SOLUBILIZATION AND COMPARATIVE ANALYSIS OF MAMMALIAN ERYTHROCYTE MEMBRANE GLYCOPROTEINS

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SUMMARY. SDS-acrylamide gel electrophoresis reveals the presence of a major glycoprotein in human, ox, horse, swine, and sheep erythrocyte membranes. Their apparent molecular weights differ among the various species. The major glycoproteins and additional minor glycoproteins can be recovered in the aqueous phase after extraction of the membranes with a mixture of $CHCl_3$-CH_3OH at room temperature. The extracted glycoproteins remain in the supernatant after centrifugation at 100,000 g for 60 min. in Tris-EDTA buffer. These glycoprotein preparations possess high activities for the phaseolus vulgaris phytohemagglutinin receptor, the infectious mononucleosis heterophile antigen, and the myxovirus receptor. Their specific activities and yields differ markedly from species to species.

Mammalian erythrocyte membrane glycoproteins play an important role as red cell surface antigens[1-4]. It has, furthermore, been demonstrated that the human erythrocyte membrane glycoproteins extend through the membrane barrier from the outer to the inner surface[5,6]; they may, therefore, be regarded as integral proteins of the erythrocyte membrane[7]. Characterization of mammalian erythrocyte membrane glycoproteins may aid in the further elucidation of the structure and function of biological membranes. In this report, a simple solubilization procedure of mammalian erythrocyte membrane glycoproteins is described and data on their electrophoretic and immunological analysis are presented.

MATERIAL AND METHODS. Citrated animal blood was purchased from Animal Blood Centre, Syracuse, New York. Mammalian erythrocyte membrane glycoproteins were extracted and solubilized by a method adapted from Kornfeld and Kornfeld[8].

The erythrocytes were washed with 0.9% NaCl-5mM Tris-HCl buffer, pH 7.4. The washed erythrocytes were hemolyzed with nine volumes of 10mM Tris-0.1mM EDTA-HCl buffer, pH 7.4, and centrifuged at 20,000 g for 20 min. Ghosts were subsequently washed four times under the same conditions. The membranes were mechanically homogenized and diluted to 2 mg/ml protein concentration with 10mM Tris-0.1mM EDTA-HCl buffer, pH 7.4. All steps of this procedure were carried out at a temperature of 0° to 4°C. To one volume of the membrane suspension, nine volumes of a $CHCl_3$-CH_3OH mixture (2:1, v/v) were added. The mixture was stirred vigorously at room temperature for 30 min. After centrifugation of the mixture at 1,500 rpm for 10 min. at room temperature, the aqueous layer was carefully aspirated and then concentrated to one-tenth the volume of the original membrane suspension in a rotary evaporator at 37°C. The concentrated materials were centrifuged at 100,000 g for 60 min. at 4°C. The supernatant was clear and contained glycoproteins.

SDS-acrylamide gel electrophoresis was performed in 0.5% SDS-0.1M sodium phosphate buffer, pH 7.1. 7.5% acrylamide, 0.2% N,N'-methylenebisacrylamide, 0.5% SDS and 0.1M phosphate buffer were used for the preparation of gels, 5mm in diameter and 12cm in length. Samples were dissolved in 2% SDS, 1% 2-mercaptoethanol, 10% glycerol, 10mM phosphate buffer and 0.001% bromphenol blue (BPB), followed by incubation at 70°C. for 20 min. Electrophoresis was carried out at 10mA per tube and stopped as soon as the BPB reached the 8.0cm mark from the origin. Gels were stained with either Coomassie brilliant blue or periodic acid Schiff (PAS) according to Fairbanks et al.[9].

Phaseolus vulgaris phytohemagglutinin (PHA) receptor activity, infectious mononucleosis (IM) heterophile antigen, and myxovirus receptor activity were measured by hemagglutination inhibition tests according to Kornfeld and Kornfeld[8], Fletcher and Woolfolk[3], and Kathan et al.[10], respectively. Total hemagglutination inhibition units (HAI units) were determined according to Gardas and Kościelak[11]. PHA-P was purchased from Difco Laboratories, Detroit, Michigan. For the assay of IM heterophile antigen, sera from several

patients with IM were used. Influenza A_2 virus (Jap 305) was kindly provided by Dr. J.F. Woodruff of Cornell University Medical College.

Protein concentration was measured by the method of Lowry et al.[12] and sialic acid was assayed by the method of Warren[13].

