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CHARACTERIZATION OF A NEW TYPE OF GLYCOPROTEIN SACCHARIDES CONTAINING POLYSIALOSYL SEQUENCE

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SUMMARY The occurrence of polysialosyl sequence has been shown for the first time in the carbohydrate units of glycoprotein isolated from the eggs of rainbow trout. The saccharide chains accounting for 80 % of the weight of the glycoprotein were O-glycosidically linked to the peptide moiety. The reduced saccharides obtained by alkaline borohydride treatment of the glycoprotein contain various amounts of N-glycolylneuraminic acid and were fractionated by a column of DEAE-Sephadex. Analyses of the saccharides containing large amounts of N-glycolylneuraminic acid show that 2 → 8 linked homopolymers of more than 15 N-glycolylneuraminic acid residues were attached to the core composed of 2 galactose, 1 N-acetylgalactosamine, and 1 N-acetylgalactosaminitol residues.

INTRODUCTION In carbohydrate chains of glycoproteins so far characterized, sialic acid usually occurs only in the terminal position as a single residue. Recently we reported the occurrence of disialosyl (α-N-glycolylneuraminyl-(2 → 8)-N-glycolylneuraminyl) groups in a glycoprotein isolated from the eggs of rainbow trout and characterized the saccharide units containing the disialosyl groups (1,2). We now report that this glycoprotein also contains 2 → 8 linked homopolymers of more than 15 N-glycolylneuraminic acid residues in its carbohydrate chains. Homopolymers of N-acetylneuraminic acid are known in bacterial products (3,4). This report will be the first to show not only the occurrence of polymeric chains of sialic acid in an animal glycoprotein but also the occurrence of polymers of N-glycolylneuraminic acid in nature.

EXPERIMENTAL A glycoprotein with high content of N-glycolylneuraminic acid was isolated from ovulated eggs of rainbow trout (Salmo irideus) as reported previously (2). The reduced saccharides obtained by alkaline borohydride treatment (M $NaBH_4$ - 0.1 M NaOH, 72 h, 37°) of the glycoprotein were fractionated on a DEAE-Sephadex column equilibrated with 0.01 M Tris-Cl (pH 8.0) by a linear concentration gradient of NaCl.

Total sialic acid was determined by the resorcinol method (5). Free sialic acid released by hydrolysis was determined by the thiobarbituric acid method (6). In both determinations, N-glycolylneuraminic acid was used as the standard. For enzymatic liberation of sialic acid, samples containing 0.02 μmol of sialic acid were incubated with 0.002 unit of neuraminidase from

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Clostridium perfringens (EC 3.2.1.18, Boehringer-Mannheim) in 40 µl of 0.1 M acetate buffer (pH 4.7) for 48 h at 37°. Under these conditions, 95 % of N-acetylneuraminic acid was released from colominic acid (Nakarai chemicals, Ltd). The identification of the type of sialic acid and the determination of other sugars were made as reported previously (1). The type of linkage of sialic acid and the proportion of the terminal to internal sialic acid residues in the saccharide chains were determined by methylation analysis as described previously (2).

RESULTS AND DISCUSSION As shown in Fig. 1, DEAE-Sephadex chromatography of the reduced saccharides from the trout egg glycoprotein resulted in resolution of more than 20 sialic acid-containing peaks. Peaks 1 and 2 contained only monosialosyl species, whereas materials eluted in other peaks contained larger oligosialosyl groups. The ratio of N-glycolylneuraminic acid to N-acetylgalactosaminitol was found to increase with peak number. We are particularly interested in materials showing high ratios of N-glycolylneuraminic acid to N-acetylgalactosaminitol, and the composition of those eluted in each peak from 15 to 22 is given in Table 1. Each saccharide chain was composed of 1 mol each of N-acetylgalactosamine and N-acetylgalactosaminitol, 2 mol of galactose, less than 1 mol of fucose, and 10 - 24 mol of N-glycolylneuraminic acid. More than 90 % of the sialic acid in each fraction was released by neuraminidase under the conditions described, indicating that the linkage of the sialic acid is of α configuration and that the sialic acid was not substituted by other sugars and/or other alkali-stable substituent(s). From the

