Glycoconjugate Journal (1993) **10**:425–434

A structural study of the asparagine-linked oligosaccharide moiety of duck ovomucoid

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Received 10 June 1993, revised 23 July 1993

Asparagine-linked oligosaccharides of duck ovomucoid were released quantitatively from the protein by digestion with glycoamidase A (from almond), the reducing ends of the oligosaccharide chains thus obtained were aminated with a fluorescent reagent, 2-aminopyridine, and the mixture of pyridylamino derivatives of the oligosaccharides was separated using two different types of high performance liquid chromatography (HPLC) on a reversed phase column and an amide adsorption column. More than sixteen different oligosaccharides were separated and the structures were characterized by a combination of the 2-dimensional sugar mapping technique using HPLC, exoglycosidase digestion, and proton nuclear magnetic resonance measurements (1- and 2-dimensional). Furthermore, the HPLC profile of duck ovomucoid oligosaccharides was compared with previously reported profiles obtained from quail and chicken ovomucoids.

Keywords: Oligosaccharide structure, duck ovomucoid sugar chain

Abbreviations: COSY, chemical shift-correlated spectroscopy; DQF-COSY, double quantum filtered COSY; DSS, sodium, 4,4-dimethyl-4-silapentane 1-sulfonate; Gal, D-galactose; GlcNAc or GN, *N*-acetyl-D-glucosamine; HOHAHA, homonuclear Hartmann–Hahn spectroscopy; Man or M, D-mannose; NOE, nuclear Overhauser enhancement; ODS, octadecylsilyl; PA, pyridylamino; ROESY, rotating frame nuclear Overhauser effect spectroscopy; SDS/PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis.

Avian ovomucoids are present in all bird egg whites studied to date and account for about 10% of egg white proteins. Ovomucoids from chicken, quail, and duck are known as protease inhibitors and as multifunctional glycoproteins. They have homologous amino acid sequences of relative molecular mass 28 000 ± 1500, and isoelectric point 4.0–4.6. They have five glycosylation sites containing carbohydrates that constitute 20–25% of the glycoprotein [1–4]. The structures of asparagine-linked oligosaccharides from chicken ovomucoid [5, 6] and Japanese quail ovomucoid [7] have been elucidated in detail. In chicken ovomucoid, penta-antennary oligosaccharides exist as specific components. In contrast, in quail ovomucoid, a trimannosyl core structure is a major component. In this paper, we report the characteristic oligosaccharide profile of duck ovomucoid on the basis of analyses using ^{1}H NMR spectroscopy and the 2-dimensional sugar mapping technique. The occurrence of the heterogeneity of duck ovomucoid oligosaccharides will be discussed briefly.

Materials and methods

Enzymes and reference compounds

Glycoamidase A from almond (commercially available as glycopeptidase A), β-galactosidase and β-*N*-acetylhexosaminidase from jack beans were purchased from Seikagaku Kogyo Co. Pepsin was purchased from Sigma Chemical Co. Sialidase from *Arthrobacter ureafaciens* was purchased from Nacalai Tesque.

Reference oligosaccharides

The pyridylamino derivatives of isomaltotetraose–isomaltododecaose (PA-glucose oligomers) were purchased from Nakano Vinegar Co. The structures of a series of standard N-linked oligosaccharides are shown in Table 1. Code

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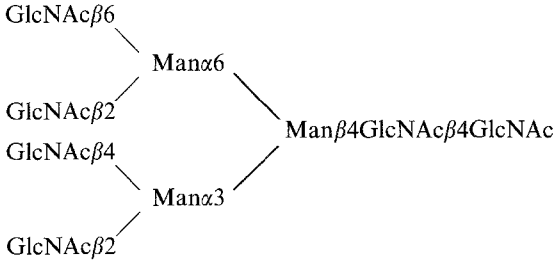
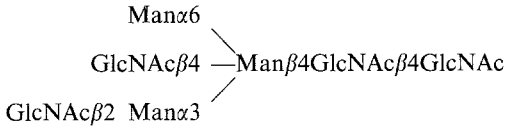
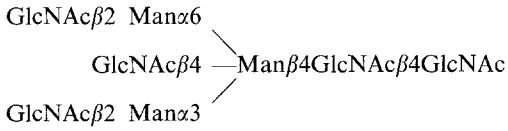
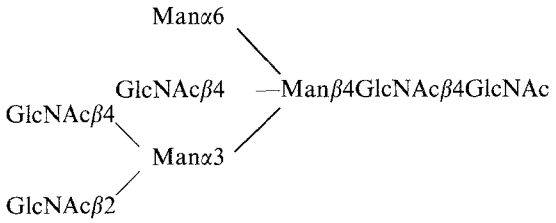
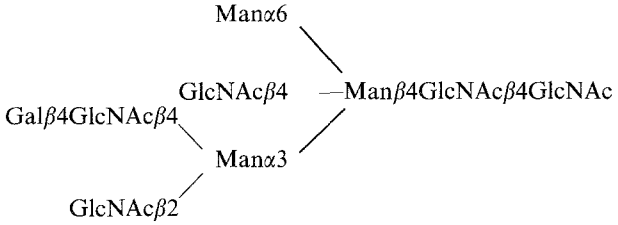
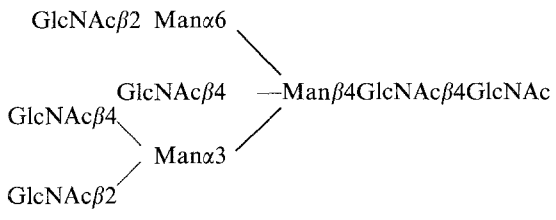
Table 1. Elution positions on HPLC and the proposed structures of PA-oligosaccharides from duck ovomucoid.

