Fish Egg Polysialoglycoproteins: Structures of New Sialooligosaccharide Chains Isolated from the Eggs of *Oncorhynchus keta* (Walbaum). Fucose-Containing Units with Oligosialyl Groups[†]

Michio Shimamura, Toshiya Endo,[‡] Yasuo Inoue,* Sadako Inoue, and Hideki Kambara

ABSTRACT: A series of acidic oligosaccharide alditols having different neutral core oligosaccharides were isolated from salmon egg polysialoglycoproteins by alkali–borohydride treatment followed by anion-exchange chromatography and Iatrobead chromatography. Their structures were determined by methylation analysis, molecular secondary ion mass spectrometry of underivatized oligosaccharides, and enzymatic desialylation. The molecular secondary ion mass spectra of intact sialooligosaccharides exhibit pronounced quasi-molecular-ion peaks, $(M + H)^+$, $(M + Na)^+$, $(M + 2Na - H)^+$, and/or $(M + K)^+$, as well as some diagnostic sequence ion peaks. Of a number of oligosaccharide alditols, the following are novel: Fucα1→3GalNAcβ1→3Galβ1→4Galβ1→3[(→8NeuGcα2)$_n$→6]GalNAcol (n = 1–6). The proton nuclear magnetic resonance spectra of these oligosaccharides are also reported and discussed.

Polysialoglycoproteins, a novel type of glycoproteins, first isolated from the eggs of rainbow trout (Inoue & Iwasaki, 1978) have also been isolated from the eggs of Pacific salmon, *Oncorhynchus keta* (Walbaum). A neutral oligosaccharide chain and acidic sugar chains have been isolated from the polysialoglycoproteins of salmon eggs by their alkali–borohydride treatment, and the structure of the neutral carbohydrate chain has recently been elucidated as Fucα1→3GalNAcβ1→3Galβ1→4Galβ1→3GalNAcol[1] (Shimamura et al., 1983). This paper deals with the isolation and identification of a homologous series of the major acidic oligosaccharide chains present in salmon egg polysialoglycoproteins. The structures have been determined to be Fucα1→3GalNAcβ1→3Galβ1→4Galβ1→3[(→8NeuGcα2)$_n$→6]GalNAcol by the conventional methods (methylation analysis; enzymatic or chemical degradation followed by chromatographic analyses of the products) as well as by instrumental techniques (secondary ion mass spectrometry and high-resolution ^{1}H NMR spectroscopy). The last two techniques have made possible the analysis of underivatized sialooligosaccharides. In addition to these fucose-containing units, the salmon egg polysialoglycoproteins contain short-core and long-core carbohydrate units with oligosialyl groups. The latter two units have been found to occur in the trout egg polysialoglycoproteins (Nomoto et al., 1982; Iwasaki et al., 1984).

Materials and Methods

Materials and methods followed closely those described in previous papers (Shimamura et al., 1983; Nomoto et al., 1982; Inoue & Matsumura, 1979; Inoue et al., 1982) unless otherwise stated.

Preparation and Fractionation of Oligosaccharide Alditols from Polysialoglycoproteins. The oligosaccharide alditols released by the alkali–borohydride treatment of polysialoglycoproteins (1.3 g) were applied to a 1.5 × 132 cm DEAE-Sephadex A-25 column, preequilibrated with 0.01 M Tris-HCl (pH 7.6) and eluted with 6 L of a 0–0.8 M NaCl linear gradient in 0.01 M Tris-HCl at pH 7.6. A typical elution profile is shown in Figure 1. This procedure not only yields a rather good separation with respect to the number of sialic acid residues but also shows a differentiation within the fraction of oligosaccharides with a given number of sialic acid residues by the types of their core oligosaccharides. Tubes under selected peaks designated as in Figure 1 were separately pooled, desalted, and rechromatographed on DEAE-Sephadex A-25.

Peaks S6, S7, and S9 gave predominantly a single component whereas peaks S3, S4, S10, S11, S13, S14, ... gave two or more compounds as judged by TLC. Separation into homogeneous acidic oligosaccharide alditols was achieved by column chromatography on Iatrobeads (Iatron Chemical Products Co.). The elution profile for S3, monitored by the thiobarbituric acid determination after mild acid hydrolysis, and that monitored by TLC (eluent of 1-propanol–concentrated NH₄OH–H₂O, 6:1:2; resorcinol stain) are shown in Figure 2. Fractions S3-1 and S3-2 were pooled as illustrated in Figure 2. The other acidic oligosaccharide fractions were also purified in this way and designated by two numbers indicating the elution order from the columns of DEAE-Sephadex A-25 and Iatrobeads. Some fractions were purified by rechromatography on Sephadex A-25, and fraction S11, for example, gave two fractions, each of which comprised predominantly one spot on TLC (S11-1 and S11-2 in Figure 3). As a final step in purification, each peak was chromatographed on Sephadex G-25.

