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A comparative study of carbohydrate component of hen and human glycophorins

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Sialoglycoproteins (SGPs) from plasma membranes of hen red blood cells were isolated by preparative SDS-PAGE and their carbohydrate composition and some immunochemical properties were investigated and compared with that of human glycophorin A. It was shown, that two principal SGPs with molecular weights of 28 kDa and 55 kDa are monomeric and dimeric forms of the same molecule and are immunochemically closely related. The amount of carbohydrate component consists about 33 % of molecular mass (in human glycophorin A it is 52.3 %). The analysis of monosaccharide composition of hen SGPs indicates, that they possess O-linked and N-linked oligocaccharide chains. In comparison with human glycophorin A hen SGPs have a higher amount of N-glycosidic chains and lower amount of O-glycosidic chains, the last are tentatively of more branched structure.

Introduction. Glycophorins are major integral sialoglycoproteins of red blood cell (RBC) membrane, which play an important role in the formation of carbohydrate structure of cell surface. Their characteristic feature is a large carbohydrate moiety, consisting about a half of the molecular mass, which is composed for a number of richly sialilated O-linked oligosaccharide chains. The structures of genes, polypeptide and carbohydrate chains of glycophorins are well studied in man and some other mammals [1—4]. Recently we have communicated about the presence and some properties of glycophorin-like molecules in nucleated hen erythrocytes [5]. In present article we report results of the investigation of carbohydrate component of hen glycophorin as well as some immunological study.

Materials and Methods. Fresh blood from white leghorn hens (*Gallus domesticus*) was obtained on poultry farm during slaughter. 0.1 M sodium oxalate was used, as anticoagulant, in relation 1:10. Blood cells were separated by centrifugation for 10 min at 1000 *g* and buffy coat and supernatant were removed by aspiration. Erythrocytes were washed 3 times with phosphate-buffered saline (PBS) — 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.2 with careful removal of buffy coat at each washing.

Plasma membranes were isolated as we described in [5].

Sialoglycoproteins were obtained from the membranes by extraction with chloroform-isopropanol system according to modified method of Hamagushi [6]. In our modification of the method isopropanol was used instead of methanol and the final proportion of water:alcohol:chloroform was 1:2:4 [7].

Electrophoretically pure glycophorin fractions were obtained from extracted material by preparative electrophoresis in polyacrylamide gel. The procedure of preparative electrophoresis and electroclution of glycoprotein fractions was carried out as described in [8]. Isolated hen glycophorin fractions were used for re-electrophoresis, analysis of carbohydrate composition and immunization of rabbits.

Analytical electrophoresis was carried out in polyacrylamide slab gels in the buffer system of Laemmli [9] with gradient of acrylamide concentration 5—17.3 %. Gels were stained consequently by periodic acid — Shiff reagent (PAS) [10] and the-

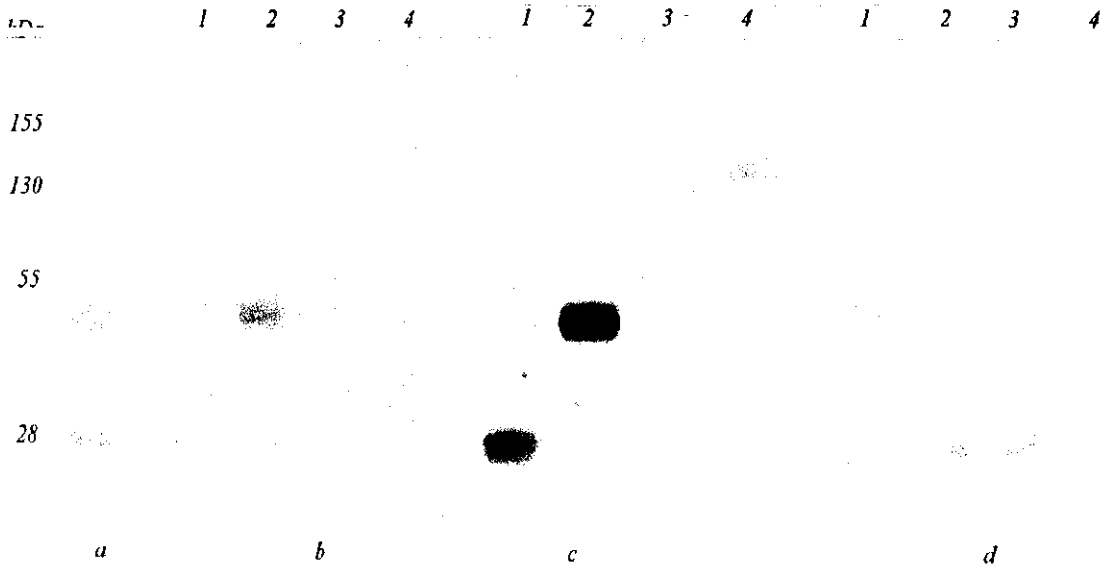
reafter with Coomassie Brilliant Blue R-250. In some experiments electrophoregramms were transferred onto nitrocellulose sheets (Millipore, HA type, 0.45 μm) by electroblotting under conditions described by Towbin et al. [11]. Glycoprotein fractions on blots were detected with HRP-labelled peanut agglutinin (PNA) after desialylation of blots by incubation in 0.05 M sulfuric acid for 50 min at 80 °C.

