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THE RELATIONSHIP BETWEEN THE N-ACETYLGALACTOSAMINE CONTENT AND THE BLOOD GROUP Sd^a ACTIVITY OF TAMM AND HORSFALL URINARY GLYCOPROTEIN

Cecilia P.C. Soh, W.T.J. Morgan, Winifred M. Watkins and A.S.R. Donald

Division of Immunochemical Genetics, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

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SUMMARY. Substances with the human blood group Sd^a character occur on red cells and are also present in secretions. In urine Sd^a activity is associated with the Tamm and Horsfall (T-H) glycoprotein. About 4% of Caucasian individuals, whose red cells are Sd^a negative, have a T-H glycoprotein that is without Sd^a activity. The two immunologically distinct forms of T-H glycoprotein have almost identical qualitative and quantitative amino acid and carbohydrate compositions. The only exception is the N-acetylgalactosamine content which falls in the range of 1-2% for preparations from Sd(a+) individuals whereas the level is negligible in the Sd^a inactive preparations. These results strongly indicate that N-acetylgalactosamine makes an important contribution to the Sd^a determinant structure in the T-H glycoprotein.

INTRODUCTION. The blood group property, called Sd^a, first clearly identified by Macvie, et al (1) and Renton, et al (2), is inherited as a dominant character and individuals may be classified into two phenotypes, Sd(a+) and Sd(a-). Considerable variations are observed in the strength of the Sd^a antigen on the red cells of different individuals. Sd^a positive cells from group O or B individuals which react strongly with human anti-Sd^a sera are also agglutinated by the lectins from Dolichos biflorus and Helix pomatia, reagents previously considered to be specific for the blood group A character (3). Moreover the immunodominant sugar in the A determinant, N-acetylgalactosamine, also inhibits the lectin mediated agglutination of Sd(a++) cells, and this finding led to the suggestion that Sd^a, like A, depends upon a terminal N-acetylgalactosamine residue (4,5).

Morton, et al (6,7) reported that substances with Sd^a activity occur in most human secretions, with the greatest concentration in urine and meconium. These workers partially purified the Sd^a active urinary substance and recorded some of its biochemical properties. The results of recent work (8,9), aimed

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at a closer identification of the Sd^a substance in urine, suggested that the Sd^a activity is closely associated with the well-known Tamm and Horsfall urinary glycoprotein (10,11) which is a component of all urines and has been extensively characterised in terms of its physical, chemical and immunological properties (12). Isolation of an Sd^a active T-H glycoprotein free from A or P blood group activity when blood group A glycoprotein or group P glycosphingolipid (globoside) were separately added to urine before salt precipitation of the T-H material and extraction with lipid solvents (9) strengthened the view that the Sd^a activity of the preparations is an integral property of the glycoprotein and does not result from co-precipitation or adsorption from urine of an independent molecular species. These findings indicated that the Sd^a blood group property is a hitherto unrecognised genetic marker on the T-H glycoprotein. The results presented in this communication provide further evidence that the Sd^a determinant is carried on the T-H glycoprotein and reveal a striking difference in the N-acetylgalactosamine content of the two variant forms of T-H glycoprotein which correlates with the Sd^a activity of the preparation.

MATERIALS AND METHODS. Strongly reacting group Sd(a++) red cells were provided by Dr. G.W.G. Bird, Blood Transfusion Centre, Birmingham, U.K. and Dr. S. Sringarm, Army Institute of Pathology, Bangkok, Thailand. Human anti-Sd^a sera and Sd(a-) red cells were supplied by Dr. C. Giles, Blood Group Reference Laboratory, London, U.K. *Dolichos biflorus* lectin was a gift of Dr. G.W.G. Bird. *Helix pomatia* lectin-Sepharose 6MB was purchased from Pharmacia (G.B.) Ltd. and *Phaseolus vulgaris* lectin (Bacto Phytohemagglutin P) from Difco Laboratories, U.S.A. Rabbit anti-human uromucoid serum was obtained from Behringwerke, A.G., Germany.

T-H glycoproteins were prepared from urine collected from persons with the Sd(a+) and Sd(a-) red cell phenotypes. The preparations from individual donors were kept separate throughout the investigation. The glycoprotein was isolated by the procedure of Tamm and Horsfall (10) which involves three precipitation steps with 0.58M NaCl. After the third precipitation the glycoprotein was dissolved in, and thoroughly dialysed against, distilled water. The solution was then centrifuged for 20 min at 30,000g and the clear supernatant was dried from the frozen state.