Enzymatic Desialylation of Oligosaccharide Alditols. A fraction of purified Fuc-containing units (S6-1, 350 μg) was incubated with 0.5 unit of sialidase (*Arthrobacter ureafaciens*, Nakarai Chemicals, Ltd.) in 1 mL of 0.1 M acetate buffer (pH 4.7) at 37 °C for 24 h. The reaction mixture was applied to a column of DEAE-Sephadex A-25 (Cl⁻), and the neutral oligosaccharide that passed through the column was desalted by gel filtration on Sephadex G-25.

In another experiment, the mixture of oligosaccharide alditols obtained from salmon egg polysialoglycoproteins was subjected to exhaustive sialidase digestion, and the products were applied to a DEAE-Sephadex A-25 column. Two neutral oligosaccharide fractions (F and S) were obtained by Sephadex G-25 column chromatography of the material that passed through the DEAE-Sephadex column (Figure 4A). An acidic
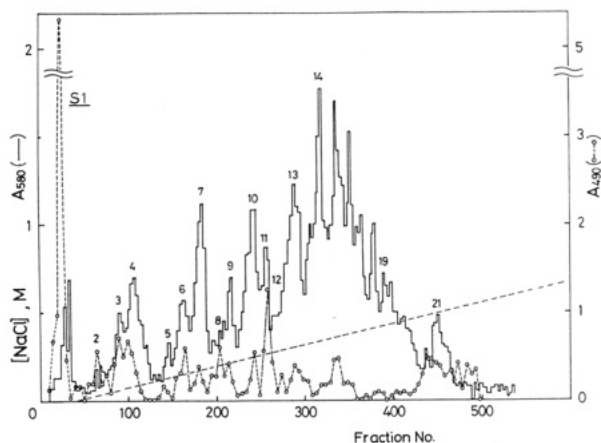
FIGURE 1: Fractionation of oligosaccharide alditols by chromatography on DEAE-Sephadex A-25. Fractions of 7.0 mL were collected and assayed for total hexose (O) and sialic acid (—).
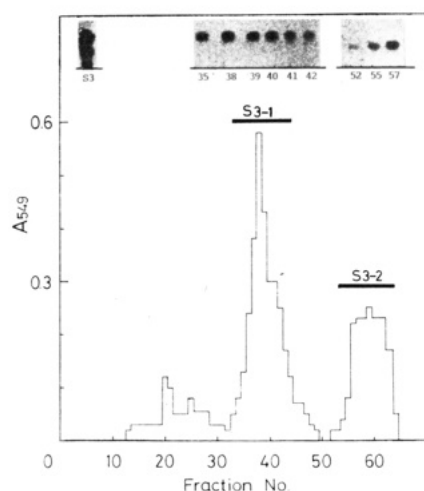

FIGURE 2: Elution pattern from column chromatography of S3. The column consisted of Iatrobeads (0.8 × 57 cm). Elution was effected by 1-propanol–concentrated NH_4OH–H_2O (6:1:2). Material was collected in 1.8-mL fractions at a rate of 4 mL/h. TLC of the sialyloligosaccharide fractions eluted from the Iatrobead column was also shown: A 2–3-μL aliquot from each fraction was spotted on a Kiesel gel 60 plate (Merck), which was then developed with solvent, 1-propanol–concentrated NH_4OH–H_2O (6:1:2), at 4 °C for 16 h. Sialyl compounds were revealed by resorcinol/HCl reagent (heated at 80 °C for 15 min after sprayed).

oligosaccharide (L) was separated from free NeuGc on the DEAE-Sephadex column (Figure 4B).

SIMS. Positive-ion SIMS spectra were obtained with a Hitachi M-80 double-focusing mass spectrometer equipped with a sputtered-ion MS system and an M-003 data processor at Central Research Laboratory, Hitachi, Ltd.

Results and Discussion

Salmon egg polysialoglycoproteins, like trout egg polysialoglycoproteins (Inoue & Iwasaki, 1978, 1980), contain as much as 80% carbohydrate by weight. On the basis of isolation, it was estimated that there are, on the average, about 180 μg [or (4–5) × 10^{14} molecules] of polysialoglycoproteins per egg. A neutral oligosaccharide unit and a series of acidic sugar units were released by alkali–borohydride treatment of polysialoglycoproteins, and the structure of the former (S1) has recently been identified as a novel pentasaccharide alditol, Fucα1→3GalNAcβ1→3Galβ1→4Galβ1→3GalNAcol. The
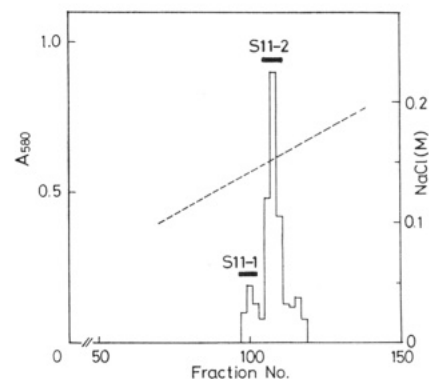