RESULTS AND DISCUSSION. Figures 1 and 2 present the SDS-acrylamide gel electrophoresis patterns of erythrocyte membranes from eight mammalian species. The protein patterns reveal the presence of more than 20 components which show extensive similarities among the different species, as previously reported[14] (Fig. 1). In contrast, the PAS stain patterns reveal marked differences from species to species (Fig. 2). The patterns suggest that human, ox, horse, swine, sheep, and goat erythrocyte membranes contain one major glycoprotein. The apparent molecular weights of the major glycoproteins were calculated from the migration rates in SDS-acrylamide gel electrophoreis. They are 58,000 for the human, 32,000 for the horse, 50,000 for the swine,

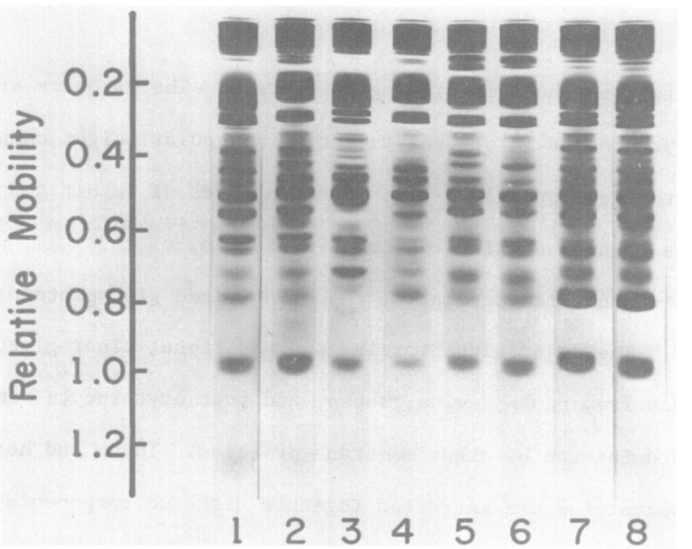

Fig. 1. SDS-acrylamide gel electrophoresis patterns of erythrocyte membrane proteins from eight mammalian species. Coomassie brilliant blue stain. 80μg of protein was applied to each gel.1-Human, 2-Ox, 3-Horse, 4-Swine, 5-Sheep, 6-Goat, 7-Rabbit, and 8-Guinea Pig. Relative mobilities were determined from the migration rate relative to BPB. The components with a relative mobility of approximately 1.0 are hemoglobin subunits.

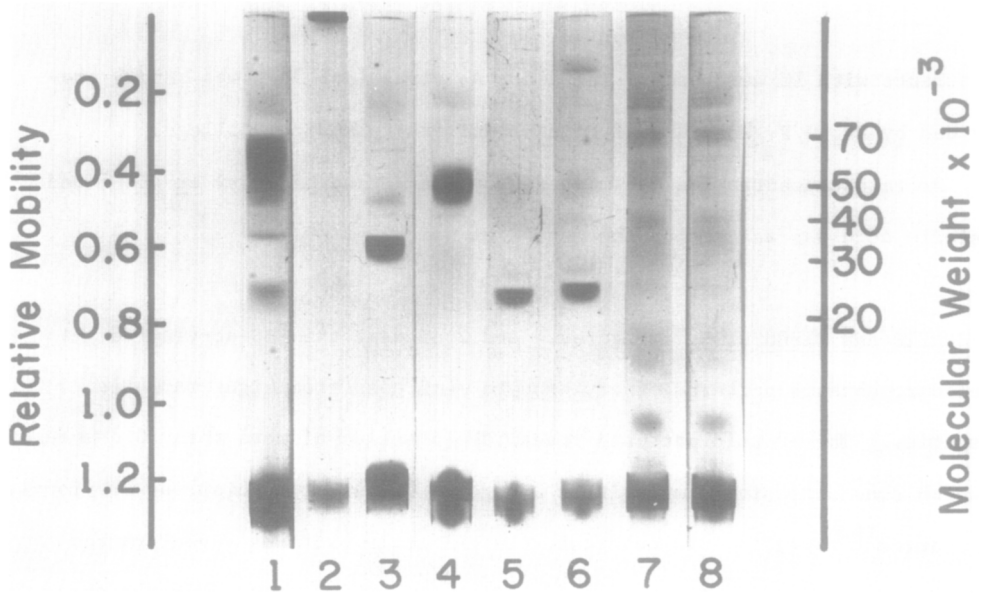

Fig. 2. SDS-acrylamide gel electrophoresis patterns of erythrocyte membrane proteins. PAS stain. The series of mammals and the amounts of protein applied are the same as in Fig. 1. For the apparent molecular weight determinations, human transferrin, bovine serum albumin, human haptoglobin β chain, human immunoglobulin light chain and bovine ribonuclease were used as marker proteins. The prominent PAS positive components with a relative mobility of approximately 1.2 are glycolipids.

