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.

(7). In this experiment 40% of the total protein was lost before analysis, presumably with the lipid layer which floats to the top of the guanidine solution when the membranes are solubilized in this medium. However, a large fraction of the remaining protein was in the molecular weight range 150,000 to 200,000. In contrast to these findings are the results of Demus and Mehl (8) who used a phenol-formic acid-water solvent and found no high molecular weight components in erythrocyte ghosts, and Laio et al. (9) who claim that erythrocyte membranes are composed primarily of a polypeptide chain of approximately 6000 molecular weight. These latter authors observe the same pattern in SDS gel electrophoresis as Berg (1) and Lenard (2) but believe that the higher molecular weight components are aggregates of low molecular weight material.

Zwaal and Van Deenen (10) solubilized protein-lipid complexes from human red blood cell membranes using 1-butanol and demonstrated heterogeneity of these complexes by disc gel electrophoresis at low pH. This method did not allow the calculation of actual molecular weights of the complexes. There are only two solvent systems known at the present time in which all proteins thus far investigated are dissociated to their constituent polypeptide chains, and in which these polypeptides with disulfide bonds reduced adopt a conformation such that their hydrodynamic size is a unique function of molecular weight. Reduced polypeptide chains are true random coils in 6 M GuHCl (11). In SDS, at equilibrium concentrations greater than 5 x 10^-4 M, they can be described as rods of constant diameter with the long axis proportional to the molecular weights (12). It is only in these two solvent systems that the molecular weight of polypeptide chains can be unequivocally determined from a measurement of the effective hydrodynamic radius (e.g. gel filtration, intrinsic viscosity, electrophoresis in SDS). SDS gel electrophoresis is the preferred analytical method for a mixture of polypeptides because of the sharp resolution obtained. However, this is a poor method for low molecular weight components because the hydrodynamic size is not a good discriminant for rod-shaped particles as the length approaches the diameter. Gel filtration in GuHCl provides very low resolution, particularly with molecular weights greater than 100,000, but is suitable for use to very low molecular weights (5). (Both of these methods are uncertain when applied to polypeptide chains containing substantial amounts of carbohydrate. For example, the hydrodynamic behavior of a linear random coil with fairly long lateral extensions is not readily predictable.)

We have re-examined human red blood cell membranes in both GuHCl and SDS and find our results compatible with Berg (1) and Lenard (2) on a semiquantitative basis. We report...
Table 1

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<th>Molecular Weight (kDa)</th>
<th>Myosin</th>
<th>β-Galactosidase</th>
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Fig. 1. Mobilities of polypeptide-SDS complexes on a 5% polyacrylamide gel.

Fig. 2. Spectrophotometric scan of SDS-polyacrylamide gel-poly peptide chains from human red blood cell membranes.

Fig. 3. Elution profile of polypeptide chains from human red blood cell membranes-4% agarose, 6 M GuHCl.

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 overall 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 (6), 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

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H. Trayer, unpublished results.

Table II

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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)
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

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

Glycine (5 mm)
EDTA (1 mm)
β-Mercapto-
ethanol (5 mm)

Mixed overnight at 4°
Centrifuged at 30,000 rpm for 2 to 3 hours

Supernatant III

Pellet III

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REFERENCES

Polypeptide Chains from Human Red Blood Cell Membranes
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