of the number and distribution of spots that are reproducibly present in a map of whole lysate. Figure 3 shows a two-dimensional map of lysate proteins following the removal of hemoglobin by DEAE-cellulose chromatography. All enzymes identified to date, with the exception of carbonic

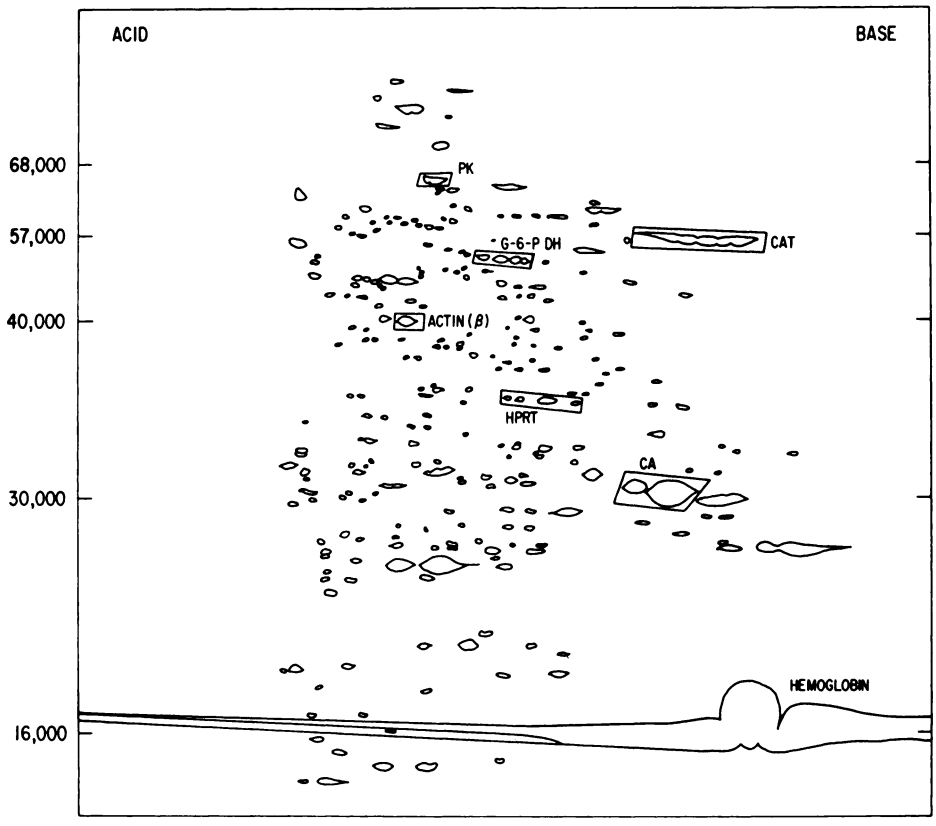
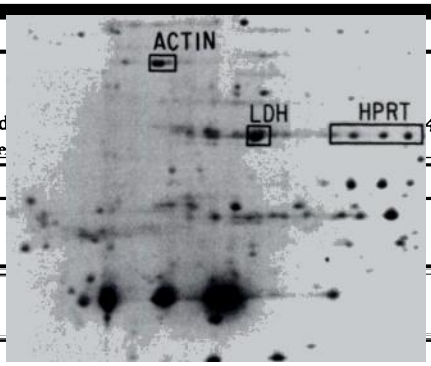


Fig. 2. Diagrammatic representation of protein spots drawn from the gel in Fig. 1. Erythrocyte actin and hemoglobin are labeled along with identified enzymes as in Fig. 1.



