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Author	友田, 正司(Tomoda, Masashi) 北村, 正子(Kitamura, Masako)
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Pseudomyxoma peritonei による粘質物（第2報）
シアロムコ多糖体の単離と性質*

友田正司，北村正子

The Mucous Substance in Pseudomyxoma Peritonei. II.
Isolation and Properties of a Sialomucopolysaccharide.*

Masashi TOMODA and Masako KITAMURA

The glycoprotein obtained from a peritoneal pseudomyxomatous gelatinous substance was treated with a protease and fractionated with ethanol, and a fraction rich in sialic acid was purified by the gel filtration on Sephadex G-50 and G-100. The sialomucopolysaccharide so obtained is an active Le^a-specific substance and it contains 31.8% of N-acetyl neuraminic acid, 18.2% of D-galactosamine, 13.4% of D-glucosamine, 13.7% of D-galactose, 3.7% of L-fucose and 12.0% of peptide composed of ten kinds of amino acids. It is concluded that the both hexosamines exist as N-acetyl derivatives. The major part of peptide moiety is taken by threonine, proline and phenylalanine. The molar ratio of component carbohydrates and amino acids was as follows; sialic acid: galactosamine: glucosamine: galactose: fucose: threonine: proline: phenylalanine was about 4 : 4 : 3 : 3 : 1 : 1 : 1 : 0.8.

Introduction

In the previous paper[1], the chemical properties of a glycoprotein isolated from the gelatinous substance of a patient of Pseudomyxoma peritonei were described. There are a few reports[1~4] on the glycoprotein obtained from peritoneal pseudomyxomatous gelatinous mucus, but in point of homogeneity, the problem of their composition had not been solved. The glycoprotein has now been treated with a protease, fractionated with ethanol, and the sialic acid-rich fraction was treated on Sephadex columns and a sialomucopolysaccharide was obtained in good yield. This substance has been shown to be homogeneous on paper-electrophoresis, and its chemical properties are described in the present paper.

Materials and Methods

Material : The glycoprotein was isolated as described previously.[1]

Enzymic Digestion and Ethanol Fractionation : 10 g of the glycoprotein was made in

* Japan. J. Exp. Med., **36**, 335 (1966) に発表

1) Part I ; M. Tomoda and M. Kitamura : Japan. J. Exp. Med., **35**, 255 (1965).
2) S. Takahashi and K. Turumi : Fukushima J. Med. Sci., **3**, 1 (1956).
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suspension with 200 ml of 15 mM calcium acetate solution, then 0.2 g of Pronase (Kaken-Kagaku Co.) was dissolved and the mixture was layered with toluene and incubated at 37°C for 24 hrs. The resulting solution, which contained some insoluble material, was adjusted to about 20% (v/v) concentration with ethanol and the separated precipitate was removed. The supernatant liquid was prepared to 80% ethanol concentration and the formed precipitate was obtained by centrifuging, and freeze-dried. The enzymic digestion was repeated and the fraction separated between 20 and 50% of ethanol was obtained. The digestion for 6 hrs. and fractionation process were similarly carried out once more. The fraction finally obtained was freeze-dried. (yield, 1.6 g).

Gel Filtration on Sephadex Columns : Sephadex G-50 and G-100 (Pharmacia Co.) were stirred and decantated to remove fines and repeatedly washed with distilled water. For the separation of 1 g of the sample, a Sephadex column (3×100 cm) was used, and fractions were collected at 20 ml for 1 hour. The mucopolysaccharides in eluates were measured by resorcinol method[5] (E at 580 mμ) and phenol-sulfuric acid method[6] (E at 490 mμ). Peptides were estimated by ninhydrin method[7] (E at 570 mμ).

Paper Electrophoresis : This was carried out with Tôyô-Roshi No. 51 paper and the following buffers : A, 0.1 M borate buffer of pH 9.0; B, pyridine acetic acid buffer of pH 4.3 (pyridine : acetic acid : water, 1 : 2 : 97); C, 0.1 M formate buffer of pH 3.1. The condition was 10 volt/cm for 2 hrs. Toluidine blue[8], periodate-permanganate[9] and periodate-*o*-toluidine reagents were used for detection.

