ISOLATION AND PARTIAL CHARACTERIZATION OF THE MAJOR GLYCOPROTEINS OF HORSE AND SWINE ERYTHROCYTE MEMBRANES

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SUMMARY

The major glycoproteins of horse and swine erythrocyte membranes were isolated and examined chemically and immunologically. The major glycoprotein of horse erythrocyte membranes had a molecular weight of 33 000 and consisted of 46.2 % protein and 53.8 % carbohydrate, of which 9.4 % was hexose, 10.1 % hexosamine and 33.7 % sialic acid. This glycoprotein was associated with activity for the infectious mononucleosis heterophile antigen.

There were two different major glycoproteins in swine erythrocyte membranes. One major glycoprotein had a molecular weight of 46 200 and consisted of 34.2 % protein and 65.8 % carbohydrate, of which 18 % was hexose, 19 % hexosamine and 27.2 % sialic acid. This glycoprotein had phytohemagglutinin (*Phaseolus vulgaris*) binding activity. The other glycoprotein had a molecular weight of 29 000 and consisted of 50.4 % protein and 49.6 % carbohydrate, of which 6.4 % was hexose, 7.0 % hexosamine and 36.3 % sialic acid. This glycoprotein had weak or absent phytohemagglutinin binding activity.

INTRODUCTION

Mammalian erythrocyte membrane glycoproteins carry various cell surface antigens and receptor sites [1–3]. These glycoproteins can be recovered in the aqueous phase after extraction of membranes with a mixture of chloroform and methanol [1]. The aqueous phase of chloroform/methanol extracts of erythrocyte membranes of several different species such as human, ox, horse, swine, sheep and goat contain one major component and several minor components [1]. The major glycoproteins of these different species differed from each other in molecular size and antigenic specificities. The major glycoprotein of human erythrocyte membranes has been studied extensively [4–11] and is known to play an important role for the membrane structure as an integral protein [12] as well as a site for various receptors. Information

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on the major glycoproteins of other species is more limited. The preparation and the chemical and immunological characterization of the major glycoproteins of horse and swine erythrocyte membranes are the subject of this report.

MATERIALS AND METHODS

Separation of major glycoproteins from horse and swine erythrocyte membranes. Units of whole horse and swine blood were purchased from the Animal Blood Center, Inc. (Syracuse, N.Y.). Swine blood was taken from three different breeds, Duroc, Yorkshire and Hampshire. Preparation of erythrocyte membranes and extraction of glycoproteins from erythrocyte membranes with chloroform/methanol were performed according to Hamaguchi and Cleve [1]. The major glycoproteins were isolated from the aqueous phase of chloroform/methanol extracts by preparative gel electrophoresis on 0.5 % sodium dodecylsulfate - 7.5 % polyacrylamide gels as described previously for human erythrocyte membranes [13].

Analytical disc gel electrophoresis was carried out on 0.5 % sodium dodecylsulfate–7.5 % polyacrylamide gels in 0.5 % sodium dodecylsulfate/0.1 M sodium phosphate buffer at pH 7.1. Proteins were separated at 3 V/cm for 8 h in tubes which were 5 mm in diameter and 12 cm in length. Gels were stained with Coomassie brilliant blue or with the periodic acid–Schiff reagent [14].

Protein concentrations were determined by the method of Lowry et al. [15] using three times crystallized bovine pancreatic ribonuclease as the standard. Phosphorus was measured by the method of Bartlett [16]. Cholesterol was measured according to Zlatkis et al. [17].

Amino acid analysis was carried out on a Beckman model 120-B automatic analyzer (Beckman Instruments, Inc., Calif.) using the system of Moore and Stein [18].

Carbohydrates were analyzed by gas-liquid chromotography using 3 % OV-17 on chromosorb W (80–100 mesh) as described previously [13]. Methanolysis and trimethylsilyl derivatization of carbohydrates of glycoprotein were performed according to the method of Reinhold [19].