Peak and code No.[a]	*Glc units*		*PA-oligosaccharides*	*Relative quantity* (%)
	ODS	*Amide observed (reported*[a]*)*		
Without bisecting *N*-acetylglycosamine				
B M2.1	7.4 (7.4	3.3 3.3)	Manα6 \ Manβ4GlcNAcβ4GlcNAc	9.9
A 000.1	7.3 (7.4	4.3 4.3)	Manα6 \ Manβ4GlcNAcβ4GlcNAc / Manα3	23.3
G 100.1	9.3 (9.5	4.6 4.7)	GlcNAcβ2 Manα6 \ Manβ4GlcNAcβ4GlcNAc / Manα3	3.7
H1 100.3	9.7 (9.9	5.5 5.6)	Galβ4GlcNAcβ2 Manα6 \ Manβ4GlcNAcβ4GlcNAc / Manα3	2.2
C 100.4	7.9 (8.0	5.6 5.7)	Manα6 \ Manβ4GlcNAcβ4GlcNAc / Galβ4GlcNAcβ2 Manα3	3.1
D 100.7	8.3 (8.3	4.6 4.5)	Manα6 \ GlcNAcβ4 Manβ4GlcNAcβ4GlcNAc / Manα3	16.2
E 100.9	8.5 (8.5	5.7 5.8)	Manα6 \ Galβ4GlcNAcβ4 Manβ4GlcNAcβ4GlcNAc / Manα3	3.7
F1 200.1	8.9 (8.9	5.0 5.1)	GlcNAcβ2 Manα6 \ Manβ4GlcNAcβ4GlcNAc / GlcNAcβ2 Manα3	1.0
H2 200.3	9.7 (9.6	6.0 6.1)	GlcNAcβ2 Manα6 \ Manβ4GlcNAcβ4GlcNAc / Galβ4GlcNAcβ2 Manα3	2.9
J 300.1	10.8 (10.9	5.6 5.5)	GlcNAcβ2 Manα6 \ GlcNAcβ4 Manβ4GlcNAcβ4GlcNAc / Manα3 / GlcNAcβ2	3.3

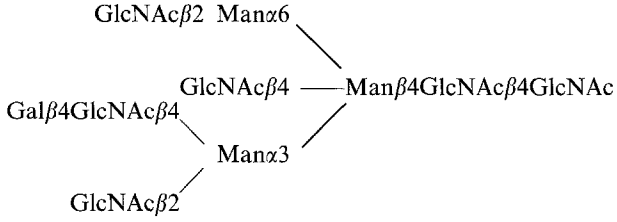
continued

Table 1. *Continued.*

Peak and code No.[a]	*Glc units*		*PA-oligosaccharides*	*Relative quantity* (%)
	ODS	*Amide*		
	observed (*reported*[a])			
F2 400.1	8.9 (9.0	6.1 6.3)		5.5
With bisecting *N*-acetylglucosamine				
I 101.2	10.4 (10.4	5.1 5.1)		3.6
K 201.1	12.2 (12.3	5.3 5.4)		2.1
L 201.15	14.8 (14.8	5.3 5.3)		5.8
M 201.16	15.3 (15.3	6.3 6.3)		0.8
N 301.1	16.7 (17.1	5.6 5.7)		10.5

continued

Table 1. *Continued.*

Peak and code No.[a]	*Glc units*		*PA-oligosaccharides*	*Relative quantity* (%)
	ODS	*Amide*		
	observed (*reported*[a])			
O 301.7	17.2 (17.5	6.6 6.7)		10.5

[a] Elution positions of standard PA-oligosaccharides expressed as glucose units and the code Nos. are cited from [8, 9].

numbers are cited from the references [8, 9]. Standard PA-oligosaccharides of code numbers M2.1, 000.1, 100.1, 100.3, 100.4, 101.2, 200.1, 200.3, 201.1, 300.1, and 400.1 were prepared as described previously [8]. Triantennary oligosaccharides with bisecting *N*-acetylglucosamine, 301.1 and 301.2 were obtained from chicken ovalbumin or nicotinic acetylcholine receptor of *Torpedo californica* [10]. Monoantennary oligosaccharide 100.9 was prepared by *β-N*-acetylhexosaminidase and α-L-fucosidase digestion of oligosaccharide 410.3, which was obtained from porcine pancreatic kallidinogenase [11]. Oligosaccharide 100.7 was obtained by *β*-galactosidase digestion of oligosaccharide 100.9. Oligosaccharides 201.15 and 201.16 were obtained from chicken ovomucoid [6].