Amino acid and hexosamine analyses were performed on an amino acid analyser as described by Donald (13). Neutral sugars were determined by g.l.c. according to the procedure of Yang and Hakomori (14). Sialic acid was estimated by the method of Warren (15). SDS polyacrylamide gel electrophoresis was carried out as described by Laemmli (16) and the gels were

stained for protein with Coomassie Blue R250 reagent and for carbohydrate with periodic acid-Schiff (PAS) reagent. Dolichos biflorus lectin was coupled to Sepharose 4B by the method of Pereira and Kabat (17). Haemagglutination inhibition tests were carried out as described previously (18).

RESULTS AND DISCUSSION. Tamm and Horsfall (T-H) glycoproteins were isolated from the urine of three persons of the Sd(a+) red cell phenotype and four of the Sd(a-) red cell phenotype. The glycoproteins were recovered from the urine by the classical method of Tamm and Horsfall (10) and were judged to be essentially homogeneous since they each gave one major band on SDS polyacrylamide gel electrophoresis. The bands formed by different preparations had the same electrophoretic mobility and stained with both Coomassie blue and PAS reagents, indicating their glycoprotein nature. Similarly in double-diffusion tests in agar gel the glycoproteins each gave precipitin lines with a commercial rabbit anti-human urinary mucoid serum and the lines coalesced to give reactions of identity irrespective of whether the preparations were derived from Sd(a+) or Sd(a-) individuals. However, in serological inhibition tests with human anti-Sd^a serum, and in precipitation tests with the Dolichos biflorus lectin, the specimens were sharply divided into two groups; the T-H glycoproteins from Sd(a+) individuals were strongly Sd^a active and precipitated with Dolichos biflorus lectin whereas those from Sd(a-) individuals had no significant Sd^a activity and failed to precipitate with the lectin.

Since the salt precipitation method used for the isolation of T-H glycoprotein results in the formation of giant aggregates of macromolecular subunits (12) the possibility that an independent molecular species with Sd^a activity is adsorbed onto, or coprecipitated with the T-H glycoprotein, has to be excluded. Extraction with lipid solvent mixtures did not lower the Sd^a activity of the T-H preparations (9). The fractionation of T-H glycoprotein from solution in organic solvents such as 95% phenol, formamide or diethyleneglycol (to be described elsewhere) failed to separate the T-H preparation into Sd^a active and inactive components but did result in some lowering of Sd^a serological activity. However, changes in the physical properties of the T-H

preparation had also occurred, as evidenced by the fact that the glycoprotein no longer precipitated in the presence of 0.58M NaCl. It was therefore thought possible that loss of Sd^a activity had arisen from a change in conformation of the molecule. A lowering of the Sd^a activity was also observed on heating solutions of the glycoproteins for 10 min in phosphate-buffered saline (pH 7.0) at temperatures above 70°. Loss of viral inhibitory activity had been observed earlier (11) when solutions of T-H glycoprotein in phosphate buffer were heated at 70°. The atypical physical properties of the T-H glycoproteins preclude the use of many of the procedures that would normally be employed to determine whether a particular structure is an integral part of a macromolecule. Affinity chromatography is, however, a relatively gentle procedure and attempts were therefore made to fractionate the Sd^a active T-H preparations on columns of Dolichos biflorus lectin-Sepharose 4B and Helix pomatia lectin-Sepharose 6MB. A small part of the material retained on the Dolichos column could be eluted with 2% N-acetylgalactosamine and the remainder was eluted with 0.15M NaCl. With the Helix column all the bound material was eluted with 2% N-acetylgalactosamine. All of the fractions, including the material that passed straight through the columns, had identical levels of Sd^a activity when tested on a dry weight basis and also each retained the property of reacting with the rabbit anti-human urinary mucoid serum which is used as a measure of T-H glycoprotein. These experiments thus support the conclusion that the Sd^a blood group determinant is carried on the T-H urinary glycoprotein.

Analytical figures for a T-H preparation from an Sd(a+) individual and an Sd(a-) individual are given in Table 1. The amino acid compositions are very similar to each other and also to the values quoted by others for T-H glycoproteins prepared from pooled urine specimens (12). The carbohydrate analyses are also very similar to each other and to those found previously with the exception of the N-acetylgalactosamine content which was negligible in the T-H preparation from the Sd(a-) individual. Analyses on the seven T-H

Table 1. Analytical composition of T-H glycoproteins isolated from the urine of individuals with the Sd(a+) and Sd(a-) phenotypes