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and 24,000 for the sheep membrane glycoprotein. The major ox erythrocyte membrane glycoprotein is present as a high molecular weight component which penetrates the gel for only 1-2mm. The membranes of rabbit and guinea pig erythrocytes do not contain a major glycoprotein.

The electrophoretic patterns of the solubilized glycoproteins are shown in Figure 3. The major glycoproteins and additional minor glycoproteins are extracted from human, swine, sheep, and goat erythrocyte membranes almost without contamination by other membrane proteins. In ox and horse, the major glycoptroteins are extracted together with two components which react strongly with Coomassie brilliant blue and only weakly with the PAS reagent. The glycoproteins solubilized represent 2-4% of the total ghost protein (Table I). The sialic acid recoveries are particularly high in preparations from human, ox, and sheep red cell membranes (Table I).

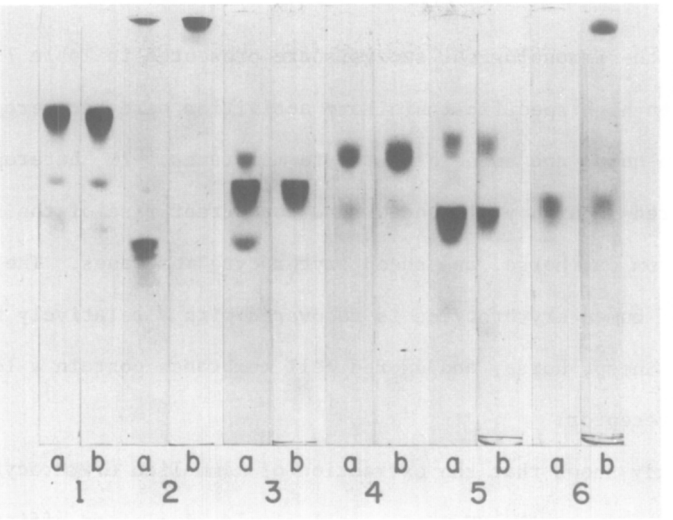

Fig. 3. SDS-acrylamide gel electrophoresis patterns of the glycoprotein preparations from erythrocyte membranes. a-Coomassie brilliant blue stain; b-PAS stain. 1-Human, 2-Ox, 3-Horse, 4-Swine, 5-Sheep, and 6-Goat.

Table I: CHEMICAL AND IMMUNOLOGICAL ANALYSIS OF SOLUBILIZED MEMBRANE GLYCOPROTEINS

	PROTEIN	SIALIC ACID	PHA		ANTIBODY FOR IM ANTIGEN		MYXOVIRUS	
	recovery percent	recovery percent	activity (µg/ml)	recovery percent	activity (µg/ml)	recovery percent	activity (µg/ml)	recovery percent
HUMAN								
ghost			360		N.A.		105	
glycoproteins	3.7	83	13	94	N.A.		47	8.5
OX								
ghost			2,000		590		3,500	
glycoproteins	3.3	71	700	10	66	100	1,700	6.8
HORSE								
ghost			6,600		880		8,100	
glycoproteins	2.2	26	300	64	29	82	590	62.0
SWINE								
ghost			1,300		N.A.		N.A.	
glycoproteins	2.8	42	23	100	N.A.		N.A.	
SHEEP								
ghost			6,700		1,700		1,900	
glycoproteins	2.4	61	330	60	98	50	390	16.0

N.A. No inhibitory activity at 7,500 µg/ml.

Figures are mean values from three preparations in humans and from two preparations in the other mammals. Specific inhibitory activity is the minimum amount of protein in µg/ml necessary to inhibit completely the hemagglutination by four hemagglutinating doses of reagent. The recovery of immunological activities is expressed as percentage of HAI units of the glycoprotein preparation against HAI units of ghosts. Activity for IM antigen was tested with horse erythrocytes. For the other tests human O Rh_o erythrocytes were used.

Data on the immunological analysis are presented in Table I. PHA receptors with high specific inhibitory activities were recovered in high yields from human and swine erythrocyte membranes. IM heterophile antigen was recovered in high yield and with a concurrent rise of the specific activities from ox, horse, and sheep erythrocyte membranes. The myxovirus receptor of human erythrocytes is recovered with a relatively high specific activity. Sheep, horse, and ox red cell membranes contain a less potent myxovirus receptor.

This study shows that the extraction of mammalian erythrocyte membranes with a mixture of $CHCl_3$-CH_3OH is a relatively simple and efficient procedure for the solubilization of membrane glycoproteins. Work carried out in this laboratory indicates that the major glycoproteins can be purified further by gel filtration on Sephadex G-100 columns in the presence of 1% SDS or on Sepharose 4B columns in the presence of 6M guanidine hydrochloride[4,15].

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