Miscellaneous chemicals

The following materials were purchased from the sources indicated: Sephadex G-15 (Pharmacia LKB Biotechnology Inc.); Bio-Gel P-4, 200–400 mesh (Bio-Rad); sodium cyanoborohydride (Aldrich). 2-Aminopyridine (Wako Pure Chemical Industries) was recrystallized from hexane solution.

Purification of ovomucoids

Fresh eggs of duck (Khaki Campbell), quail (Japanese quail), and chicken (White Leghorn) were obtained from the farm of Nagoya University. Ovomucoid was isolated from each egg white following a reported method [12]. All fractions with trypsin inhibitory activity recovered from SP-Sephadex-50 column chromatography were pooled, dialysed against distilled water, and lyophilized. The purity of the isolated ovomucoids was confirmed by SDS/PAGE (12% acrylamide gel) according to the method of Laemmli [13].

Preparation of oligosaccharides from three different ovomucoids

Oligosaccharides were released from each 200 mg of duck, quail and chicken ovomucoid protein by sequential digestion with 4 mg pepsin, pH 2.0, and with 100 mU of glycoamidase A, pH 5.0, as described previously [14]. After the oligosaccharide fraction was collected by gel filtration on a Bio-Gel P-4 column, it was reductively aminated with 2-aminopyridine and sodium cyanoborohydride [15]. PA-oligosaccharides were purified by gel filtration on a Sephadex G-15 column and analysed using the following HPLC procedure.

Detection method of sialic acid

The sialic acid released from protein by sialidase digestion was analysed by isocratic elution with 100 mM NaOH including 20 mM sodium acetate on a CarboPac PA-1 column (4.6 mm × 250 mm, Dionex) with a flow rate of 1 ml min^{-1}. The sialic acid was monitored with a pulsed amperometric detector.

Isolation and characterization of PA-oligosaccharides by HPLC (2-dimensional sugar mapping technique) [8]

First, the sample PA-oligosaccharide was separated by HPLC on a reversed phase column (Shim-pack HRC-ODS 6 mm × 150 mm, Shimadzu). Elution was performed at a flow rate of 1.0 ml min^{-1} at 55 °C using two solvents, A and B. Solvent A consisted of 10 mM sodium phosphate buffer (pH 3.8), and solvent B consisted of 10 mM sodium phosphate buffer (pH 3.8) containing 0.5% 1-butanol. After the injection of a sample, the ratio of solvent B to A was increased using a linear gradient from 20:80 to 50:50 in 60 min. Each oligosaccharide fraction that separated as a peak was collected and evaporated to dryness. Second, the residue was dissolved in solvent C, and injected on an amide-adsorption column (TSK-GEL Amide-80, 4.6 mm ×

260 mm, Tosoh). Solvent C was composed of mixture of 3% acetic acid in water with triethylamine, pH 7.3:acetonitrile, 35:65 by vol. Solvent D was composed of 3% acetic acid in water with triethylamine, pH 7.3:acetonitrile, 50:50 by vol. Elution was performed at a flow rate of 1.0 ml min^{-1} at 40 °C using solvents C and D. After injection of a sample, the ratio of solvent D to C was increased with a linear gradient from 0 to 100% in 50 min. In both HPLC systems, PA-oligosaccharides were detected by fluorescence using excitation and emission wavelengths of 320 and 400 nm, respectively. Third, the values of the elution positions of the PA-oligosaccharides were expressed as glucose units, as defined by comparing the elution positions with those of the reference PA-glucose oligomers (isomaltotetraose–isomaltododecaose). The coordinate, expressed as glucose units of each sample oligosaccharide on the ODS and amide silica column, was plotted on the 2-dimensional sugar map and compared with the coordinates for the standard *N*-linked oligosaccharides which include 180 different compounds [9].

^{1}H-NMR measurements

Prior to NMR measurements, PA-oligosaccharide (10–100 μg each as neutral sugar) isolated by HPLC was desalted by gel filtration on a Sephadex G-15 column.