FIGURE 3: Rechromatography of fraction S11 of Figure 1 on DEAE-Sephadex A-25 column (0.9 × 70 cm).
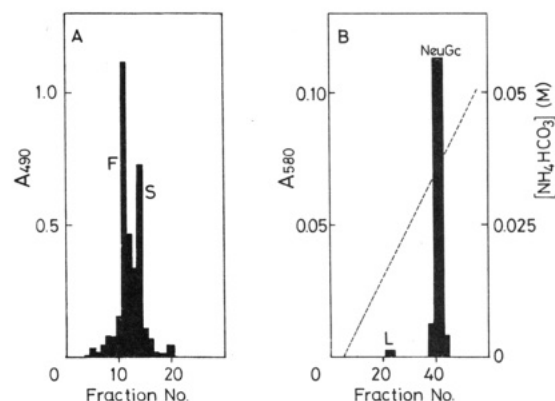

FIGURE 4: Isolation of neutral and acidic oligosaccharide alditols after release and subsequent enzymatic desialylation of the carbohydrate chains present in salmon egg polysialoglycoproteins. See text for further details.

principal aim of the present study is to examine the structures of a number of acidic heterooligosaccharide units that are linked O-glycosidically to serine and threonine residues. Of the numerous acidic oligosaccharide alditols, those listed in Table I were isolated and purified to homogeneity as judged by TLC and composition analysis. The structures of the acidic oligosaccharide alditols have been finally established as those given in Table II, and the principal steps for the determination are described in the following sections.

Methylation Analysis of Sialooligosaccharide Alditols. Some representative fractions of Fuc-containing units and short-core units were subjected to methylation analysis. The results demonstrated that the Fuc-containing unit, say, S6-1, contains 3,6-di-O-substituted GalNAcol, 3-O- and 4-O-substituted galactoses, 3-O-substituted GalNAc, and the terminal Fuc residue and that the short-core unit, say, S7-1, contains 3,6-di-O-substituted GalNAcol and the terminal Gal. The results showed the linkage of the sialic acid residues to the proximal GalNAcol and suggested the presence of oligosialyl groups except for S3-2 and S3-1. The methylation analysis of sialyl groups showed the molar ratio of 8-O-substituted NeuGc to terminal NeuGc residue for S3-2, S6-1, S9-1, S11-2, S13-2, and S14-2 was 0, 1.0, 2.0, 3.3, 3.7, and 4.5, respectively [cf. Inoue & Matsumura (1979) and Inoue et al. (1982)].

Structure of Fucose-Containing Core. The asialo-S6-1 had the same migration rate on TLC as the authentic fucopentasaccharide (S1), Fucα1→3GalNAcβ1→3Galβ1→4Galβ1→3GalNAcol (Shimamura et al., 1983). Methylation analysis of the asialo-S6-1 showed the presence of 3-O-substituted GalNAcol, 3-O- and 4-O-substituted galactoses, 3-O-substi-

Table I: Carbohydrate Composition of Oligosaccharide Alditols Derived from Salmon Egg Polysialoglycoproteins

type of core oligosaccharide	reduced oligosaccharide	mol/mol of GalNAcol				
		NeuGc	Fuc	Gal	GalNAc	GalNAcol
Fuc-containing units	S1	0.0	1.1	2.0	1.1	1.0
	S3-2	1.3	1.1	2.2	1.1	1.0
	S6-1	2.0	1.0	2.1	1.0	1.0
	S9-1	3.0	0.9	1.8	1.0	1.0
	S11-2	4.3	1.1	2.1	1.1	1.0
	S13-2	5.0	1.0	2.4	1.1	1.0
	S14-2	6.6	0.9	1.9	1.1	1.0
short-core units	S7-1	2.2	0.0	1.0	0.0	1.0
	S10-1	3.1	0.0	1.1	0.0	1.0
	S13-1	4.1	0.0	1.0	0.0	1.0
	S14-1	5.3	0.0	0.9	0.0	1.0
long-core units	S3-1	1.1	0.0	2.2	1.9	1.0

Table II: Proposed Structures of Three Classes of Homologous Sialocarbohydrate Units

	n	

I. Fucose-Containing Units

S3-2 1 Fucα1 → 3GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3GalNAcol
S6-1 2   6
S9-1 3   ↑
S11-2 4   (→ 8NeuGcα2)$_n$
S13-2 5
S14-2 6

II. Short-Core Units

S7-1 2 Galβ1 → 3GalNAcol
S10-1 3   6
S13-1 4   ↑
S14-1 5   (→ 8NeuGcα2)$_n$

III. Long-Core Units[a]

S3-1 0 GalNAcβ1 → 4GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3GalNAcol
                 3   6
                 ↑   ↑
              NeuGc2   (→ 8NeuGcα2)$_n$

[a] For detailed information concerning the long-core units with oligosialyl groups isolated from rainbow trout egg polysialoglycoproteins, see the preceding papers in this issue.

tuted GalNAc, and the terminal Fuc residue in approximately equivalent molar ratio. Thus the asialo-S6-1 was identified as the neutral fucopentasaccharide (S1). The identity of the core structure of the other Fuc-containing sialooligosaccharide alditols with S1 was confirmed by TLC behavior after enzymatic desialylation of each parent oligosaccharide.