cytic leukemia lines), and

Analysis of Leukemias

K-562 and Reh[22] were te

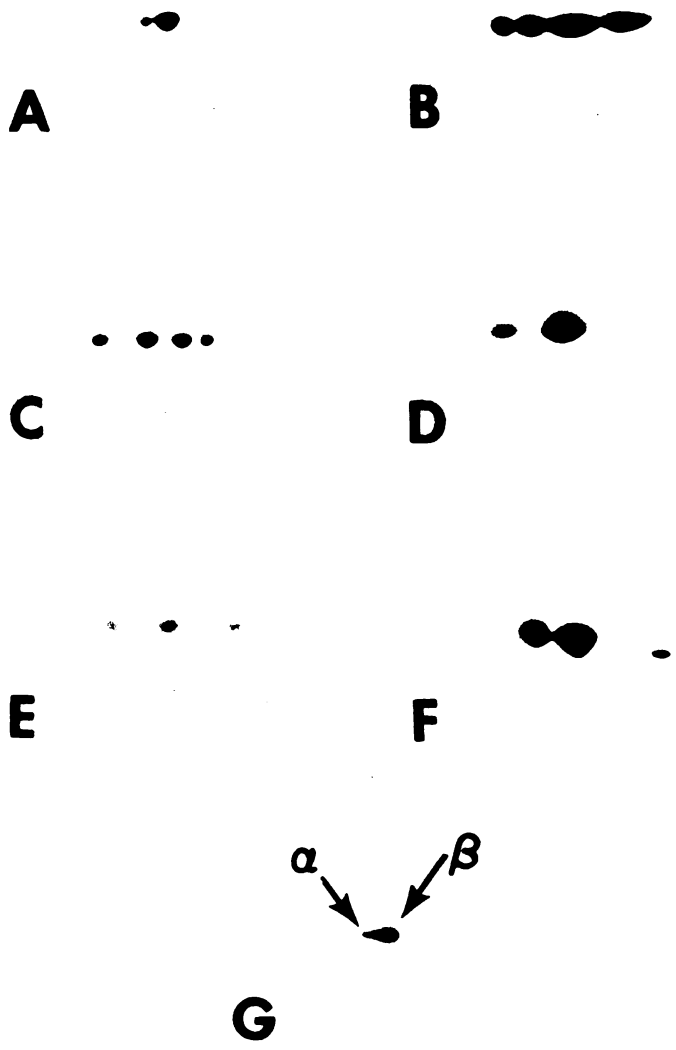


Fig. 4. Sections from two-dimensional gels showing the protein subunit patterns of seven purified erythrocyte lysate proteins. A: pyruvate kinase; B: catalase; C: glucose-6-phosphate dehydrogenase; D: lactate dehydrogenase; E: hypoxanthine phosphoribosyltransferase; F: carbonic anhydrase; G: actin.

(over 100 per day), it is now feasible to search systematically for new human red blood cell protein variants.

Although individual proteins purified from lysate samples have been successfully characterized, attempts to characterize the non-hemoglobin-soluble proteins of the erythrocyte have been complicated by the vast amount of hemoglobin present. However, the techniques for resolving all or a large fraction of the proteins are now available. The high-resolution protein mapping technique employing two-dimensional electrophoresis makes this resolution feasible.

The removal of hemoglobin from lysate preparations followed by two-dimensional electrophoresis results in the unmasking of a few new protein spots not seen in the whole lysate. However, the techniques for removing hemoglobin also remove some other proteins in varying amounts. In order to obtain hemoglobin-free lysates for two-dimensional analysis, carefully controlled procedures must be used to prepare samples that are identical in protein composition.

True isozymes have been demonstrated for a number of enzymes, and multiple electrophoretic forms of some enzymes have been demonstrated in the human erythrocyte.[18,24,25] The existence of multiple electrophoretic forms of subunits for a number of enzymes is also apparent from inspection of the electrophoretic patterns shown here. The origin of these multiple forms remains to be discovered. Isozymes may be due to multiple genetic loci, multiple alleles at a single locus, or secondary isozymes that arise by posttranslational modification.[27]

Crude lysates or partially purified glucose-6-phosphate dehydrogenase revealed a pattern of 7–11 bands of activity following isoelectric focusing.[25] The results of the study reported here show that the enzyme is composed of at least four subunits demonstrating charge heterogeneity. All of the subunits had similar molecular weights of 56,000 d. The charge heterogeneity of undenatured protein is therefore greater than that of the subunits, as expected. No differences were observed in the pattern of the enzyme purified by affinity chromatography, as compared with that observed in the whole-lysate preparation.