All NMR spectra were recorded on a JEOL JNM-GSX500 spectrometer. ^{1}H-NMR spectra were recorded with 32K data points and spectral widths 7000 Hz at 35 °C. An exponential window function with a broadening factor of 0.5 Hz was used for sensitivity enhancement. DQF-COSY [16], 2D HOHAHA [17], and ROESY [18] were measured in the phase-sensitive mode [19] with special widths of 3000 Hz. The mixing times used for 2D HOHAHA and ROESY experiments were 100 ms and 300 ms, respectively. The probe temperature was set to 65 °C throughout the 2D experiments. 2K data points were used in the t_2 dimension and 64 transients were acquired for each of 512 t_1 blocks. Prior to 2D Fourier transformation, the acquired data were multiplied by a Gauss function in t_2 and by a shifted sine-squared function in t_1 and zero-filled to yield a matrix 1024 (F_2) × 1024 (F_1) of the real data points. The solvent resonance was suppressed by selective irradiation during the preparation period of 1.2 s.

Results and discussion

Purification of duck, quail and chicken ovomucoid

The electrophoretogram of each purified ovomucoid is shown in Fig. 1. All three ovomucoids gave a broad band. It might be due to heterogeneity in their carbohydrate moiety, since extensive heterogeneity of sugar moiety was reported within chicken ovomucoid preparations [2]. No band was detected corresponding to the other major egg white glycoproteins such as ovalbumin and ovotransferrin, indicating that these ovomucoid preparations were pure enough for their oligosaccharide analysis. In spite of having the same molecular mass for the polypeptide portion of the three ovomucoids, the mobility of quail ovomucoid was slightly faster than that of the duck and chicken ovomucoids, suggesting that the oligosaccharide moiety of quail ovomucoid is smaller than that of the duck and chicken ovomucoids.

Preparation of the oligosaccharide fraction and comparison of oligosaccharide profiles among duck, quail and chicken ovomucoids

Sialic acid was not detected in these ovomucoid oligosaccharides. The neutral sugar content of the oligosaccharide fraction obtained from each ovomucoid protein (duck, quail and chicken) by digestion with glycoamidase A (from almond) was determined with orcinol–H_2SO_4 reagent [20]. It was confirmed that less than approximately 10% of the total carbohydrates of the original ovomucoid proteins were not recovered by this procedure.

On reversed phase HPLC, each 500 pmol of PA-oligosaccharide fraction prepared from duck, quail, and chicken ovomucoids was separated into multiple peaks. The area of each peak reflects the mole number of the

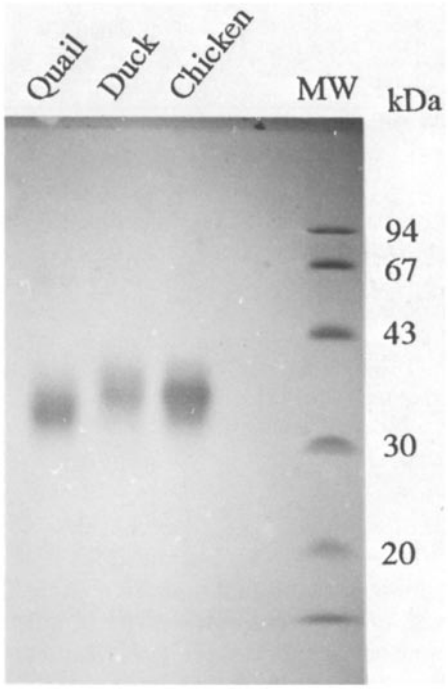


Figure 1. SDS-polyacrylamide gel electrophoresis of three different ovomucoids (duck, quail, and chicken). Each ovomucoid purified from duck, quail, and chicken egg white was subjected to SDS-polyacrylamide gel electrophoresis and visualized by Coomassie brilliant blue staining: Q, from quail; D, from duck, and C, from chicken.

