A Comparison of the Carbohydrate Composition of Serumorosomucoid from Man, Cattle, Horse, and Sheep

CURT ENDRESEN and
TERJE B. CHRISTENSEN

Department of Biochemistry, University of Oslo, Blindern, Oslo 3, Norway

Orosomucoid, which is also called a_1-acid-glycoprotein, is one of the serum proteins with the highest content of carbohydrate. In the past, the results of carbohydrate analyses carried out on orosomucoid from the same species have shown considerable variations [1] and this fact may be due to unreliable analytical methods as well as to varying degree of purity of the protein preparations analysed. With the improved methods available for the analysis of carbohydrates and for the testing of protein purity it should now be possible to obtain results of higher accuracy.

In the present work, serum-orosomucoid from the species man, cattle, horse, and sheep have been purified and their carbohydrate compositions were determined using gas chromatographic analysis.

Experimental. Orosomucoids from man, horse, and sheep were isolated from the sera of healthy individuals. Pooled, dried beef serum from Difco Laboratories, U.S.A., was used as the source of bovine orosomucoid. Rabbit antiserum against human orosomucoid was obtained from Behringwerke, Germany. DEAE-cellulose was purchased from Serva, Germany, CM-cellulose from Whatman, England, Dowex 50XX-2 (200/400 mesh) from Fluka, Switzerland, and silylation reagents from Pierce, U.S.A.

Carbohydrate analysis was carried out by gas chromatography of the trimethylsilyl derivatives.[2] Release of the protein bound sugars was effected by two different methods of acid hydrolysis employing, respectively, 1 N HCl (in dry methanol) [2] and 0.01 HCl (aqueous) in the presence of Dowex 50.[3] The latter method was used mainly in order to determine the content of mannose because methanolysis did not give reproducible results for this sugar.

In the protein purification procedure the method of chromatography on CM-cellulose was a modification from that of Bezkorovainy and Winzler.[6] The technique of using barium acetate and ethanol at low temperatures was adapted from the method of Schmid.[7]

The purification of orosomucoid was carried out at 4° unless otherwise is mentioned.

Pure human and bovine orosomucoids were obtained by subjecting 50 ml serum to the following method of purification:

1 A. Chromatography on a column of DEAE-cellulose according to the method of Whitehead *et al.*[8]

2 A. Ammonium sulphate was added to the solution of partially purified orosomucoid (in 0.1 M acetate buffer, pH 4.0) until 50 % saturation. The solution was left for 12 h and the precipitated protein was removed by centrifugation and discarded.

3 A. The supernatant was dialysed against distilled water, freeze-dried and the lyophilized material dissolved in 0.015 M acetate buffer (pH 4.1) and applied to a CM-cellulose column equilibrated with the same buffer. Elution was carried out with 0.025 M acetate buffer (pH 4.1) using a sodium chloride gradient from 0.03 M to 0.10 M, and this gave rise to two protein peaks. Examination of the fractions by disc electrophoresis revealed that the first peak consisted of pure orosomucoid. The appropriate fractions were pooled, dialyzed against distilled water and freeze-dried. The yield of pure orosomucoid was approximately 15 mg.

Because the above method did not give orosomucoid of the same high purity from horse and sheep sera the following modified procedure was used. 50 ml serum was used as starting material.

1 B. Identical to 1 A.

2 B. Identical to 2 A except for the use of 60 % saturation of ammonium sulphate.

3 B. After dialysis of the supernatant against distilled water and freeze-drying, approximately 20 mg protein was dissolved in 10 ml of 0.1 M acetate buffer (pH 4.0) which was 0.02 M with respect to barium ions. Ethanol was added to a concentration of 20 % (v/v) and after 12 h at $-5°$ the precipitated protein was collected by centrifugation and discarded.