Component	Sd^a active (W.M.)	Sd^a inactive (A.S.)
	% of dry weight	
Fucose	1.13	1.19
Mannose	6.63	6.63
Galactose	5.88	5.61
Glucose	0.37	0.49
N-Acetylglucosamine	9.83	9.92
N-Acetylgalactosamine	1.38	0.07
Sialic acid	3.8	5.5
Total amino acids	65.6	69.0
	Residues per 100 residues amino acids	
Asp	12.4	12.3
Thr	8.3	8.2
Ser	8.8	8.8
Glu	8.6	8.4
Pro	5.0	4.8
Gly	8.2	8.3
Ala	7.1	7.2
½Cys	7.6	8.4
Val	5.8	5.8
Met	2.1	2.1
Ile	2.1	2.1
Leu	7.7	7.6
Tyr	3.7	3.8
Phe	3.2	2.7
His	2.6	2.5
Lys	2.4	2.4
Arg	4.5	4.4

preparations revealed a consistent pattern (Table 2); the content of N-acetylgalactosamine in the Sd^a active preparations fell within the range 0.8-1.5% whereas in the preparations from individuals of the Sd(a-) phenotype the figure was 0.2% or less. Values in this range are probably too low for accurate measurement by the methods used and N-acetylgalactosamine may in fact be absent from A.S. and R.D. T-H glycoproteins. Values for N-acetylgalactosamine in T-H preparations quoted by other workers (12,19) are also in the range of 1-2%. Since these glycoproteins were isolated from pooled urine samples, and theoretically 96% of the donors would have been Sd(a+), this

Table 2. The relationship between the Sd^a phenotype of the donors and the N-acetylgalactosamine content of their T-H urinary glycoprotein

Donor	Sd^a red cell phenotype	Anti-Sd^a in serum	T-H glycoprotein	
			Sd^a activity*	GalNAc content %
W.M.	Sd(a+)	-	0.04	1.38
S.D.	Sd(a+)	-	0.04	1.41
A.Y.	Sd(a+)	-	0.08	0.84
C.G.	Sd(a-)	-	250	0.19
B.M.	Sd(a-)	-	500	0.22
A.S.	Sd(a-)	+	>1000	0.07
R.D.	Sd(a-)	+	>1000	0.05

*Minimum amount of substance (μg/ml) giving complete inhibition of agglutination of Sd(a++) cells by human anti-Sd^a serum.

figure is in general agreement with that found for the Sd^a active T-H glycoproteins in the present investigation.

The strength of the Sd^a antigen on red cells varies greatly in different individuals (3). In our experience the Sd^a activity of the red cells and of the T-H glycoprotein from the same donor are not closely related, for example A.Y. red cells react more strongly with anti-Sd^a than do W.M. or S.D. red cells but the T-H preparation from A.Y. is slightly less active than the other two Sd^a preparations (Table 2). It is interesting to note, however, that the N-acetylgalactosamine content of A.Y. is less than that in W.M. or S.D., and hence there does seem to be a direct correlation between the content of N-acetylgalactosamine and the Sd^a activity of the T-H preparation. The cells of C.G. and B.M. failed to react with anti-Sd^a sera yet there are no detectable Sd^a antibodies in their sera indicating that these donors are not entirely lacking in Sd^a antigenic activity. In fact the T-H glycoproteins from C.G. and B.M. have very weak inhibitory activity (Table 2) and it seems probable that a spectrum of N-acetylgalactosamine levels may be found in the T-H glycoproteins and that these two preparations represent the lower end of this spectrum.

Recently Serafini-Cessi, et al (20) described the interaction of T-H glycoprotein with the leucoagglutinin and haemagglutinin from Phaseolus vulgaris and the inhibition of the precipitation reaction of the leucoagglutinin by N-acetylgalactosamine. On testing the seven T-H preparations with Bacto Phytohemagglutinin P it was found that precipitation was heavier with some preparations than others but the degree of precipitation could not be correlated with the Sd^a activity, or with the N-acetylgalactosamine content, of the glycoproteins. In our studies, however, no attempt was made to separate the leucoagglutinin from the haemagglutinin.

On the evidence currently available the kidney is the only organ of origin of the T-H glycoprotein (12). Since Sd^a activity is also found in other tissues (21), as well as on red cells, it appears that the Sd^a determinant must resemble the ABH and Lewis blood group determinants in that it may be carried on different types of macromolecules, according to the site of synthesis. The finding that T-H glycoprotein in the kidney exists in two immunologically distinct forms, Sd^a active and Sd^a inactive, suggests that the Sd^a property might be a factor of importance to be considered in the selection of renal allografts since statistically one in twenty five recipients will be Sd^a negative.

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