Our hypoxanthine phosphoribosyltransferase analysis shows four electrophoretically distinct subunits present in the whole lysate. The purified enzyme gave an identical pattern. It has been reported that in the mature erythrocyte in humans there are at least seven isozymes that arise by posttranslational modification of the enzyme and are functions of in vivo aging.[26,27] In our study the subunit molecular weight was approximately 34,000 d, the same value reported by Arnold and Kelley,[24] but significantly higher than the value of 26,000 d reported by Olsen and Milman.[20]

The pattern exhibited by catalase subunits following two-dimensional separation of whole lysate frequently shows some horizontal streaking, possibly due to effects of overloading or to co-focusing of the enzyme with hemoglobin. However, when purified samples of catalase are electrophoresed, when lysate loading is decreased, or when hemoglobin-free lysate is electrophoresed, a number of subunits can be distinguished. Aebi et al.[18] have shown that erythrocyte catalase can be separated into three fractions of equal specific activities, but they differ in electrophoretic mobility because of oxidation of sulfhydryl groups. The results reported here show that following denaturation and reduction a degree of electrophoretic heterogeneity remains, resulting in the separation of five distinct subunits of molecular weight 57,000 d.

Erythrocyte pyruvate kinase also shows some degree of charge heterogeneity of its subunits following two-dimensional separation. This enzyme is considered by many to be similar to the L-type isozyme of hepatocytes. Erythrocyte pyruvate kinase may be a hybrid between L-type (liver) and M-type (muscle) subunits,[28,29] or the erythrocyte isozyme may be a heterotetramer designated L_2L_2', with the molecular weight of the L_2' subunits being slightly greater than that of the L_2 subunits.[30] Alternatively, interconversion of isozymes may occur during isolation.[31] Although these conflicting ideas are not reconciled here, our results with whole

lysate show two electrophoretic subunit types, with the more basic form being in excess, as determined by staining intensity. No differences were found in the subunit molecular weights, as both types migrated in SDS as 66,000-d proteins.

Analysis of the pattern of pyruvate kinase in hemoglobin-free lysate shows the appearance of a third distinct and more acidic spot in relatively low concentration. The appearance of this new spot may be due to modification of the enzyme during the procedures used to remove hemoglobin.

Lactate dehydrogenase appears as only a minor spot when whole lysate is electrophoresed, and exact localization in the map is difficult to determine. However, in hemoglobin-free lysates the enzyme subunits are easily seen, and they have a molecular weight of approximately 34,000 d. Only one form of the enzyme has been identified in the two-dimensional maps. The LDH-1 form is the major isozyme of human red cells. Purification of the enzyme yielded two electrophoretically distinct bands, possibly representing the LDH-1 form and the minor LDH-2 isozyme.

The resolving power and the applicability of two-dimensional electrophoresis to the study of isozymes are demonstrated by the separation achieved with erythrocyte carbonic anhydrase. This enzyme has been shown to exist in two isozymic forms designated CA I and CA II.[12] Isozymes of this monomeric enzyme appear in the map of whole lysate at molecular weight positions of approximately 29,000 d for the CA I form and 30,000 d for the CA II form, with the CA I form being slightly more basic. Removal of hemoglobin from lysate by DEAE-cellulose chromatography removes all carbonic anhydrase isozymes.

The use of high-resolution mapping techniques provides a method for the analysis of a large number of erythrocyte protein gene products. Work is now under way in this laboratory to use the multiple-sample capabilities of the ISO-DALT system to identify more enzyme subunits and to analyze known mutant proteins.

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