Paper Chromatography of Sugars : The procedure was similar to that described in the previous paper[1], but the following solvent systems were used : A, bntan-1-ol : pyridine : water (5 : 3 : 1, by vol.); B, butyl acetate : acetic acid : ethanol : water (3 : 2 : 1 : 1, by vol.); C, butan-1-ol : propan-1-ol : 0.1 N-hydrochloric acid (1 : 2 : 1, by vol.); D, water saturated collidine.

Determination of Sugars : Galactose was determined by phenol-sulfuric acid method[6] and carbazole method[10]. Fucose was measured by thioglycolic acid method[11]. Quantitative hexosamine analysis was carried out by the method of Pearson[12] using Amberlite CG-120 column with 0.3 N-hydrochloric acid after 2 N-hydrochloric acid hydrolysis. For the quantitative analysis of sialic acid, resorcinol method[5] was employed. Determination of acetyl group was carried out by a titration method

5) L. Svennerholm : Acta Chem. Scand., **12**, 547 (1958).
6) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith : Anal. Chem., **28**, 350 (1956)
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10) F. B. Seibert and J. Atno : J. Biol. Chem., **163**, 511 (1946).
11) M. N. Gibbons : Analyst, **80**, 268 (1955).
12) C. H. Pearson : Biochem. J., **88**, 540 (1963).

after hydrolysis[13].

Paper Chromatography and Determination of Amino Acids: These were done as described previously[1]. The determination was kindly carried out by Mr. I. Motoyama, the Institute for Infectious Diseases, University of Tokyo.

Results

Gel Filtration: Fig. 1-1 shows the gel filtration on a Sephadex G-50 column of the preparation by the enzymic digestion and ethanol fractionation. The ratio of the concentration of sialic acid to neutral sugars in each fraction was generally equal. The latter half of sugar-containing fractions showed the existence of peptides. The eluates obtained from tubes 12 to 25 in Fig. 1-1 were collected and concentrated in vacuo. After one more gel filtration on a Sephadex G-50 column, re-separation of the fraction with Sephadex G-100 was carried out. In this case no ninhydrin-positive substance was detected and a sialomucopolysaccharide was obtained from tubes 16 to 24 in Fig. 1-2. The yield of this was 0.3 g from 1 g of the starting fraction. The sialomucopolysaccharide was obtained as white powder and easily soluble in

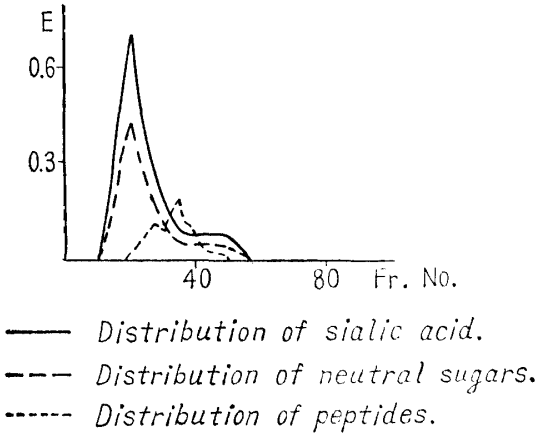


Fig. 1-1.
Gel filtration on a Sephadex G-50 column.

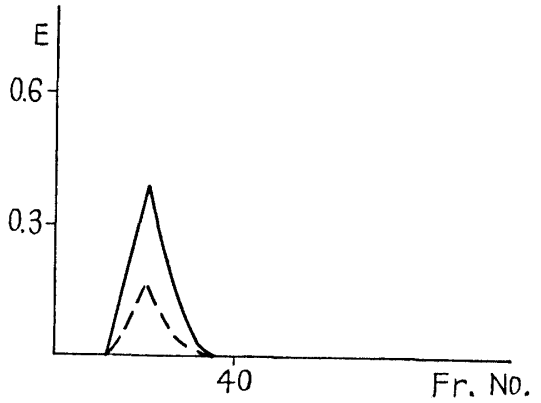
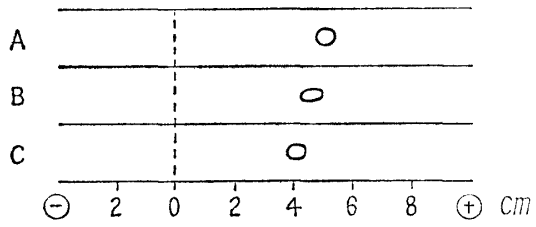


Fig. 1-2.
Gel filtration on a Sephadex G-100 column.