Core Types of Carbohydrate Units Present in Salmon Egg Polysialoglycoproteins. In order to characterize the core types of all the carbohydrate units found in salmon egg polysialoglycoproteins, the mixture of oligosaccharide alditols obtained by alkali–borohydride treatment was first digested exhaustively with sialidase, and the products were fractionated as shown in Figure 4. The neutral fractions denoted as F and S in Figure 4A were identified as fucopentasaccharide (S1) and Galβ1→-3GalNAcol, respectively, on the basis of composition analysis and TLC behavior. The minor acidic component, L, in Figure 4B was suggested to have a structure identical with that unambiguously determined for the long-core unit having a single NeuGc residue derived from trout egg polysialoglycoproteins (Iwasaki et al., 1984), on the evidence of its composition analysis, sialidase-resistant nature, and its behavior on TLC. Insufficient material was obtained for further studies of this component. The relative proportions of the three types of core oligosaccharides found in salmon egg polysialoglycoproteins are (short-core unit) 8.3, (Fuc-containing unit) 7.5, and
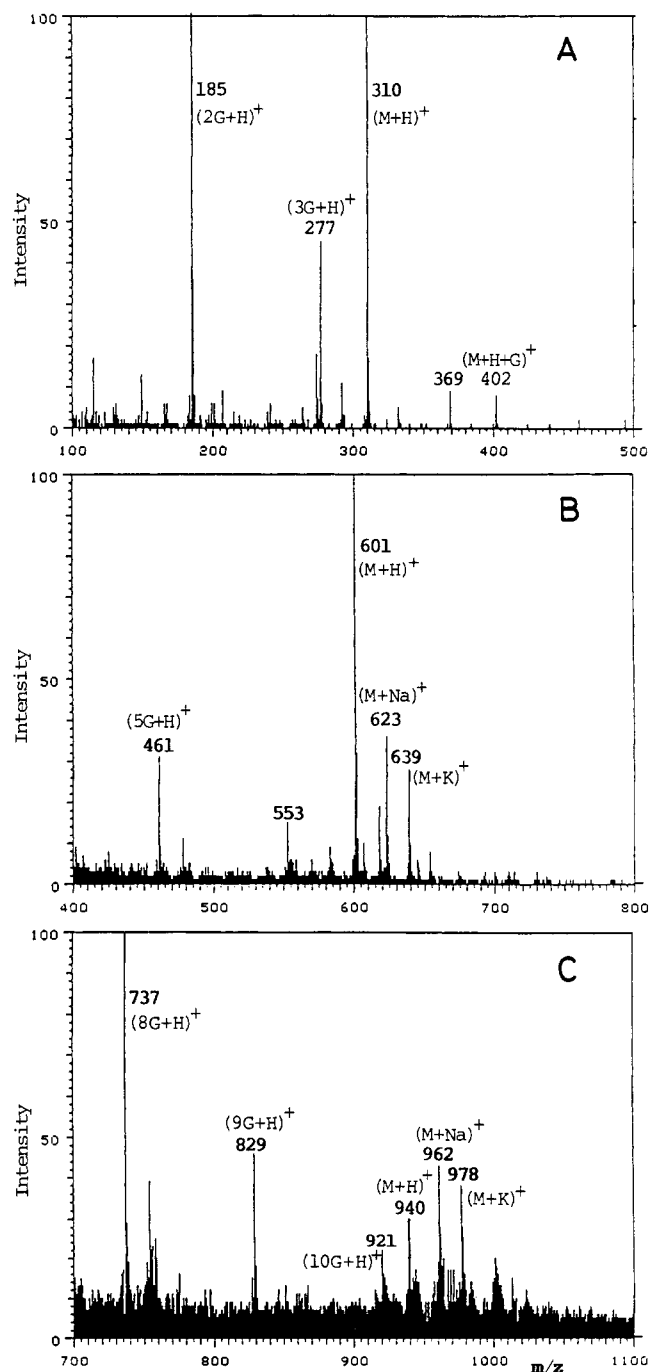

