Polypeptide Chains from Human Red Blood Cell Membranes*

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SUMMARY

Human red blood cell membranes contain at least 17 polypeptides of differing molecular weight ranging from 27,000 to 220,000. Previous publications have reported higher values for the largest molecular weight species, but are in error owing to incorrect calibration of sodium dodecyl-sulfate-polyacrylamide gels with unreduced proteins. The two highest molecular weight species which constitute approximately 40% of the total membrane protein have molecular weights of 200,000 and 220,000. It is shown that these polypeptides are not aggregates of a lower molecular weight species, and analysis of the entire membrane by gel filtration in 6 M guanidine hydrochloride reveals no significant amount of protein with molecular weight less than that of hemoglobin. Solubilization of a fraction of the membrane protein in EDTA according to the method of Marchesi et al. (Marchesi, S. L., Steers, E., Marchesi, V. T., and Tillack, T. W., Biochemistry, 9, 50 (1970)) produced a heterogeneous mixture of polypeptide chains. Prolonged exposure to this solvent leads to dissolution of over 90% of the membrane protein.

A number of studies of the heterogeneity and molecular weights of proteins from red blood cell membranes have appeared in the recent literature. Some of these investigations appear to be in conflict—others in agreement.

Berg (1) and Lenard (2) dissolved human erythrocyte membranes in SDS and determined the molecular weights of the constituent polypeptide chains by SDS-polyacrylamide gel electrophoresis (3, 4). Both of these authors show that a large fraction of the membrane polypeptides is in two molecular weight classes—100,000 and 200,000. Their absolute molecular weight values do not agree exactly and are both in error because of incorrect calibration of their gels with unreduced proteins (5). Gwynne and Tanford (6) dissolved human red blood cell ghosts in 6 M GuHCl, reduced and alkylated the disulfide bonds, and determined the molecular weights by gel filtration in GuHCl.

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; GuHCl, guanidine hydrochloride.
The correct molecular weights of the polypeptide chains based on calibration of SDS-polyacrylamide gels with reduced proteins. We find no evidence for any significant amount of 6000 molecular weight material and our results indicate that the highest molecular weight species, which is present as approximately 40% of the total protein, cannot be an aggregate.

In addition, we have subjected the membranes to the extraction procedure developed by Marchesi et al. (13) using EDTA and $\beta$-mercaptoethanol. These authors solubilized approximately 30% of the total membrane protein and have called this soluble fraction "spectrin." We show here that spectrin is actually a heterogeneous mixture of several polypeptide chains, and, in fact, prolonged exposure to this solvent system results in solubilization of over 90% of the protein from human red blood cell membranes.

EXPERIMENTAL PROCEDURE

Materials—Sodium dodecyl sulfate was obtained from Mann. Guanidine hydrochloride was purchased from Heico and used without further purification. Human red blood cell ghosts were prepared from freshly drawn blood by the method of Dodge, Mitchell, and Hanahan (14).

Methods—The preparation and use of gel filtration columns in 6 M GuHCl have been described previously (7). SDS acrylamide gel electrophoresis was carried out by using the method of Weber.
and Osborn (4). Calibration curves were obtained by using protein polypeptide chains of known molecular weights with disulfide bonds reduced. A typical calibration curve is shown in Fig. 1. Red blood cell ghosts were dissolved in either 6 M GuHCl or SDS, and the disulfides were reduced and alkylated prior to analysis.

RESULTS

Molecular Weights of Polypeptide Chains in Human Red Blood Cell Membranes—The polypeptide chain molecular weights of proteins from human red blood cell membranes were determined by SDS-polyacrylamide gel electrophoresis and are shown in Table I. A variety of donors were used and only minor differences were observed. For comparison, the molecular weights assigned by Lenard (2) to these species are also given. The discrepancies which are most pronounced in the high molecular weight region result from calibration of the gels with unreduced proteins by the latter author. Fig. 2 shows a spectrophotometric scan of a gel from which our molecular weights were obtained. The over-all appearance of this scan is identical with that published by Berg (1), but again the latter author's molecular weights are in error. We agree with both Lenard and Berg that over 65% of the total membrane protein is in two molecular weight classes—one around 200,000 and the other around 100,000.