4 B. Ethanol was added to the supernatant to increase the concentration to 40 % (v/v) and the solution left at $-5°$ for 12 h. The precipitated protein, which was collected by centrifugation and carefully dried, was shown to consist of pure orosomucoid. The yield was approximately 15 mg. The various orosomucoid preparations showed reactions of identity by double diffusion [4] in agar against anti human orosomucoid serum. Each orosomucoid preparation moved as a single band

Table 1. Carbohydrate composition of orosomucoid from man, cattle, horse, and sheep. Approximately 3 mg protein was used for each analysis and D-mannitol was used as internal standard. The results given are the average of five analyses and the S.D. was calculated from the five figures obtained. Glucosamine and sialic acid were quantitated using *N*-acetylglucosamine and *N*-acetylneuraminic acid, respectively, as standards. a: hydrolysis by methanolic HCl. b: hydrolysis by aqueous HCl. The results are expressed as g carbohydrate (±S.D.) per 100 g protein.

Carbohydrate		Man	Cattle	Horse	Sheep
Fucose	a	1.3±0.3	1.4±0.3	0.9±0.2	0.7±0.2
	b	1.0±0.1	1.3±0.2	1.0±0.2	0.7±0.1
Mannose	b	6.2±0.4	6.5±0.2	5.7±0.2	5.9±0.2
Galactose	a	9.2±0.5	9.7±1.2	8.4±0.7	8.5±0.8
	b	8.7±0.3	9.2±0.3	9.2±0.6	8.3±0.3
N-Acetyl-glucosamine	a	13.5±0.9	14.4±1.5	14.6±0.9	13.5±1.3
Sialic acid	a	11.5±1.1	10.5±0.8	9.7±1.0	11.2±1.1
Total carbohydrate		41.3	42.2	39.7	39.7

Table 2. Carbohydrate composition of orosomucoid; data taken from the literature. The figures represent g carbohydrate per 100 g protein.

Carbohydrate	Man [1]	Cattle [9]	Horse [10]	Sheep [11]	Chimpanzee [12]	Rat [13]	Rabbit [14]
Fucose	0.7– 1.5	1.5		0.5	1.1	0.4	0.4
Mannose	4.7– 6.5			5.55	4.8		8.7
Galactose	6.5–11.2			5.55	6.7		9.5
Total hexose	11.2–17.7	14.4	14.0	11.1	11.5	15.3	18.3
N-Acetyl-glucosamine	12.2–15.3				13.4		
N-Acetyl-galactosamine				11.2	1.2		
Total hexosamine		10.2	11.5		14.6	8.3	13.6
Sialic acid	10.8–14.7	12.5	10.8	13.5	11.9	10.0	15.6
Total carbohydrates	34.9–49.2	38.6	36.3	36.3	38.9	34.0	47.9

on disc electrophoresis, indicating homogeneity, and had the same rate of migration as human orosomucoid.

Discussion. Our results (Table 1) show that the carbohydrate compositions of the four types of orosomucoid analysed are very similar. This does not exclude the possibility that the structures and numbers of carbohydrate units may show species differences. In fact, since the molecular weights of orosomucoid from man and sheep are 44 100 [15] and 34 000,[11] respectively, it follows that the carbohydrate units must differ with regard to structure and/or number of units per protein molecule.

The differences in fucose content are quite large in a relative sense, but the inaccuracies involved in the analysis of

this minor constituent make it uncertain whether the differences are real.

The quantity and types of acyl substituents on the sialic acid residues may also be different in the four orosomucoids studied because the analytical methods used do not yield information on this point. The same reservation applies to glucosamine but in this case the general occurrence of *N*-acetylglucosamine makes such differences less likely.

A comparison with other species (Table 2) shows that orosomucoid from chimpanzee has a carbohydrate composition very similar to the preparations analysed in this investigation whereas orosomucoids from rabbit and rat have distinctly higher and lower carbohydrate content, respectively.

We would like to thank professor S. G. Laland for providing excellent laboratory facilities.