Activities for infectious mononucleosis heterophile antigen and *Phaseolus vulgaris* phytohemagglutinin binding activity of glycoproteins were determined by hemagglutination inhibition assay according to Fletcher and Woolfolk [2], and Kornfeld and Kornfeld [20], respectively. Tests were carried out on serial 2-fold dilutions of glycoprotein. *P. vulgaris* phytohemagglutinin was purchased from Difco Laboratory (Detroit, Mich.). Sera from patients with infectious mononucleosis were used for the assay of infectious mononucleosis heterophile antigen.

RESULTS

The major glycoproteins of horse and swine erythrocyte membranes were isolated from the aqueous phase of chloroform/methanol extracts by preparative electrophoresis on polyacrylamide gels. Banding patterns of unfractionated aqueous phase of chloroform/methanol extracts and of the isolated major glycoproteins in polyacrylamide gels are shown in Figs 1–4.

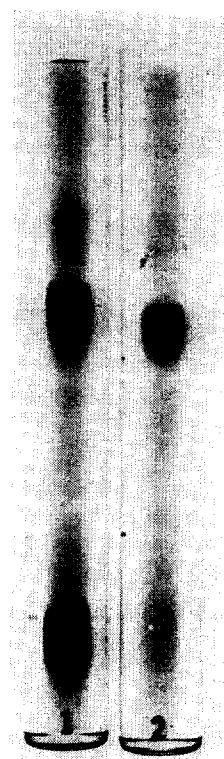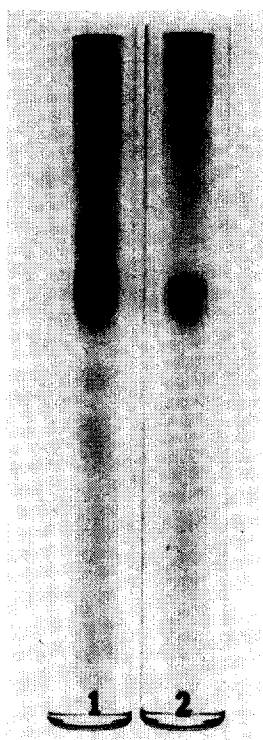The aqueous phase of chloroform/methanol extracts of horse erythrocyte

Fig. 1. Banding pattern of horse erythrocyte membrane glycoproteins on 0.5 % sodium dodecyl-sulfate-7.5 % polyacrylamide gels. Gels were stained with Coomassie brilliant blue. Gel 1 represents the aqueous phase of chloroform/methanol extracts of horse erythrocyte membranes, and gel 2 represents isolated horse major glycoprotein.

Fig. 2. Same as Fig. 1. Gels were stained with periodic acid-Schiff reagent.

membranes contain one major component and at least three minor components which stain for protein (Fig. 1) and for carbohydrate (Fig. 2) .The isolated major glycoprotein has the same migration rate as in the aqueous phase of chloroform/methanol extracts (Figs 1 and 2). The absence of additional bands indicated a relatively high degree of purity of the isolated materials. The migration rate of the major glycoprotein of horse erythrocyte membranes on polyacrylamide gels corresponded to a molecular weight of 33 000.