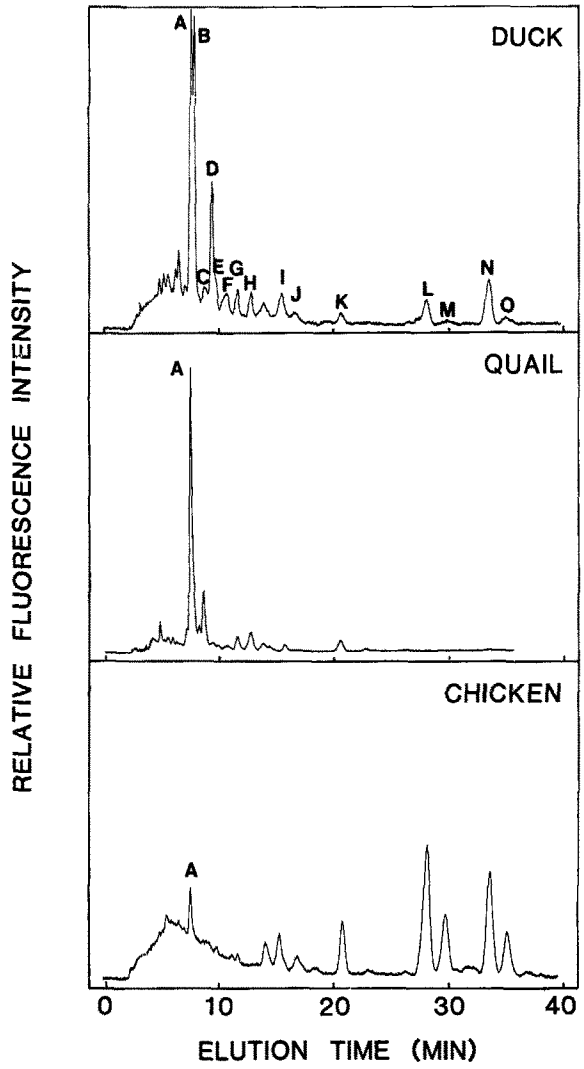


Figure 2. Comparison of HPLC profiles on an ODS column for PA-oligosaccharides derived from duck, quail, and chicken ovomucoids. PA-oligosaccharides were prepared from three different ovomucoids independently and separated by reversed phase HPLC using an ODS-silica column. At least fifteen peaks (A through O) were separated in the case of duck. Peaks F and H were further separated into two peaks, F1 and F2, and H1 and H2, on the subsequent amide silica column.

oligosaccharide (Fig. 2). Oligosaccharides A through O shown in Fig. 2 were further examined for homogeneity by use of an amide adsorption Amide-80 column. The elution positions of oligosaccharides on the Amide-80 column reflect primarily the molecular sizes of the oligosaccharides. It was revealed that oligosaccharide fractions A + B, D + E, F, and H were separated into distinct peaks, A and B, D and E, F1 and F2, and H1 and H2. Other peaks were all homogeneous not only on the ODS column but also in terms of molecular size. Figure 2, moreover, clearly shows that a significant degree of variety exists among the three different ovomucoids, duck, quail and chicken.

Characterization of oligosaccharides A–O using 2-dimensional mapping technique

The values of the elution positions of oligosaccharides, expressed as glucose units on ODS and amide silica columns were plotted on the 2-dimensional sugar map prepared using the 180 different N-linked oligosaccharide standards [8, 9], as illustrated in Fig. 3. The elution positions of oligosaccharides A, B, C, D, E, F1, F2, G, H1, H2, I, J, K, L, M, N and O coincided, respectively, within allowable error, with those of the reference compounds 000.1, M2.1, 100.4, 100.7, 100.9, 200.1, 400.1, 100.1, 100.3, 200.3, 101.2, 300.1, 201.1, 201.15, 201.16, 301.1, and 301.2. Each PA-oligosaccharide A–O and the corresponding standard PA-oligosaccharide were coinjected into two different HPLC columns, and confirmed to give a single peak. Each PA-oligosaccharide A–O as well as the corresponding standard PA-oligosaccharide was always digested with several glycosidases, at the same time and in the same manner. After β-galactosidase and β-*N*-acetylhexosaminidase digestion, the elution position of the sample oligosaccharide was compared with that of the corresponding standard oligosaccharide. The comparison was continued until both oligosaccharides yielded the common trimannosyl core structure Manα6(Manα3)Manβ4GlcNAcβ4GlcNAc (Fig. 3). For example, in the case of oligosaccharide O, coinjection with the corresponding standard PA-oligosaccharide (301.2) gave an unambiguous single peak on the two HPLC columns. The linkage of galactose is β(1-4), because digestion with the usual amount of jack bean β-galactosidase (5 mU per 500 pmol substrate) completely

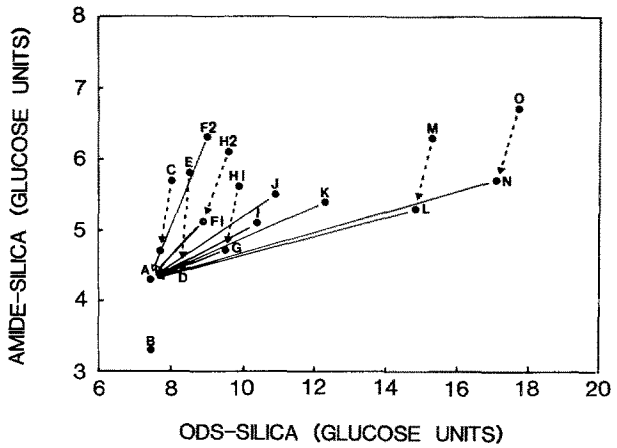
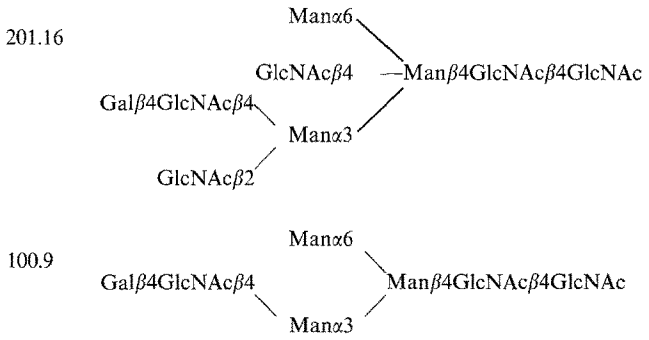