A : *borate buffer.*
B : *pyridine-acetic acid buffer.*
C : *formate buffer.*

Fig. 2. Paper electrophoresis of the mucopolysaccharide.

13) S. Mizukami and T. Ieki : Yakugaku Zasshi, **76**, 467 (1956).

water, giving a viscous solution, $[\alpha]_D^{20} = -8°$ (c=2, H_2O). It doesn't show any protein reaction and absorption maximum in ultraviolet region.

Paper Electrophoresis : Homogeneity of the sialomucopolysaccharide obtained by the gel filtrations on Sephadex was shown in Fig. 2. Detectable spots with the three kinds of reagents were found the same position in the each condition.

Sugar Components : Table 1 shows the component sugars of the sialomucopolysaccharide and their Rf values revealed by paper chromatography.

Table 1. Rf values of sugar components

	Solvent A	Solvent B	Solvent C	Solvent D
L-Fucose	0.46	0.45	0.41	0.56
D-Galactose	0.27	0.25	0.23	0.46
D-Glucosamine-HCl	0.21	0.16	0.20	0.37
D-Galactosamine-HCl	0.18	0.15	0.18	0.30
N-Acetyl neuraminic acid	0.08	0.35	0.37	

Sugar Composition : The quantitative analytical values of sugars are shown in Table 2.

Table 2. Sugar composition and molar ratio

	Composition (%)	Molar ratio
N-Acetyl neuraminic acid	31.8	3.90
D-Galactosamine	18.2	4.04
D-Glucosamine	13.4	2.97
D-Galactose	13.7	3.02
L-Fucose	3.7	1
Acetyl	14.2	
Nitrogen	5.36	

Amino Acid Composition : The amino acid composition of the sialomucopolysaccharide is given in Table 3.

Table 3. Amino acid composition (%)

Glycine	0.27	Serine	0.58
Alanine	0.10	Threonine	3.00
Valine	0.51	Phenylalanine	3.19
Isoleucine	0.61	Clutamic acid	0.60
Leucine	0.17	Proline	3.01

Discussion

The sialomucopolysaccharide now obtained has the molar proportions of N-acetyl neuraminic acid : D-galactosamine : D-glucosamine : D-galactose : L-fucose to be

4 : 4 : 3 : 3 : 1. It will be able to presume that the both hexosamines are N-acetyl derivatives on the result of determination of acetyl group. As described in the previous paper, the original glycoprotein has the higher content of sialic acid than those reported by Odin[3] and Takahashi & Turumi[2], but the molar ratio of carbohydrate constituents of the present sialomucopolysaccharide is roughly similar to that of Odin's the second type sialomucin. Hexosamine : hexose : fucose : sialic acid is about 1 : 0.43 : 0.14 : 0.56. The molecular weight of the preparation gave a value of 124,000 by the measurement of osmotic pressure.

It is well known that many mucopolysaccharides in the human epithelial mucus secretions possess blood group activity. In the carbohydrate moiety of all blood group substances, D-galactose, L-fucose and the N-acetyl derivatives of D-glucosamine and D-galactosamine have been identified. In addition to these components, the considerable high content of sialic acid has also been found in the preparations from ovarian cyst fluid[14,15]. The substance present obtained by the authors showed blood group Le^a activity of $1 : 8 \times 10^3$ as minimum giving inhibition and no group A, B and H serological activity.

Adams[4] obtained the two fractions from pseudomyxomatous mucin with phenol extraction and trypsin treatment, but the analytical data of these fractions differ from those of our sialomucopolysaccharide especially in glucosamine/galactosamine ratio. He reported the presence of glycosidic linkage of carbohydrate to the hydroxyl groups of threonine and serine in various animal mucin.

The sialomucopolysaccharide obtained by us contained 12% of peptides. Ten kinds of amino acids were detected but only three kinds of amino acids, i. e., threonine, proline and phenylalanine formed about 80% amounts of the peptide moiety. As compared with the component carbohydrate, their molar ratios were as follows; fucose : threonine : proline : phenylalanine was 1 : 1.06 : 1.10 : 0.78. There is every possibility of linking the primary hydroxyl group of threonine to carbohydrates but the mutual linking sites of peptides and carbohydrates remain unsolved.

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15) N. Hiyama : Biochem. & Med. of Mucopolysac., 161 (1962, Maruzen, Tokyo.).