The broad band observed in Fig. 2 with a peak centered near 100,000 varies slightly with different donors (see Table I). This band contains at least one glycoprotein (2). Since, as we have already pointed out, polypeptide chains with long branches may not behave hydrodynamically in either SDS or GuHCl in the same manner as linear polypeptide chains, this variation in peak position may be related to different carbohydrate contents. The 100,000 value assigned to the molecular weight should then be considered as an apparent molecular weight, and the polypeptide molecular weight of the glycoprotein may actually be quite different.

Human red blood cell membranes were also dissolved in 6 M GuHCl, the disulfide bonds were reduced and alkylated, and in one experiment the lipid layer was removed by centrifugation prior to application to a gel filtration column (6). In a second experiment the entire solution was placed on the gel filtration column. The elution diagram obtained in the first case was in essential agreement with that previously published by Gwynne and Tanford in which a large fraction of the material was eluted in a broad band with a peak at 200,000 molecular weight. Sedimentation equilibrium of the peak tube also gave a molecular weight of 200,000. The results of application of the entire membrane solution including lipid to the gel filtration column are shown in Fig. 3. Again, over 65% of the total protein is found at molecular weights above 100,000, and the broad, high molecular weight band has a peak position corresponding to 210,000 molecular weight. The fraction between 45 and 60 g of effluent shown in Fig. 2 was pooled, the guanidine was removed by dialysis, and the reduced and alkylated protein was dissolved in SDS. The molecular weights of the polypeptide chains in this sample were then determined by SDS-polyacrylamide gel electrophoresis. Five bands were found with molecular weights corresponding to the five highest molecular weight species in Table I with the overwhelming portion of the material in two bands at 200,000 and 220,000. No protein was found in the void volume peak in Fig. 2. No low molecular weight material of measurable quantity was eluted from the gel filtration column.

The two highest molecular weight components from human red blood cell membranes which comprise close to 40% of the total membrane protein have the same molecular weight when determined as random coils in 6 M GuHCl and as rods in SDS. Sedimentation equilibrium studies of Gwynne and Tanford (6) also give an identical molecular weight for these species. The latter method gives a true molecular weight of the solute species. The other two methods effectively measure a linear dimension of the molecular domain, which would be much smaller for most types of aggregates than the linear dimension of a single polypeptide chain of the same molecular weight. It is therefore

![Graph](https://via.placeholder.com/150)

**Table II**

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*H. Trayer, unpublished results.*

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**Fig. 4.** Polypeptide chains from human red blood cell membranes solubilized by EDTA and chromatographed on 4% agarose, 6 M GuHCl. ---, Supernatant 1—see text for explanation; ----, Supernatant 2—see text for explanation; ~~~, Supernatant 3—see text for explanation.
highly improbable that the 200,000 and 220,000 molecular weight polypeptide chains found in human red blood cell membranes are aggregates of some smaller polypeptide.

Ghosts suspended in cold, distilled water
EDTA (5 mM) pH 7.5, with NaOH
β-Mercaptoethanol (5 mM)
Dialyzed for 1 to 2 days 4° against above
Centrifuged at 30,000 rpm for 90 min in Spinco No. 30 rotor

Supernatant I
Pellet I

Suspended in
Glycine (5 mM)
EDTA (1 mM)
β-Mercapto-
ethanol (5 mM)
Mixed overnight at 4°
Centrifuged at 30,000 rpm for 90 min

Supernatant II
Pellet II

Suspended in
distilled water
Mixed for 2 to 3 hours at 4°
Centrifuged at 30,000 rpm for 2 to 3 hours

Supernatant III
Pellet III

Diagram I

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REFERENCES