For immunoblotting the following buffers were used: PBS with 0.05 % Tween-20, pH 7.4 (PBS-T); and 0.05 M Tris-HCl buffer, 0.15 M NaCl, 0.05 % Tween-20, pH 8.4 (TBS-T). Treatment of nitrocellulose blots with antibodies included the following steps: 1) incubation in PBS-T for 30 min at room temperature; 2) overnight incubation at 4 °C in the rabbit anti-hen glycophorin antiserum diluted 1:40 with PBS-T; 3) washing with TBS-T; 4) incubation in hen anti-rabbit IgG antibodies conjugated with HRP in concentration 2 μg/ml in TBS-T; 5) washing of the unbound anti-IgG antibodies with TBS-T; 6) detection of peroxidase activity by 4-chloro-1-naphtol reagent, as described in [9].

Anti-rabbit IgG antibodies were obtained by immunization of hens with rabbit IgG, purification of antibodies by affinity chromatography, followed by labelling with HRP according to the method of Nakane et al. [12].

Protein was determined by the method of Lowry et al. [13] or the BCA method [14]. Sialic acid was quantified according to Jourdian [15]. Monosaccharides were determined by gas-liquid chromatography (GLC-MS) as alditol acetates [16], after hydrolysis in 4 M trifluoroacetic acid (TFA) for 4 h at 100 °C, reduction with sodium borohydride and peracetylation in pyridine/acetic anhydride 1:1 (v/v) at 100 °C for 35 min. For GLC-MS a Hewlett-Packard 5890 instrument was used, equipped with a mass selective detector 5971A. Separations were performed on a capillary column HP-1 (0.2 mm × 12 m) with a temperature gradient 150—230 °C (8 °C/min).

Results and Discussions. Glycoproteins of hen red blood cells, extracted from isolated plasma membranes with chloroform-isopropanol mixture, resolved in SDS-PAGE into four principal fractions with molecular weights (m. w.) of 28 and 55 kDa as well as 130 and 155 kDa (Figure, *a*). As we have shown earlier [5], the properties of sialoglycoproteins with m. w. of 28 and 55 kDa, including an interaction with lectins, most closely resemble that of human and other mammalian glycophorins.



a — SDS-PAGE of hen red blood cell sialoglycoproteins, 200 μg of substance per lane, staining with PAS-reagent; *b* — sialoglycoprotein fractions obtained by preparative SDS-PAGE, 20 μg of protein per lane, staining with PAS-reagent, thereafter with Coomassi Brilliant Blue R-250; *c* — lectinoblotting with PNA-HRP, monomer (28 kDa) was obtained from dimer (55 kDa) (*1*) and vice versa (*2*) by repetitive preparative electrophoresis, 5 μg of protein per lane; *d* — immunoblotting with rabbit antiserum to 28 kDa glycoprotein, 3 μg of protein per lane. Specimens of glycoproteins with m. w. of 28 kDa (*1*), 55 kDa (*2*), 130 kDa (*3*), 155 kDa (*4*)

The main sialoglycoproteins of hen RBCs with m. w. 28 and 55 kDa were obtained in pure state by preparative SDS-PAGE. During re-electrophoresis of isolated individual fractions the interconvertion of 28 and 55 kDa bands was observed (Figure, *b*). In preparation of 28 kDa fraction small amount of 55 kDa band was always present due to the formation of dimer, as well as in 55 kDa fraction a small band of 28 kDa was formed due to disintegration of dimer. The property of interconvertion was employed for further purification of 28 and 55 kDa glycoproteins by preparative electrophoresis.

Electrophoretically isolated glycoprotein fractions 28 kDa and 55 kDa were submitted to repetitive electrophoresis, during which 28 kDa fractions formed some 55 kDa dimer, while 55 kDa fractions partially decomposed and formed 28 kDa monomeric fractions. Isolated in such way glycoproteins of 28 kDa and 55 kDa were pure enough for analytical investigation (Figure, *c*).