Figure 3. Characterization of structures of oligosaccharides A through O obtained from duck ovomucoid (Fig. 2) using the 2-dimensional mapping technique described in the 'Materials and methods' section. The coordinates for oligosaccharides A through O were superimposed on the coordinates for the reference compounds on the map. Arrows indicate the direction of changes in the coordinates of oligosaccharides after digestion with exoglycosidases: – – – –, β-galactosidase; ———, β-*N*-acetylhexosaminidase.

released the galactose residue. Under this condition, β(1-3) galactoside cannot be released. For the determination of the linkage position of the galactose residue, β-*N*-acetylhexosaminidase digestion was used. After β-*N*-acetylhexosaminidase digestion of the oligosaccharide O, both 201.16 and 100.9 were obtained.



On the basis of these results, it is concluded that oligosaccharide O has the structure of 301.2. The elution positions on HPLC columns and the proposed structures of duck ovomucoid oligosaccharides are illustrated in Table 1. Mono-, bi-, tri- and tetra-antennary *N*-acetyllactosamine-type oligosaccharides exist in duck. The trimannosyl core structure (oligosaccharide A) is predominant in both duck and quail, whereas the amount of this oligosaccharide is low in chicken (Fig. 2). The oligosaccharide of the smallest molecular size, oligosaccharide B (Manα6Manβ4GlcNAcβ4GlcNAc) is characteristic in duck. Oligosaccharide structures of A, G, H1, C, F1 and H2 have also been reported to exist in quail ovomucoid [7]. Oligosaccharide structures of A, G, H1, C, and N were also found in chicken ovomucoid [6]. Oligosaccharides with a bisecting *N*-acetylglucosamine residue make up about 25% of the oligosaccharides in duck. Quail oligosaccharides, by contrast, were found not to contain bisecting *N*-acetylglucsamine residue while most chicken oligosaccharides do contain bisecting *N*-acetylglycosamine residue. Penta-antennary oligosaccharides found in chicken are absent in duck and quail. As in quail [7] and chicken ovomucoid [5, 6], in duck ovomucoid there is no oligosaccharide with a fucose residue (Table 1).

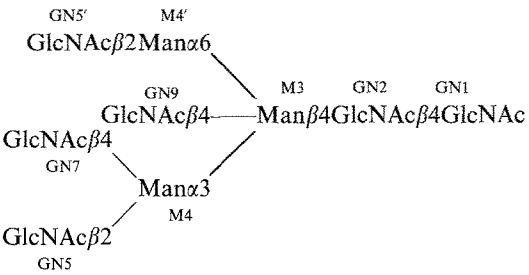
^{1}*H-NMR analyses of oligosaccharides L and N*

The chemical shifts of the structural reporter groups of oligosaccharides L and N are summarized in Table 2. On the basis of the chemical shifts and intensities observed in the ^{1}H-NMR spectra, oligosaccharide L is shown to lack one *N*-acetylglucosamine residue compared with oligosaccharide N (code No. 301.1).

The cross-peak regions between the anomeric proton resonances and other sugar proton resonances of the 2D HOHAHA spectra of oligosaccharides L and N are shown in Fig. 4(a, b), respectively. The magnetization transfer from H1 of Man(M) to H3 of M was observed for M4 and M4′ in the 2D HOHAHA spectra of oligosaccharides L and N. The assignments of H2 of M and H3 of M were determined by observation of the COSY cross-peaks between H1 of M and H2 of M and between H2 of M and H3 of M in the DQF-COSY spectra (data not shown). It should be noted that a significant difference was observed in the chemical shift of H2 of M4′ between oligosaccharide L and N (see Fig. 4a, b).

In order to identify the linkage of the mannose residues and the *N*-acetylglucosamine, ROESY spectra were measured for oligosaccharides L and N (Fig. 4c, d). As shown in Fig. 4(c, d), no ROESY cross-peaks between H1 of M4 and protons of GlcNAc-7 (GN7) were detected. However, in the case of oligosaccharide N, ROESY cross-peaks between H1 of M4 and H1 of GN5 and between H1 of M4′ and H1 of GN5′, in addition to those due to intraresidue NOEs, were detected (Fig. 4d). These results are consistent with the existence of the linkage of GlcNAc-**5**(β1-2)Man-**4**(α1-3) and GlcNAc-**5′**(β1-2)Man-**4′**(α1-6) in the structure of oligosaccharide N. In the case of oligosaccharide L, the ROESY cross-peak was detected only between H1 of M4 and H1 of GN5, and no ROESY cross-peak due to intraresidue NOE was detected for M4′ (Fig. 4c).

