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PROPERTIES OF THE MAJOR GLYCOPROTEIN OF THE BEEF ERYTHROCYTE MEMBRANE

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SUMMARY

The major glycoprotein of the beef erythrocyte membrane has been isolated It has a molecular weight of about 180000, of which 38% is protein and 62% is carbohydrate. The carbohydrate portion consists of hexosamine (24% of the dry weight of the glycoprotein), hexose (22%) and sialic acid (16%). The polypeptide portion is characterized by an abundance of serine and threonine residues.

INTRODUCTION

Glycoproteins account for a significant portion of the intrinsic protein of the red cell membrane[1]. Their carbohydrate moieties are antigenic determinants[2] and receptors for plant agglutins and viruses[3].

The major glycoprotein of human red cells has been isolated using a number of reagents including pyridine[4], hot 75% alcohol[5] and lithium diiodosalicylate[6]. Molecular weights of 26000[4,7] and 58000[5,6] have variously been reported for this molecule.

There is considerable evidence of variation in the size of glycoproteins from different species of red cells[5,8]. Reports indicate that the major glycoprotein of beef red cells has a much higher molecular weight than its human counterpart[1,7,8]. This has been confirmed in this study of the isolated and purified beef erythrocyte glycoprotein. Other differences in properties between the beef and human erythrocyte glycoprotein have been noted.

METHODS

General methods

Protein was estimated by the method of Lowry *et al.*[9], using bovine serum albumin as a standard. Lipid was measured as described by Chen *et al.*[10]. Sialic acid was measured according to Warren[11] using *N*-acetylneuraminic acid as a standard. Release of the sugars was complete after hydrolysis for 1 h in 0.05 M H_2SO_4 at 80 °C. Total neutral hexose was determined by the phenol–H_2SO_4 method of Dubois *et al.*[12]. Hexosamine was estimated after hydrolysis in 3 M HCl for 4 h as described by Allison

and Smith[13]. Amino acids were determined after hydrolysis for 22 h in constant boiling HCl (6 M) at 110 °C in sealed evacuated tubes using a Technicon autoanalyzer.

Gel electrophoresis

Gel electrophoresis was performed and the gels were fixed and stained as described by Fairbanks *et al.*[14].

Column chromatography

Samples were chromatographed through Biogel A-5m (Bio-rad Laboratories Inc.) in 1% sodium dodecyl sulfate, 0.5 mM dithiothreitol, 0.02% sodium azide and 10 mM Tris–HCl, pH 7.4, on a 3.0 cm × 80 cm column. Fractions were monitored for absorbance at 280 nm. The molecular weight of the glycoprotein was estimated as described by Fish *et al.*[15], using myosin, β galactosidase, bovine serum albumin, chymotrypsinogen and cytochrome *c* as standard proteins for calibrating the column. Cytochrome *c* was added to all samples as an internal marker.

Sedimentation studies

Equilibrium sedimentation studies for molecular weight estimation were performed with a Spinco Model E analytic ultracentrifuge. The glycoprotein was dissolved in 6 M guanidine·HCl and 5 mM β-mercaptoethanol, and this solution was dialyzed against the same solvent for at least 4 days prior to each run. A 12-mm cell was used at a speed of 13410 rev./min.

The partial specific volume of the glycoprotein was calculated from its composition using partial specific volumes of 0.62 for hexose and hexosamine, 0.59 for sialic acid and 0.74 for protein[16]. A value of $\bar{v}$ of 0.67 was obtained by this method for the purified glycoprotein, and this value is unlikely to be changed significantly in 6 M guanidine·HCl[17,18].

Preparation of ghosts

Beef erythrocyte ghosts were prepared by the method of Burger *et al.*[19] using 2 mM Ca^{2+} to prevent disruption of the ghosts during hemolysis.

Isolation of the glycoprotein

The glycoprotein was separated from the bulk of the membrane components by the method of Hamaguchi and Cleve[8]. Erythrocyte ghosts (in 50 mg portions) were shaken vigorously in a solution which was 6 parts chloroform, 3 parts methanol and 1 part water. On standing, 3 layers developed: a bottom chloroform layer containing the majority of the lipid, an upper aqueous layer, and an interfacial layer of insoluble protein. The upper aqueous layer contained most of the glycoprotein relatively free of other proteins as shown in the gel traces in Fig. 1. Further purification was achieved by gel filtration on Biogel A-5m in 1% sodium dodecyl sulfate, 0.5 mM dithiothreitol, 0.02% sodium azide and 10 mM Tris–HCl, pH 7.4. The glycoprotein eluted close to the void volume and was thus separated from smaller protein contaminants and the bulk of phospholipid which remained associated through the first purification step. Sodium dodecyl sulfate was removed from the glycoprotein fraction by dialysis against a large volume of 90% acetone in the cold room (4 °C) for 48 h. Acetone was removed