In the aqueous phase of chloroform/methanol extracts of swine erythrocyte membranes, two different banding patterns were observed (Figs 3 and 4, gel 1 and gel 3). The banding pattern of gel 1 in Figs 3 and 4, was obtained from erythrocyte membranes from several individual pigs of breeds Yorkshire and Duroc. The molecular weight of this major glycoprotein was calculated to be 46 200. The banding pattern of gel 3 in Figs 3 and 4 was observed in Hampshire breed of swine. The molecular weight of major glycoprotein in this breed was 29 000. The isolated glycoproteins have migration rates identical with the glycoproteins in the unfractionated aqueous phase of chloroform/methanol extracts.
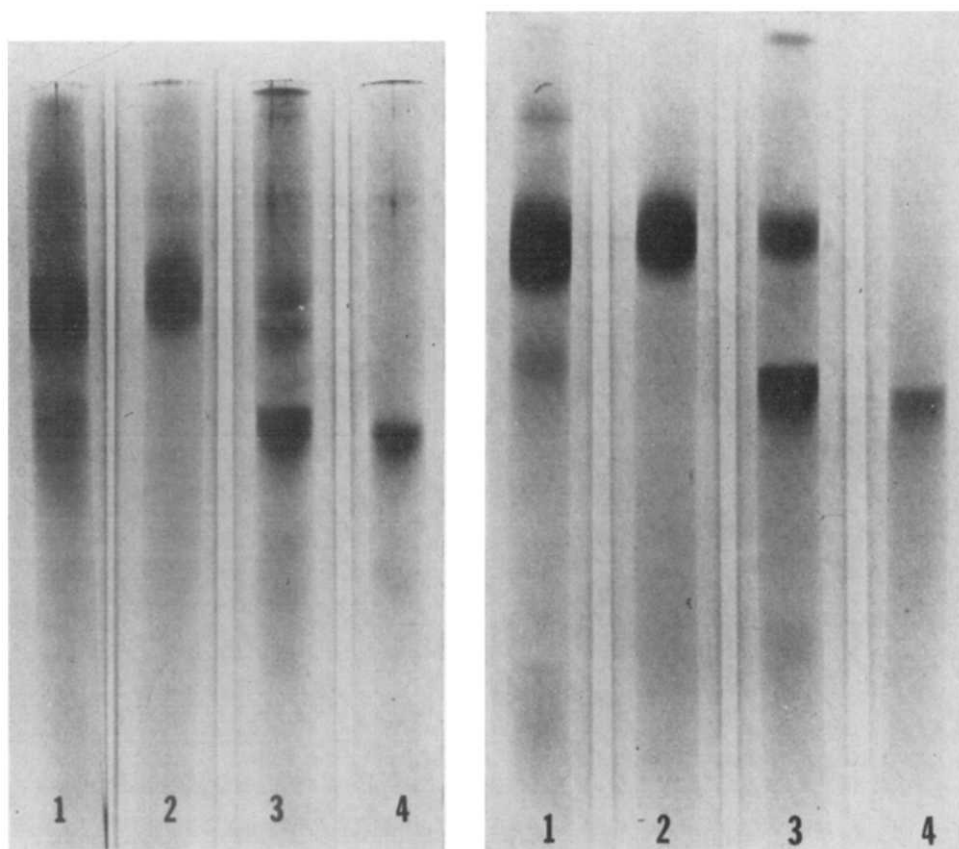
Fig. 3. Banding patterns of swine erythrocyte membrane glycoproteins on 0.5 % sodium dodecyl-sulfate-7.5 % polyacrylamide gels. Gels were stained with Coomassie brilliant blue. Gel 1 represents the aqueous phase of chloroform/methanol extracts of erythrocyte membranes of Yorkshire and Duroc breeds of swine. Gel 3 represents the aqueous phase of chloroform/methanol extracts of erythrocyte membranes of Hampshire breed of swine. Gels 2 and 4 represent the isolated major glycoproteins.

Fig. 4. Same as Fig. 3. Gels were stained with periodic acid-Schiff reagent.

Chemical composition of the glycoproteins

The chemical composition of isolated major glycoproteins of horse and swine erythrocyte membranes is shown in Table I: the protein and carbohydrate concentrations given were calculated from the sum of the protein and carbohydrate contents in the lyophilized material. As reported previously [13] the lyophilized material contained inert material as a result of contamination with polyacrylamide which occurs during elution of glycoproteins from the gel segments. Table I indicates a carbohydrate content of 50 % in the major glycoprotein of horse and of Hampshire breed of swine, respectively, and of 65 % in the major glycoprotein of Yorkshire and Duroc breeds of swine. In the major glycoproteins of horse and Hampshire breed of swine, galactose N-acetylgalactosamine and sialic acid were the main carbohydrate components. In the major glycoprotein of Yorkshire and Duroc breeds of swine, galactose, N-acetyl-

TABLE I

CHEMICAL COMPOSITION OF ISOLATED MAJOR GLYCOPROTEINS OF HORSE AND SWINE ERYTHROCYTE MEMBRANES

The results are expressed as g/100 g.