FIGURE 5: Positive-ion molecular SIMS spectra of (A) NeuAc, (B) NeuAcα2→8NeuAc, and (C) NeuGcα2→8NeuGcα2→8NeuGc.
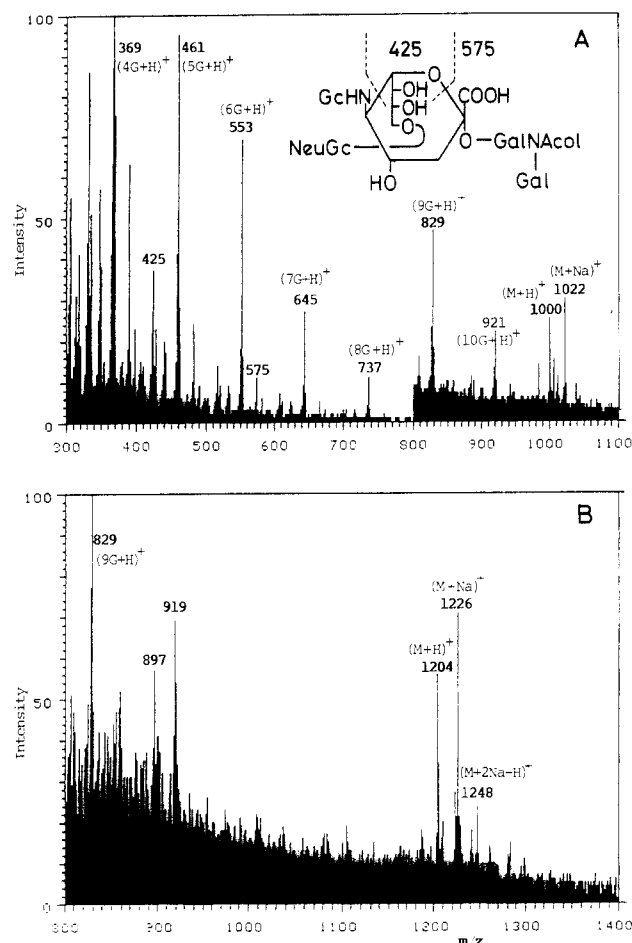
FIGURE 6: Positive-ion molecular SIMS spectra of (A) S7-1 and (B) S3-2. Masses of the fragment ions are indicated by cleavage sites.

(long-core unit) 1.0. It should be noted that the same three core types of oligosaccharides were found as the major carbohydrate units of polysialoglycoproteins from the eggs of both salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri irideus*). A significant difference has been observed, however, in the relative amounts of the three core types: salmon egg polysialoglycoproteins are rich in Fuc-containing units and almost lack long-core units, whereas the latter units are most abundant in trout egg polysialoglycoproteins (S:F:L = 0.5:0.1:1.0; S. Inoue et al., unpublished results). The presence of neutral fucopentasaccharide in polysialoglycoproteins from salmon eggs but not in those from trout eggs has already been reported (Shimamura et al., 1983).

SIMS of Sialooligosaccharides. The method of molecular SIMS has been successful in obtaining spectra of a novel oligosaccharide, S1, in a previous study (Shimamura et al., 1983). Here we would like to test the feasibility of facile desorption ionization of underivatized sialic acid containing oligosaccharides using SIMS. Figure 5 shows the positive SIMS spectra of NeuAc, NeuAcα2→8NeuAc, and NeuGcα2→8NeuGcα2→8NeuGc. We believe that the spectra are the first reported demonstration of the feasibility of observing the pseudo molecular ions of simple oligosialyloligosaccharides without derivatization. The underivatized mono-, di-, and trisialyl compounds gave spectra (Figure 5) compatible with the molecular weights of the respective materials and some structural detail. In the parent-ion region, peaks at m/z 962, $(M + Na)^+$, and at m/z 978, $(M + K)^+$, are present in addition to the peak, $(M + H)^+$, in Figure 5C. These ionic species are often observed in the positive SIMS

spectra (Kambara & Hishida, 1981; Busch et al., 1982). Peaks at m/z 185, 277, 369, 461, 553, 645, 737, 829, and 921 are due to multiple glycerol addition to the protonated glycerol, $(G + H)^+$.

The SIMS spectra of S3-2 and S7-1 are shown in Figure 6 as the examples of Fuc-containing and short-core units with sialyl groups. The positive SIMS of S3-2 is again characterized by the familiar pseudo-molecular-ion series: $(M + H)^+$ at m/z 1204, $(M + Na)^+$ at m/z 1226, and $(M + 2Na - H)^+$ at m/z 1248 (Figure 6B). It is of interest to note that the m/z 897 peak is due to the loss of NeuGc from the parent molecule by the rupture of the ketosidic bond, accompanied by the concomitant addition of hydrogen, to give the protonated molecular ion corresponding to neutral core pentasaccharide. Furthermore, the peak at m/z 919 is believed to be formed by replacement of proton with sodium ion. In the positive SIMS of underivatized sialooligosaccharides, diagnostic sequence ions are very weak or seldom observed. The fragment ions containing the structural information are seen at m/z 425 and 575 (Figure 6A). Sites of bond cleavages leading to the formation of these ions are depicted in Figure 6A. The present study, though preliminary, strongly shows that SIMS is a promising new technique for structural elucidation of underivatized oligosialylated oligosaccharides.