Table 2. Chemical shifts[a] of anomeric protons for oligosaccharides L and N at 65 °C.



Residue	*Oligosaccharide*[b]	
	L	N
Man-3	4.727	4.699
Man-4	5.058	5.062
Man-4′	4.960	4.986
GlcNAc-2	4.660	4.670
GlcNAc-5	4.547	4.549
GlcNAc-5′	—	4.556
GlcNAc-7	4.523	4.533
GlcNAc-9	4.450	4.478

[a] Chemical shifts expressed in ppm relative to DSS.
[b] ●, GlcNAc; ◆, Mannose.

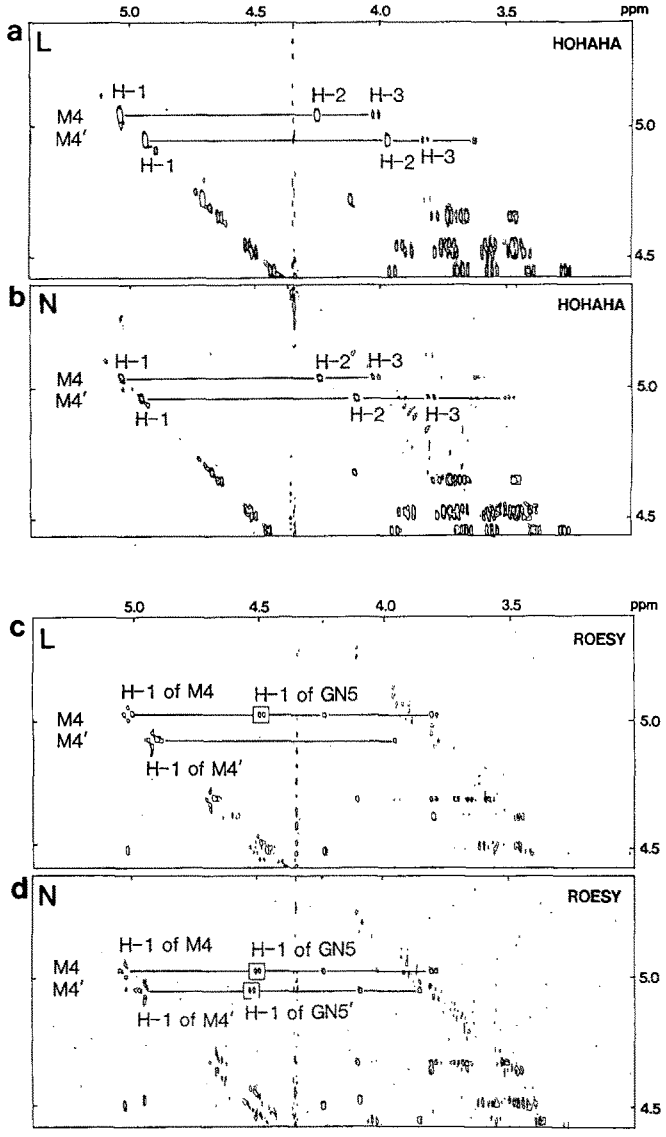
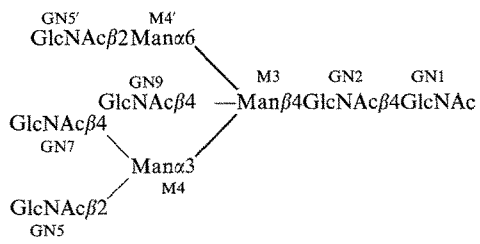


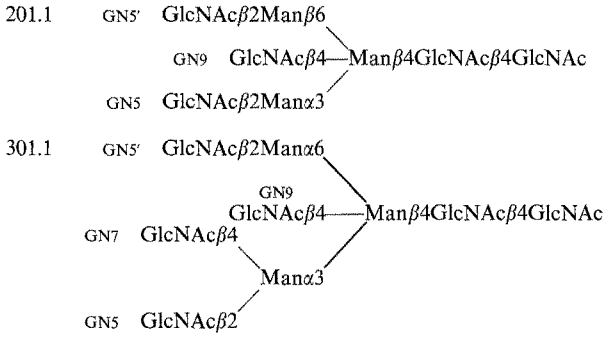
Figure 4. 2D HOHAHA spectra of oligosaccharides (a) L and (b) N, and ROESY spectra of oligosaccharides (c) L and (d) N at 65 °C. The spin systems for Man-4 and Man-4′ were connected by the solid lines. ROESY cross-peaks due to intraresidue interaction were boxed.