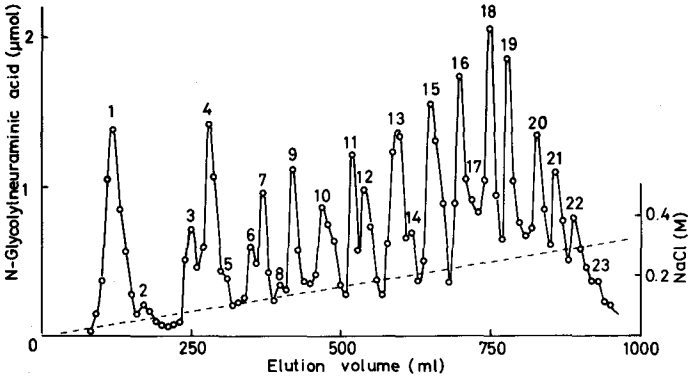

Fig. 1. DEAE-Sephadex chromatography of reduced saccharides obtained by alkaline borohydride treatment of trout egg glycoprotein.

Table 1. Composition of reduced saccharides containing large amounts of N-glycolylneuraminic acid obtained by DEAE-Sephadex chromatography.

	Peak number					
	15	16	19	20	21	22
N-Acetylgalactosaminitol	1.0[a]	1.0	1.0	1.0	1.0	1.0
N-Acetylgalactosamine	1.0	1.0	1.1	1.0	1.0	0.9
Galactose	2.1	2.2	2.5	2.4	2.6	2.6
Fucose	0.3	0.4	0.1	0.1	0.1	0
N-Glycolylneuraminic acid	10.7	11.8	14.4	17.1	21.7	24.0

a. Molar ratios are given relative to N-acetylgalactosaminitol.

sugar composition, it is suggested that each saccharide chain has a common core structure composed of 2 mol of galactose and 1 mol each of N-acetylgalactosamine and N-acetylgalactosaminitol. Various numbers of sialic acid residues, most probably in polymeric forms, are linked to the core at one or multiple positions. The type of the linkage of sialic acid and the degree of polymerization of the sialosyl groups were studied by methylation analysis. Yield of 8-O-acetyl derivatives of neuraminic acid relative to the fully methylated one in each fraction was determined by gas liquid chromatography and the results are given in Table 2. Since 8-O-acetyl derivatives arise from 2→8 linked internal residues of sialic acid whereas the fully methylated one arises from the terminal sialosyl group, the ratio of these derivatives is indicative of the size of polysialosyl chains. It should be noted,

Table 2. Ratio of 8-O-acetyl derivatives[a] of neuraminic acid[b] relative to the fully methylated one.

Peak number	15	16	19	20	21	22
Ratio	3.1	5.0	6.9	9.5	14.6	11.0

a. Methyl 8-O-acetyl-4,7,9-O-methyl-N-acetyl-N-methyl and methyl 8-O-acetyl-4,7,9-O-methyl-N-glycolyl-N-methyl neuraminate methyl glycosides.
b. Methyl 4,7,8,9-O-methyl-N-glycolyl-N-methyl neuraminate methyl glycoside.

however, that methanolysis used in the analysis did not give quantitative recovery of the monomeric units as discussed before (2) and the values given in Table 2 may only show the minimum numbers of monomeric units in each polysialosyl chain. Thus colominic acid of molecular weight ca. 10,000 gave this value of only about 10. It is therefore concluded that in some of the saccharide units of the trout egg glycoprotein, highly polymeric sequences of N-glycolylneuraminic acid of more than 15 residues linked each other by $\alpha 2 \rightarrow 8$ linkage are attached to the core oligosaccharides.

At present we do not know the biological significance of the occurrence of saccharide chains having various sizes of oligo- or polysialosyl groups. It should be emphasized that the variation in the size of polysialosyl groups does not occur during isolation. We could not detect free N-glycolylneuraminic acid in amounts sufficient to indicate the degradation of the intact glycoprotein or the reduced saccharides derived from it in any of the fractions. The results of the present study may add another novel feature to the unique chemical structure of the trout egg glycoprotein.

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REFERENCES

1. Inoue, S., and Iwasaki, M. (1978) Biochem. Biophys. Res. Commun. 83, 1018 - 1023.
2. Inoue, S., and Matsumura, G. (1979) Carbohydr. Res. 74, 361 - 368.
3. McGuire, E. J., and Binkley, S. B. (1964) Biochemistry 3, 247 - 251.
4. Liu, T.-Y., Gotchlich, E. C., Dunne, F. T., and Jonssen, E. K. (1971) J. Biol. Chem. 246, 4703 - 4712.
5. Svennerholm, L. (1963) Methods Enzymol. 6, 459 - 462.
6. Aminoff, D. (1961) Biochem. J. 81, 384 - 392.