^1H *NMR Studies of Fuc-Containing Units.* The 270-MHz ^1H NMR spectra of S1, S6-1, and S13-2 are shown in Figure 7. While exact assignments cannot be made in the region of the spectra (3.5–4.2 ppm) where many of the sugar ring proton resonances overlap, most of the resolved signals have been assigned to specific monosaccharide residues with confidence. The anomeric region of each of the spectra in Figure 7 (4.4–5.0 ppm) contains four well-resolved proton signals of equal intensity. For convenience in discussing the assignments, the subscript designating the position of the sugar residue is used (see Table III). According to the structure of the Fuc-containing units (S3-2 to S14-2 in Table II), the ^1H NMR spectra of this series are expected to contain four anomeric proton resonances corresponding to two Gal, one GalNAc, and one Fuc residues. It is evident from internal intensity comparison that four resonances are present at 4.504, 4.608, and 4.995 ppm (23 °C) and 4.792 ppm (60 °C) (Figure 7B).

(a) *Anomeric Proton Resonances of Gal[1] and Gal[2] Residues.* The resonance at 4.504 ppm is assigned to the anomeric proton of Gal[1] by a comparison with those observed for Galβ1→3[(→8NeuGcα2)$_n$→6]GalNAcol. This is fully supported by the fact that the 4.504 ppm signal was broadened and shifted bit downfield (4.54 ppm) on addition of borate (Figure 7A), presumably due to the formation of a borate complex across positions 4 and 5 of the nearby GalNAcol residue. It is known that α-*threo*-glycerols react readily with borate (Frahn & Mills, 1959, 1964). It is reasonable to assign the 4.608 ppm resonance in Figure 7B to the H-1 proton of Gal[2] in the 3Gal[2]β1→4Galβ1→ sequence: this signal remained almost unchanged upon addition of borate (cf. Figure 7A).

(b) *H-1 Resonance of Fuc Residue.* The presence of an α-L-fucopyranosyl linkage was confirmed by specific hydrolysis with α-L-fucosidase *Charonia lampas*, so that the lowest field resonance at 4.995 ppm can be assigned to the H-1 signal of the nonreducing terminal α-L-fucopyranosyl group. The 4.998 ppm resonance of the Fuc residue in S1 was broadened with shifting to 4.98 ppm on addition of borate as expected for the favorable structure of the complex formed at a vicinal 3,4-cis-diol of the Fuc residue.

(c) *H-1 Resonance of GalNAc Residue.* The remaining signal at 4.792 ppm at 60 °C (Figure 7B), totally hidden under
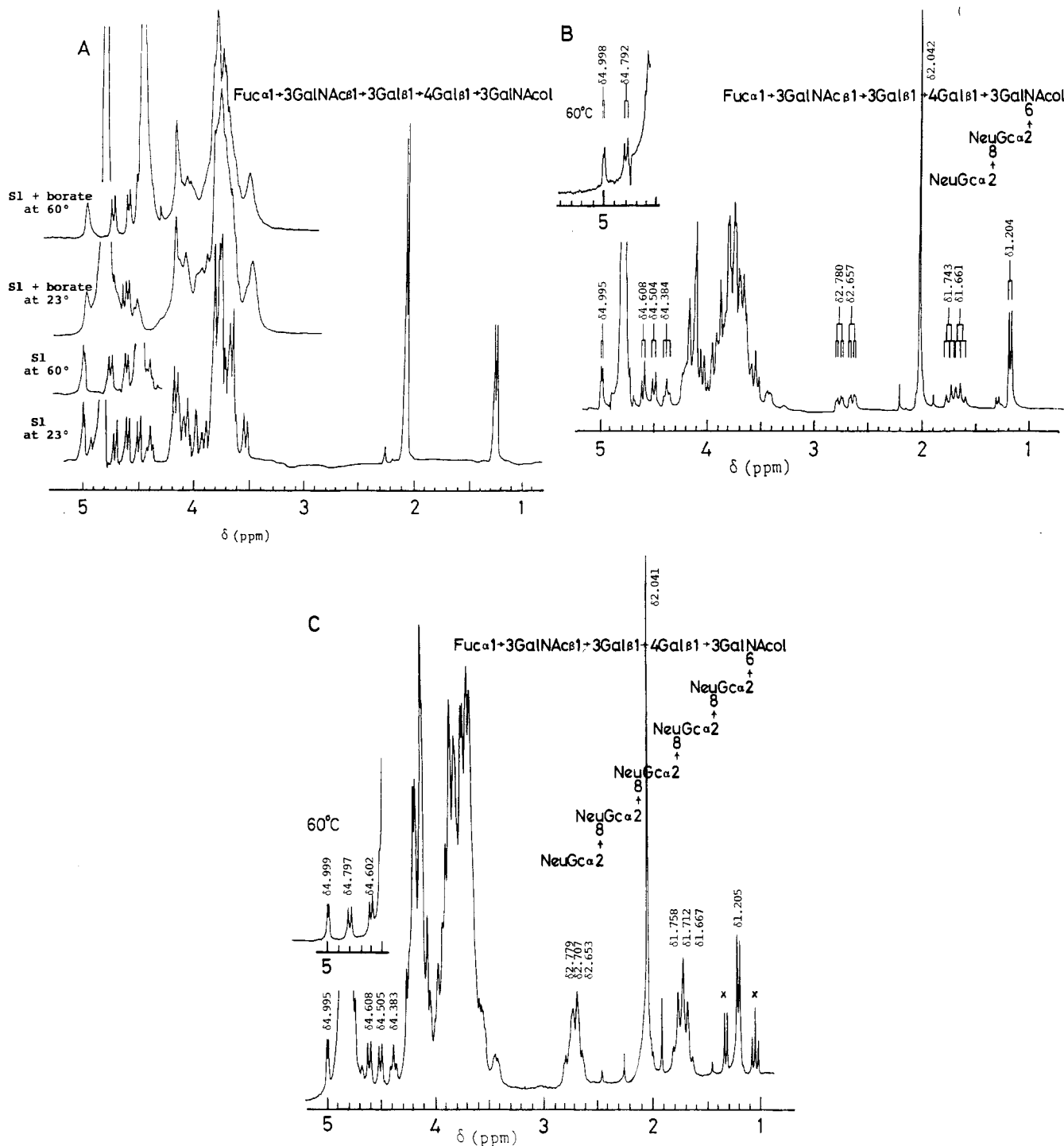
FIGURE 7: 270-MHz ^{1}H NMR spectra of (A) S1, (B) S6-1, and (C) S13-2 in D_2O at 23 and 60 °C together with the structures proposed. In (A), the effect of addition of sodium borate (final concentration, 43 mM) is shown. Anomeric regions of the spectra at 60 °C are also shown because in the 23 °C spectra of S6-1 and S13-2 the H-1 resonance of the GalNAc residue is hidden under the HDO line. Double irradiation confirmed the assignments of the NeuGc H-3$_{ax}$ and H-3$_{eq}$ resonances.