The carbohydrate composition of glycoproteins with m. w. 28 and 55 kDa is presented in the Table. The data distinctly indicates that hen SGPs contain both O-type and N-type oligosaccharide chains, the last are much more numerous, as in human glycophorin A. This is evident from the proportion of Man:GalNAc, which is equal 1.7 in avian glycophorin and only 0.2 in human glycophorin A. Additionally, the proportion of GlcNAc:GalNAc is higher in hen SGPs, then in human glycophorin A (1.6 against 0.3, respectively), which also may be caused by a higher number of N-type glycosidic chains. It may be noted also a higher relation of Gal:GalNAc in hen SGPs, as compared to human glycophorin A, which may be explained by more branched structure of O-glycosidic chains rich in lactosamine fragments in hen SGPs. The quantification of sialic acid indicates, that the number of O-glycosidic chains in hen SGPs is distinctly lower, then in human glycophorin A. According to analytical data carbohydrate component in hen glycophorin consisted about 33 % of masse of molecule.

Polyclonal antibodies against 28 kDa hen glycophorin bound on immunoblots with monomeric and dimeric fraction of the glycoprotein, to a lesser extent with 130 and 155 kDa SGPs also (Figure, *d*). The nature of last SGPs is obscure to us. By relatively high molecular weight they resemble leukosialin, a major SGP of mammalian leukocytes [17]. Nevertheless, binding of these glycoproteins with antibodies against 28 kDa SGP suggests some immunological relations between these glycoproteins. Furthermore, after re-electrophoresis of 130 and 155 kDa glycoproteins weak bands with m. w. 28 and 55 kDa appeared on immunoblots.

The results of investigation of hen RBC sialoglycoproteins showed that SGPs with m. w. of 28 and 55 kDa resembles closely glycophorins of mammals. However, they differ from the last by higher amount of N-glycans and more branched glycosidic chains of O-type.

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Monosaccharide	Fraction 28 kDa		Fraction 55 kDa		Human glycophorin A	
	%*	mol%**	%	mol%	%	mol%
Fucose	1.3	5.8	1.6	6.4	0.5	1.4
Mannose	4.6	18.4	3.9	14.1	1.6	4.3
Galactose	6.0	23.8	7.2	26.1	9.3	24.7
GlcNAc	5.7	18.4	5.9	17.5	3.4	7.2
GalNAc	3.3	10.7	3.9	11.5	10.3	21.9
Sialic acid	10.0	22.8	11.6	24.4	27.2	40.4
Protein	68.8	—	65.7	—	47.7	—

*Sum of carbohydrates and protein estimated by BCA method was taken as 100 %; **number of moles of each carbohydrate per 100 moles of all carbohydrates.

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Порівняльне дослідження вуглеводного компонента глікофоринів курей і людини

Резюме

Одержано сіалоглікопротеїни (СГП) плазматичних мембран еритроцитів курей за допомогою препаративного електрофорезу в поліакриламідному гелі в присутності DS-Na. Досліджено їх вуглеводний склад у порівнянні з глікофорином А людини, а також деякі імунологічні властивості. Показано, що головні СГП з молекулярною масою 28 і 55 кДа є мономерною і димерною формами однієї молекули, які імунологічно близькоспоріднені. Вуглеводний компонент складає біля 33 % молекулярної маси цього глікопротеїну (у глікофорині А людини — 52,3 %). Аналіз моносахаридного складу СГП курей свідчить, що вони містять як О-, так і N-зв'язані олігосахаридні ланцюги. У порівнянні з глікофорином А людини СГП курей вміщують більшу кількість N-глікозидних і меншу кількість О-глікозидних ланцюгів, при цьому останні є більш розгалуженої будови.

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Сравнительное исследование углеводного компонента гликофоринов кур и человека

Резюме

Получены сиалогликопротеины (СГП) плазматических мембран эритроцитов кур с помощью препаративного электрофореза в полиакриламидном геле в присутствии DS-Na. Исследован их углеводный состав в сравнении с гликофорином А человека, а также некоторые иммунологические свойства. Показано, что главные СГП с молекулярной массой 28 и 55 кДа являются мономерной и димерной формами одной и той же молекулы и иммунологически близкородственны. Углеводный компонент составляет около 33 % молекулярной массы этого гликопротеина (в гликофорине А человека — 52,3 %). Анализ моносахаридного состава СГП курицы свидетельствует о том, что они содержат как О-, так и N-связанные олигосахаридные цепи. По сравнению с гликофорином А человека СГП кур содержат большее количество N-гликозидных и меньшее количество О-гликозидных цепей, при этом последние имеют более разветвленную структуру.

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