Constituent	Horse	Swine (Yorkshire and Duroc)	Swine (Hampshire)
Protein (percent of dry weight)	18.4	16.9	32.5
Protein	46.2	34.2	50.4
Carbohydrate	53.8	65.8	49.6
Fucose	0.62	1.72	0
Mannose	0.41	2.61	0.17
Galactose	8.96	15.36	6.20
N-acetylgalactosamine	8.83	7.48	7.00
N-acetylglucosamine	1.24	11.48	0
Sialic acid	33.73	27.18	36.25

galactosamine, N-acetylglucosamine and sialic acid were found in high concentrations, while the major glycoprotein of Hampshire breed lacked N-acetylglucosamine. Chromatographic elution patterns indicated that the sialic acid in the major glycoprotein of horse, and of Yorkshire and Duroc breeds of swine was mostly present as

TABLE II

AMINO ACID COMPOSITION OF ISOLATED MAJOR GLYCOPROTEINS OF HORSE AND SWINE ERYTHROCYTE MEMBRANES

Values were means of four preparations for horse and two preparations each for swine analyzed in duplicate. The results are expressed as mol %.

Amino acid	Horse	Swine (Yorkshire and Duroc)	Swine (Hampshire)
Lys	2.32	5.39	3.10
His	1.16	1.73	1.41
Arg	4.16	1.14	4.04
Asp	8.00	9.52	4.44
Thr	9.73	11.59	7.62
Ser	9.59	8.57	12.37
Glu	8.83	11.55	11.77
Pro	10.90	9.84	11.21
Gly	8.05	8.22	8.80
Ala	9.54	8.62	9.89
Cys/2	0.63	0.51	0.87
Val	6.40	6.41	6.14
Met*	0.78	1.43	0.60
Ile	5.10	4.78	5.39
Leu	7.64	3.80	7.73
Tyr	1.87	1.58	1.35
Phe	4.97	5.29	3.02

* Methionine after performic acid oxidation [24]: 1.36 for horse, 1.95 for Yorkshire and Duroc breeds of swine and 1.31 for Hampshire breed of swine.

the N-glycolyl derivative, while the major glycoprotein of Hampshire breed of swine contained approximately equal amounts of the N-acetyl derivative and the N-glycolyl derivative. Fucose was present in the major glycoprotein of horse, and of Yorkshire and Duroc, but could not be detected in the major glycoprotein of Hampshire breed.

The amino acid composition of the glycoproteins is shown in Table II. Common to all three glycoproteins was the high proportion of threonine and serine residues, which represented approx. 20 % of all amino acids. The ratio of threonine and serine was, however, different between the three glycoproteins and was calculated to be 1.0 in horse, 1.4 in Yorkshire and Duroc breeds of swine, and 0.6 in Hampshire breed of swine. Small but significant amounts of half cystine residue were found in all three glycoproteins.

Phosphorus was detected in the preparations of major glycoproteins of horse, and Yorkshire and Duroc breeds of swine. The amount corresponded to a phospholipid content of 5.16 % by weight in the horse major glycoprotein and 6.85 % in the glycoprotein of Yorkshire and Duroc breeds. Cholesterol was not detected.

TABLE III

IMMUNOLOGICAL ACTIVITIES OF MAJOR GLYCOPROTEINS OF HORSE AND SWINE ERYTHROCYTE MEMBRANES

Hemagglutination inhibition activities were expressed by the minimum protein concentration (μg/ml) which inhibited completely the agglutination of erythrocytes at four hemagglutinating doses. For assay of infectious mononucleosis antigen, horse erythrocytes were used. For assay of phytohemagglutinin binding activity, human erythrocytes (0.Rh$^-$) were used. The figures for activities were mean values. The number of preparations tested are given in parentheses following the activity. N.A., no inhibitory activity: W.I., weak inhibition; N.T., not tested.