the HDO peak at 23 °C, then corresponds to GalNAc H-1. It should be noted that the oligosialyl residue, α-2→6 linked to GalNAcol residue, gives rise to a marked downfield shift of the H-1 signal of the GalNAc residue (4.755 → 4.779 ppm for S1 vs. S3-2 at 60 °C; 4.755 → 4.800 ppm for S1 vs. S11-2 at 60 °C). The observed changes must reflect some conformational differences since the H-1 resonances of the penultimate GalNAc residue, three sugar residues away from the site of the structural alteration, are affected. Specific environment-dependent changes in the H-1 signal of the GalNAc have been observed on going from 23 to 60 °C (4.718 → 4.755

ppm for S1; 4.739 → 4.779 ppm for S3-2). This unusual behavior of the GalNAc H-1 resonances in the Fuc-containing units could be related to the unique conformation of this series of compounds but needs further examination for the sake of completeness.

(d) *Methyl Proton Resonances.* The presence of two acetyl groups in the core oligosaccharide (S1) is indicated by the appearance of two methyl resonances at 2.032 and 2.048 ppm. Substitution at the 6-hydroxy group of the GalNAcol residue causes a small but definite downfield shift of the N-acetyl methyl signal of the GalNAcol residue (2.032 → 2.042 ppm).

Table III: Proton (Anomeric, GalNAcol H-2, Fuc Methyl, NeuGc H-3, and N-Acetyl Protons) Chemical Shifts of Fuc-Containing Units with Oligosialyl Groups[a]

$$\text{Fuc}\alpha 1 \to 3\text{GalNAc}\beta 1 \to 3\text{Gal}[2]\beta 1 \to 4\text{Gal}[1]\beta 1 \to 3\text{GalNAcol}$$
$$6$$
$$\uparrow$$
$$(\text{NeuGc})_{\text{distal}}\alpha 2 \to 8\,[\,(\text{NeuGc})_{\text{internal}}\,]_n\,\alpha 2 \to 8\,(\text{NeuGc})_{\text{proximal}}\alpha 2$$

