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THE STRUCTURE OF THE CARBOHYDRATE UNITS OF THE 36K GLYCOPROTEIN DERIVED FROM THE LUNG LAVAGE OF A PATIENT WITH ALVEOLAR PROTEINOSIS BY HIGH RESOLUTION ^{1}H-NMR SPECTROSCOPY

H. Munakata[a], R.B. Nimberg[a], G.L. Snider[a,b], A.G. Robins[a,b], H. Van Halbeek[c], J.F.G. Vliegenthart[c], and K. Schmid[a]

[a]Department of Biochemistry and [b]The Pulmonary Medicine Section, Boston University School of Medicine and The Veterans Administration Medical Center, Boston, MA 02118 and [c]Department of Bio-Organic Chemistry, University of Utrecht, NL-3522 AD Utrecht, The Netherlands

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Earlier studies on the carbohydrate moiety of the 36K glycoprotein derived from the lung lavage of patients with alveolar proteinosis suggested the existence of 2 unusual biantennary N-glycosidic heteroglycans. These glycans did not possess the trimannosyl core and the diacetylchitobiose. Reinvestigation of these heteroglycans by 500-MHz ^{1}H-NMR spectroscopy, however, demonstrated the chemical structure of the major carbohydrate components of this protein to be typical triantennary glycans.

In alveolar proteinosis, a disease of unknown pathogenesis, the alveoli and terminal bronchioles of the lung are filled with excessive amounts of periodate-Schiff-positive amorphous material. Employing the lung lavage from patients with this disease, Lynn and his coworkers purified and characterized several glycoproteins with molecular weights of 36K, 62K, 80K and 250K (1-5). These investigators demonstrated that the 80K glycoprotein is the precursor of the 62K glycoprotein which can be converted to the 36K glycoprotein by proteolysis (6). The polypeptide moiety of the latter glycoprotein has been extensively characterized by Lynn et al (6). However, there are some ambiguities relative to the carbohydrate portion of the 36K glycoprotein. This protein was reported to possess two biantennary heteroglycans which did not contain either the expected trimannosyl core nor the diacetylchitobiose of N-glycosidic heteroglycans (5). Therefore, a reinvestigation of these oligosaccharide structures appeared appro-

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priate. In this paper we present the structures of the carbohydrate units derived from the 36K glycoprotein utilizing high resolution ^{1}H-NMR spectrometry.

MATERIALS AND METHODS

Lavage (5 liters) was obtained from the lung of a patient with alveolar proteinosis, mixed with protease inhibitors (0.1 M ε-aminocaproic acid, 0.005 M benzamidine-HCl, 0.005 M EDTA and 0.001% sodium azide) and kept frozen until used. The lavage was centrifuged at 20,000 x g for 30 minutes. The precipitate was collected and washed two times with saline and then with distilled water, suspended in water and extracted three times with 400 ml of chloroform-methanol (2:1).

Reduction and Alkylation of the delipidated precipitate was carried out as described by Lynn et al (3). For the fractionation of the modified lavage proteins Sephadex G-200 and G-150 gel filtration was employed. For the preparation of the glycopeptide fraction the modified 36K protein was exhaustively digested first with papain and then with pronase E followed by gel filtration through Sephadex G-25 to remove amino acids and peptides.

Chemical Analysis was carried out after appropriate hydrolysis of the glycoprotein or glycopeptides (const. boiling 6 N HCl, 110°C, 24 h, sealed tube, N_2) with the aid of a Beckman amino acid analyzer, Model 119 CLW/126. Fucose and neutral hexoses were determined by gas-liquid chromatography as sugar alcohol acetates (7,8). For the latter analyses the glycoprotein and glycopeptides were hydrolyzed with 2 N HCl at 100°C for 4 h in sealed tubes under nitrogen (9). Hexosamines were quantified after hydrolysis of the samples with 4 N HCl at 110°C for 6 h. Sialic acid was determined by the thiobarbituric acid method (10).

For ^{1}H-NMR Spectrometry approximately 1 mg of the glycopeptide fraction was repeatedly exchanged in D_2O (99.96 atom% D, Aldrich, Milwaukee) with intermediate lyophilization and analyzed with a Bruker 500-MHz WM-500 spectrometer operating in the Fourier transform mode at a probe temperature of 300°K. Resolution enhancement of the spectrum was achieved by Lorentzian to Gaussian transformation from quadrature phase detection. Chemical shifts are given relative to sodium-2,2-dimethyl-2-silapentane-5-sulphonate (indirectly to acetone in D_2O : δ = 2.225 ppm) (11).