	Activity for infectious mononucleosis antigen (μg/ml)	Phytohemagglutinin binding activity (μg/ml)
Horse		
The aqueous phase of chloroform/methanol extracts	7.4 (3)	350 (3)
Isolated major glycoprotein	10.4 (3)	W.I.* (3)
Swine (Yorkshire and Duroc)		
The aqueous phase of chloroform/methanol extracts	N.A. at 400 (2)	36.2 (5)
Isolated major glycoprotein	N.A. at 450 (3)	33.4 (5)
Swine (Hampshire)		
The aqueous phase of chloroform/methanol extracts	N.A. at 500 (1)	W.I.** (2)
Isolated major glycoprotein	N.T.	N.A. at 170 (1)

  * Very weak inhibition at 300 μg/ml. At 700 μg/ml still partial inhibition.
  ** Weak inhibition at 800 μg/ml.

Immunological activity

Immunological activities for infectious mononucleosis heterophile antigen and phytohemagglutinin receptor of the aqueous phase of chloroform/methanol extracts of membranes and of the three purified glycoproteins are shown in Table III.

The aqueous phase of chloroform/methanol extracts of horse erythrocyte membranes and the isolated horse major glycoprotein had high specific activities for infectious mononucleosis heterophile antigen and only weak phytohemagglutinin binding activity. The specific activity for infectious mononucleosis heterophile antigen decreased slightly in the purified horse major glycoprotein compared to that of the aqueous phase after chloroform/methanol extraction. This slight decrease in specific activity may have been caused by the purification procedure.

Activity for infectious mononucleosis heterophile antigen was not found in the aqueous phase of chloroform/methanol extracts of swine erythrocyte membranes, or in the major glycoproteins of swine erythrocyte membranes.

The major glycoprotein and the aqueous phase of chloroform/methanol extracts prepared from erythrocytes of Yorkshire and Duroc breeds of swine had high specific activities for the phytohemagglutinin receptor, while the major glycoprotein and the aqueous phase of chloroform/methanol extracts prepared from erythrocytes of Hampshire breed had only very weak, if any, activity for phytohemagglutinin receptor.

DISCUSSION

Several reports in recent years have dealt with the glycoproteins of mammalian erythrocyte membranes [1–3, 21–23]. Mammalian erythrocyte membranes usually contain one major glycoprotein and several minor glycoproteins [1, 22]. They can be recovered in high yields in the aqueous phase following extraction of membranes with a mixture of chloroform and methanol [1]. In this study, the major glycoproteins of horse and swine erythrocyte membranes were isolated by preparative gel electrophoresis without recognizable contamination by other membrane components.

The major glycoprotein of horse erythrocyte membranes resembles closely the material obtained by Fletcher and Woolfolk [2] who extracted horse erythrocyte membranes with hot ethanol. While the amino acid composition is in close agreement, the sialic acid content in our preparation was found to be higher than in Fletcher and Woolfolk's report [2]. Activity for infectious mononucleosis heterophile antigen is associated with this major glycoprotein.

Of interest is the observation of two different major glycoproteins in erythrocyte membranes from different breeds of swine. The major glycoprotein of erythrocyte membrane of Yorkshire and Duroc breeds differed from the major glycoprotein of erythrocyte membrane of Hampshire breed in molecular size, chemical composition and immunological activities. The differences suggest that the smaller glycoprotein, the glycoprotein from Hampshire breed, is not a subunit of the larger glycoprotein, the major glycoprotein from Yorkshire and Duroc. These differences also indicate the presence of genetic variability in membrane components of swine erythrocytes. It is still unknown at present whether the difference in membrane components may also be reflected in properties of the erythrocytes as a whole.

ACKNOWLEDGEMENTS

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