	S1	S3-2	S6-1	S9-1	S11-2	S13-2	S14-2
H-1 of							
Fuc	4.998	4.997	4.995	4.996	4.996	4.995	4.997
	(5.004)	(5.001)	(4.998)	(5.001)	(5.001)	(4.999)	(5.001)
GalNAc	4.718	4.739					
	(4.755)	(4.779)	(4.792)	(4.800)	(4.800)	(4.797)	(4.789)
Gal[2]	4.604	4.608	4.608	4.608	4.609	4.608	4.609
	(4.600)	(4.602)	(4.604)	(4.603)	(4.601)	(4.602)	(4.602)
Gal[1]	4.504	4.500	4.504	4.504	4.507	4.505	4.505
	(4.506)	(4.501)			(4.507)		(4.509)
H-3$_{\text{eq}}$ of							
(NeuGc)$_{\text{proximal}}$			2.657	2.654	2.660	2.653	2.660
			(2.658)	(2.660)	(2.663)	(2.658)	(2.657)
(NeuGc)$_{\text{internal}}$				2.706	2.707	2.707	2.709
				(2.705)		(2.706)	(2.706)
(NeuGc)$_{\text{distal}}$		2.744	2.780	2.778	2.779	2.779	2.780
		(2.747)	(2.783)	(2.782)		(2.784)	(2.783)
H-3$_{\text{ax}}$ of							
(NeuGc)$_{\text{proximal}}$			1.661	1.665	1.666	1.667	1.666
			(1.658)	(1.666)	(1.669)	(1.662)	(1.661)
(NeuGc)$_{\text{internal}}$				1.698	1.711	1.712	1.714
				(1.710)	(1.720)	(1.720)	(1.719)
(NeuGc)$_{\text{distal}}$		1.714	1.743	1.750	1.758	1.758	1.758
		(1.703)	(1.745)	(1.756)	(1.760)	(1.755)	(1.757)
CH$_3$ of							
Fuc	1.204	1.204	1.204	1.204	1.205	1.205	1.205
	(1.211)	(1.210)	(1.210)	(1.210)	(1.210)	(1.210)	(1.209)
GalNAc	2.048	2.042	2.042	2.040	2.042	2.041	2.043
	(2.051)	(2.050)	(2.041)	(2.046)	(2.045)	(2.042)	(2.043)
GalNAcol	2.032	2.042	2.042	2.040	2.042	2.041	2.043
	(2.032)	(2.050)	(2.041)	(2.046)	(2.045)	(2.042)	(2.043)
H-2 of GalNAcol	4.391	4.376	4.384	4.384	4.384	4.383	4.389
	(4.373)					(4.360)	(4.364)

[a] Chemical shifts are measured in D_2O at 23 °C and are expressed in ppm from DSS; those in parentheses are the corresponding values at 60 °C. The subscripts designating the position of the residue are shown in the above structure.

A doublet at 1.20 ppm (three protons) stems from the methyl group of the terminal Fuc residue.

(e) *H-3 Resonances of Sialyl Groups.* The oligosaccharide S6-1 differs from the neutral pentasaccharide S1 only by the presence of an additional disialyl group. This is reflected in the appearance of two additional clusters of peaks at 2.65–2.78 and 1.66–1.75 ppm in the S6-1 spectrum (Figure 7B). The H-3 resonances of oligosialyl groups can be assigned by a comparison with those observed for the homologous $\alpha 2 \to 8$-linked sialyloligosaccharides as described in the preceding paper (Kitajima et al., 1984). A pair of quartets at 2.657 and 2.780 ppm are thus from the H-3$_{\text{eq}}$ protons of the (NeuGc)$_{\text{proximal}}$ and (NeuGc)$_{\text{distal}}$ units, respectively, and the signals at 1.661 and 1.743 ppm are from the H-3$_{\text{ax}}$ protons of the respective NeuGc residues. The proton signals of all other Fuc-containing units were assigned, and the chemical shifts are summarized in Table III. Given in parentheses for each resonance are the corresponding shift values at 60 °C.

References

Busch, K. L., Unger, S. E., Vincze, A., Cooks, R. G., & Keough, T. (1982) *J. Am. Chem. Soc. 104*, 1507–1511.

Frahn, J. L., & Mills, J. A. (1959) *Aust. J. Chem. 12*, 65–89.

Frahn, J. L., & Mills, J. A. (1964) *Aust. J. Chem. 17*, 274–276.

Inoue, S., & Iwasaki, M. (1978) *Biochem. Biophys. Res. Commun. 83*, 1018–1023.

Inoue, S., & Matsumura, G. (1979) *Carbohydr. Res. 74*, 361–368.

Inoue, S., & Iwasaki, M. (1980) *Biochem. Biophys. Res. Commun. 93*, 162–165.

Inoue, S., Matsumura, G., & Inoue, Y. (1982) *Anal. Biochem. 125*, 118–124.

Iwasaki, M., Inoue, S., Kitajima, K., Nomoto, H., & Inoue, Y. (1984) *Biochemistry* (first paper of three in this issue).

Kambara, H., & Hishida, S. (1981) *Anal. Chem. 53*, 2340–2344.

Kitajima, K., Nomoto, H., Inoue, Y., Iwasaki, M., & Inoue, S. (1984) *Biochemistry* (second paper of three in this issue).

Nomoto, H., Iwasaki, M., Endo, T., Inoue, S., Inoue, Y., & Matsumura, G. (1982) *Arch. Biochem. Biophys. 218*, 335–341.

Shimamura, M., Endo, T., Inoue, Y., & Inoue, S. (1983) *Biochemistry 22*, 959–963.