RESULTS AND DISCUSSION

Isolation of the reduced and alkylated lung lavage glycoproteins by gel filtration through Sephadex G-200 and G-150 yielded a preparation which revealed a single band on SDS-polyacrylamide gel electrophoresis. The position of the protein corresponded to a molecular weight of 36K. The amino acid and

Table I

CHEMICAL COMPOSITION OF THE 36K GLYCOPROTEIN DERIVED FROM THE LUNG LAVAGE OF PATIENTS WITH ALVEOLAR PROTEINOSIS

Residue[a]	Glycoprotein described in the present study[b]	Glycoprotein described by Lynn et al.(6)[c]
Hyp	8	5
Asp	28	30
Thr	16	13
Ser	17	13
Glu	37	32
Pro	23	21
Gly	45	49
Ala	20	14
Cys	4	4
Val	19	14
Met	2	3
Ile	12	16
Leu	23	27
Tyr	9	10
Phe	10	13
Lys	10	14
His	6	8
Arg	12	18
Fuc	1	1
Man	3	5
Gal	4	4
GlcN	8	7

[a] Expressed as residues per mole of protein.

[b] Isolated from the lavage of a single patient.

[c] Derived from the lavages of several patients.

carbohydrate compositions of the purified glycoprotein was similar to that reported by Lynn et al. (5,6) (Table I).

The 500-MHz ^{1}H-NMR spectrum of the glycopeptide mixture (Fig. 1) derived from the 36K glycoprotein demonstrated the presence of predominantly triantennary N-glycosidic carbohydrate units along with a small percentage of tetraantennary carbohydrate chains. These heteroglycans possess the characteristic trimannosyl core which is linked at the reducing end to diacetylchitobiose that in turn forms the bond with the asparagine residue of the peptide chain (12). No biantennary N-glycosidic

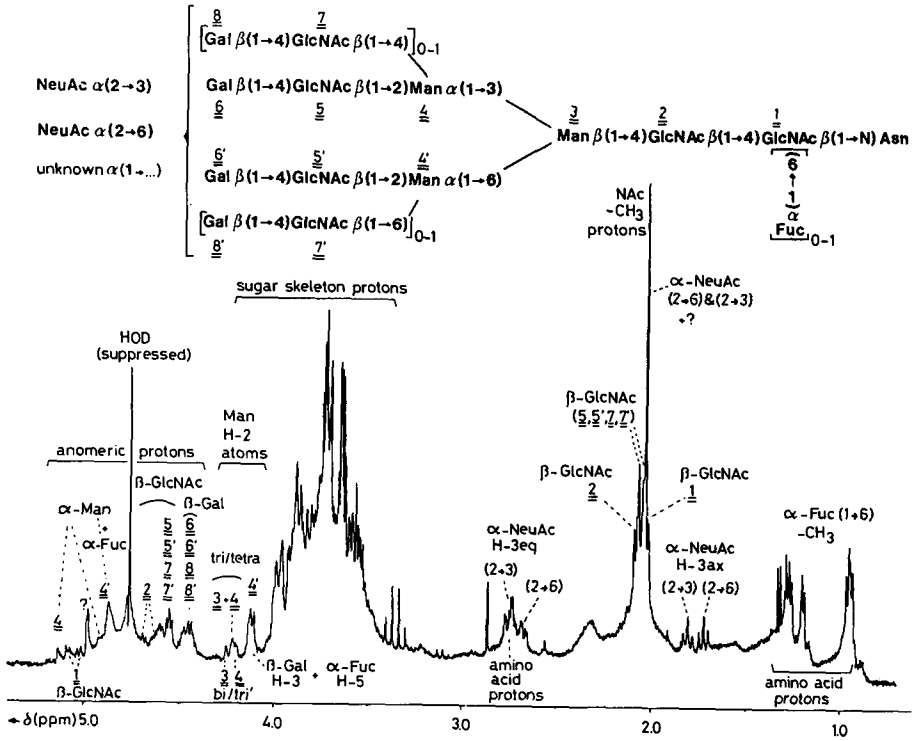

Fig. 1. Structural reporter group regions of the resolution enhanced 500-MHz ^{1}H-NMR spectrum of a mixture of predominant tri- and small amounts of tetraantennary asparagine-linked glycans of the N-acetyllactosamine type. This glycopeptide mixture was derived from the lung lavage of a patient with alveolar proteinosis.

nor O-glycosidic glycans were detected. As to the Sia-Gal linkages, 2 types were revealed: the α(2→3) and α(2→6) bonds. These two linkages were found to be present in a ratio of approximately 1 : 1.

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