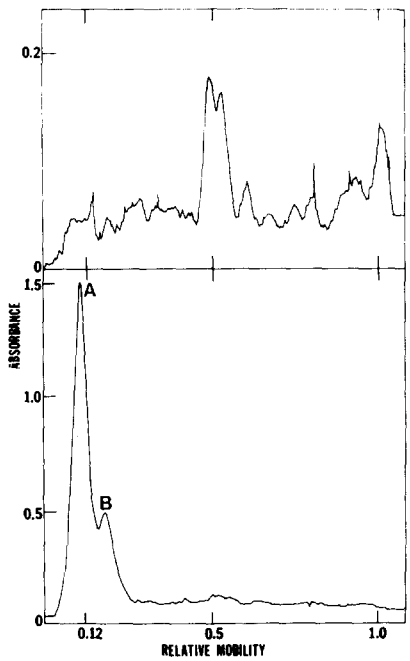

Fig. 1. Densitometric traces of gels of the aqueous supernatant after chloroform–methanol–water extraction procedure, stained for protein with Coomassie blue (top trace) and for carbohydrate with Schiff reagent (bottom trace). Peaks A and B represent a major glycoprotein (A) and a minor glycoprotein (B) which migrate with molecular weights of 285000 and 265000, respectively.

from the sample by rotary evaporation and the glycoprotein was concentrated by freeze-drying. The freeze-dried pure glycoprotein was stored at −20 °C.

RESULTS AND DISCUSSION

Column chromatography failed to separate the major glycoprotein (Peak A, Fig. 1) from a minor glycoprotein of fairly similar molecular weight (Peak B, Fig. 1). However, component B adsorbed less than 5% of the Schiff stain on gels, and the small amount of this component would not be expected to influence significantly the results of sedimentation studies and amino acid analysis reported here.

The purified glycoprotein contained 1.4 μmoles of sialic acid per mg protein, from which it was estimated that the molecule represents about 5% of the ghost protein. In all, 62% by dry weight of the molecule was carbohydrate and 38% was protein. The carbohydrate portion consisted of hexosamine (24% of the dry weight of the total glycoprotein), hexose (22%) and sialic acid (16%). The amino acid composition of the glycoprotein is listed in Table I. The polypeptide portion of the molecule was characterized by a relatively large number of serine and threonine residues.

A value of 180000 ± 15000 (5 determinations) was estimated for the molecular weight of the glycoprotein by sedimentation equilibrium studies in 6 M guanidine·HCl. This compares with a value of 150000 from column chromatography (Fig. 2 and ref. 7) and 285000 by gel electrophoresis in sodium dodecyl sulfate. The

TABLE I

THE AMINO ACID COMPOSITION OF THE BEEF ERYTHROCYTE GLYCOPROTEIN

N.D., not determined.

Amino acids	*g/100 g*	*Residues per 180000 g to the nearest integer*
Carboxymethyl cysteine	2.10	12
Aspartic acid	6.86	35
Threonine	7.53	43
Serine	9.68	63
Glutamic acid	12.08	56
Proline	5.93	35
Glycine	3.98	36
Alanine	5.30	41
Valine	5.39	31
Methionine	1.82	8
Isoleucine	4.88	25
Leucine	7.69	40
Tyrosine	5.51	21
Phenylalanine	3.86	16
Histidine	6.80	30
Lysine	3.80	18
Arginine	6.79	27
Tryptophan	N.D.	

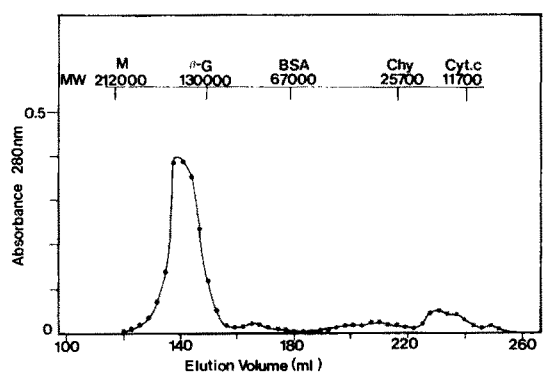

Fig. 2. Chromatographic profile of the glycoprotein fraction from the chloroform–methanol–water procedure, eluted from Biogel A-5m with 1% sodium dodecyl sulfate, 0.5 mM dithiothreitol, 0.02% sodium azide and 10 mM Tris–HCl (pH 7.4). 3-ml portions were collected and analyzed for protein at 280 nm. The positions of elution of the standard proteins are also shown. M, myosin; β-G, β-galactosidase; BSA, bovine serum albumin; Chy, chymotrypsinogen; Cyt. *c*, cytochrome *c*; MW, molecular weight.

discrepancy between results of the three methods is most likely due to a lack of binding of sodium dodecyl sulfate by the carbohydrate portion of the glycoprotein molecule. Thus the molecule would be retarded more than the standard proteins used to calibrate both columns and polyacrylamide gels.

In comparison with the major glycoprotein from human erythrocytes, the beef erythrocyte glycoprotein is larger (mol. wt 180000 against 58000 (ref. 20)), and contains considerably less sialic acid (16% by dry weight compared with 25% (ref. 20)). The two major glycoproteins are similar however in their relative proportions of carbohydrate and protein, in their amino acid compositions, and in their abundance in their respective membranes. Experiments are now in progress to determine the distribution of the beef red cell glycoprotein and its association with other proteins in the membrane.

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