On the basis of the results obtained by the NMR analysis described above, it was concluded that oligosaccharide N has the following structure:



Substrate specificity of jack bean β-N-acetylhexosaminidase on bisecting N-acetylglucosamine containing PA-oligosaccharides

In the process of making this structural study of duck ovomucoid oligosaccharides, the substrate specificity of jack bean *N*-acetylhexosaminidase was elucidated in detail. Jack bean *β-N*-acetylhexosaminidase cannot release *N*-acetylglucosamine residues completely from oligosaccharides I, K, L, M, N and O under the usual digestion conditions (20 mU enzyme per 500 pmol substrate for 20 h) because of the existence of a bisecting *N*-acetylglucosamine residue. In the standard oligosaccharides, code Nos 201.1 and 301.1, the order of susceptibility for *β-N*-acetylhexosaminidase was GN7 > GN5′ > GN9 > GN5.



In the case of 201.1, over half of GN5′ was released; however, over 40% of 201.1 remained intact under the usual conditions. When 500 mU of enzyme was added to 500 pmol of the substrate, all *N*-acetylglucosamine residues were released overnight.

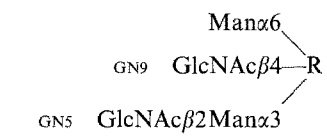
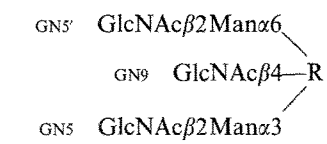
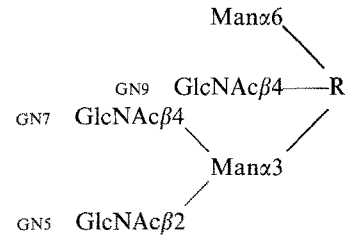
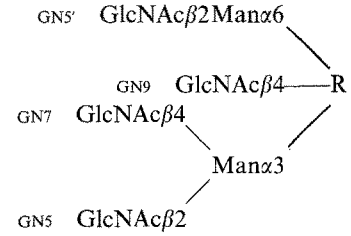
In the case of 301.1, GN7 was mostly hydrolysed under the usual conditions described above, GN5′ was about 60% hydrolysed, and GN9 and GN5 were far less hydrolysed.

As summarized in Table 3, in oligosaccharide I, both *N*-acetylglucosamine residues were completely hydrolysed and yielded the trimannosyl core structure under the usual conditions, indicating that the existence of the GN5′ residue inhibits the access of the enzyme to the oligosaccharide K. Other data in Table 3 also coincided with the results obtained with the reference compounds 201.1 and 301.1 described above.

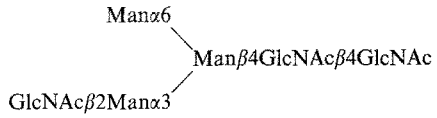
Formation of heterogeneity of duck ovomucoid oligosaccharides in vivo

At least in duck ovomucoid oligosaccharides, A, B, D, E, G and H1 are not intermediates in the oligosaccharide synthetic pathway established for animal cells [21, 22]. Some of these oligosaccharides have also been found in quail [7], and chicken ovomucoids [6]. Yamashita *et al.* suggested that the trimannosyl core structure (oligosaccharide A) was formed by an alternative biosynthetic pathway through the precursor $Glc_3Man_5GlcNAc_2$ [6]. Whether such a precursor oligosaccharide exists in avian oviducts or not is still unknown, although this structure

Table 3. Substrate specificity of jack bean β-*N*-acetylhexosaminidase.

	Oligosaccharide[a]	*Hydrolysed residue*	*Yield* (%)	*Resultant oligosaccharide*
I		GN9 and GN5	100	trimannosyl core
K		GN5′, GN9 and GN5	5	trimannosyl core
		GN5′ and GN9	15	code 100.2[b]
		GN5′	34	oligosaccharide I
		none	44	oligosaccharide K
L		GN7, GN9 and GN5	24	trimannosyl core
		GN7 and GN9	34	code 100.2[b]
		GN7	38	oligosaccharide I
		none	3	oligosaccharide L
N		GN7, GN5′, GN9 and GN5	10	trimannosyl core
		GN7, GN5′ and GN9	18	code 100.2[b]
		GN7 and GN5′	31	oligosaccharide I
		GN7	37	oligosaccharide K
		none	1	oligosaccharide N

[a] R = Manβ4GlcNAcβ4GlcNAc

[b] Code 100.2 

was found in Thy-1$^-$ mutant mouse lymphoma cells [23] and Chinese hamster ovary cells [24]. In the present study, however, an unusually small oligosaccharide without the trimannosyl core structure (oligosaccharide B in Table 1) has been discovered to be one of the main components of duck ovomucoid. We believe that at least the oligosaccharide B found in duck ovomucoid might be formed by trimming of the trimannosyl pentasaccharide with unidentified exoglycosidase digestion in the oviduct or egg white after completion of mature glycoprotein synthesis, since the formation of such a structure cannot be explained by the alternative biosynthetic pathway. The degradation of sugar chains during analytical procedures is unlikely because so far we have never observed such a degradation of a sugar moiety.

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