1. Jeanloz, R. W. and Gottschalk, A. *Glycoproteins*, Elsevier, Amsterdam 1966, p. 362.
2. Bolton, C. H., Clamp, J. R. and Hough, L. *Biochem. J.* **96** (1955) 5C.
3. Lehnhardt, W. F. and Winzler, R. J. *J. Chromatog.* **34** (1968) 471.
4. Wadsworth, C. *Int. Arch. Allergy* **10** (1967) 355.
5. Davis, B. J. and Ornstein, L. *Ann. N.Y. Acad. Sci.* **121** (1964) 404.
6. Bezkorovainy, A. and Winzler, R. J. *Biochim. Biophys. Acta* **49** (1961) 559.
7. Schmid, K. *J. Am. Chem. Soc.* **75** (1953) 60.
8. Whitehead, P. H. and Sammons, H. G. *Biochim. Biophys. Acta* **124** (1966) 209.
9. Bezkorovainy, A. and Doherty, D. G. *Nature* **195** (1962) 1003.
10. Got, R., Bourrillon, R. and Cornillot, P. *Biochim. Biophys. Acta* **58** (1962) 126.
11. Campbell, B. J., Schneider, A. L., Howe, D. N. and Durand, D. P. *Biochim. Biophys. Acta* **148** (1967) 137.
12. Yu-Teh Li and Su-Chen Li. *J. Biol. Chem.* **245** (1970) 825.
13. Kawasaki, T., Koyama, J. and Yamashina, I. *J. Biochem. (Tokyo)* **60** (1966) 554.
14. Marcais, J., Nicot, C. and Moretti, J. *Bull. Soc. Chim. Biol.* **52** (1970) 741.
15. Smith, E. L., Brown, D. M., Weimer, H. E. and Winzler, R. J. *J. Biol. Chem.* **185** (1950) 169.

Received April 21, 1972.

Ion Binding in Liquid Crystals Studied by NMR IV.* ^{23}Na NMR of Macroscopically Aligned Lamellar Mesophases

GÖRAN LINDBLOM

Division of Physical Chemistry 2, The Lund Institute of Technology, P.O. Box 740, S-220 07 Lund, Sweden

Recently it was demonstrated [1,2] that the ^{23}Na NMR spectrum of two anisotropic lyotropic liquid crystalline phases consists of three equally spaced peaks, reflecting static quadrupolar interactions. In these cases a first order quadrupole splitting was observed. Previously, in a similar liquid crystalline phase, also second order quadrupole effects have been reported.[3] Furthermore, first order quadrupole splittings with $^{7}Li^{+}$ and $^{23}Na^{+}$ NMR have recently been reported [4] for hydrated oriented deoxiribonucleic acid (DNA) fibers. We are currently investigating ionic quadrupole splitting and relaxation in lyotropic mesophases in our laboratories with the purpose of getting a better understanding of the binding and motion of small ions in liquid crystals and biological model membrane systems. Quadrupole splittings for a large number of nuclei such as ^{7}Li, ^{14}N (as NH_4^+), $^{35}Cl^-$, $^{37}Cl^-$, $^{39}K^+$, $^{79}Br^-$, $^{81}Br^-$, and $^{133}Cs^+$ have been observed. Except for F^- (^{19}F has no quadrupole moment) the method is thus generally applicable to the study of alkali and halide ions. A report concerning investigations on unoriented systems will be given shortly. In the present communication macroscopically aligned mesophases will be considered and experimental observations with the lamellar mesophases of the ternary systems sodium octanoate/decanol/water and sodium octanoate/octanoic acid/water will be presented. (Phase diagrams for these systems have been presented by Ekwall [5].) Previously, proton,[6] and deuteron [7,8] magnetic resonance and proton pulsed NMR measurements [9] have been performed on oriented liquid crystalline lyotropic phases.

The samples were prepared by mixing the appropriate amounts of the components in sealed test tubes above the transition to

*For part III. See Ref. 1.