Structures of the Asparagine-linked Sugar Chains of Subcomponent C1q of the First Component of Bovine Complement*

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Tsuguo Mizuochi, Takahiro Taniguchi, Junko Fujii-Kadowaki, Kunio Yonemasu, Takako Sasaki, and Akira Kobata

From the Department of Biochemistry, Kobe University School of Medicine, Chuo-Ku, Kobe, Japan and ∗Department of Bacteriology, Nara Medical College, Shikata, Nara, Japan

Bovine C1q, a subcomponent of the first component of complement, contains six asparagine-linked sugar chains in 1 molecule. The sugar chains are exclusively distributed in the noncollagenous regions.

The sugar chains were liberated as radioactive oligosaccharides from the polypeptide portion by hydrazinolysis followed by N-acetylation and NaBr₃/NaH reaction. In order to obtain information about their structures, they were studied by sequential exoglycosidase digestion in combination with methylation analysis. Bovine C1q was shown to contain equal amounts of neutral and acidic oligosaccharides with the following structures:

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Fucot
Galβ1→3Galβ1→4GlcNAcβ1→2Manβ1
Gaβ1→3Galβ1→4GlcNAcβ1→2Manβ1
Galβ1→3Galβ1→4GlcNAcβ1→2Manβ1
Galβ1→3Galβ1→4GlcNAcβ1→2Manβ1
Galβ1→3Galβ1→4GlcNAcβ1→2Manβ1
Galβ1→3Galβ1→4GlcNAcβ1→2Manβ1
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where NeuG1 is N-glycolylneuraminic acid.

Clq was purified to homogeneity (4). It was found that bovine Clq has a molecular structure similar to that of human Clq and can be substituted for human Clq in reconstructing active C1 with purified human C1r and C1s. However, bovine Clq, in contrast to human Clq, could not aggregate latex particles coated with human IgG (4). Because the carbohydrate composition of bovine Clq was slightly different from that of human Clq although the amino acid composition was very similar (4), structural differences in the sugar chain moieties of both Clq samples could be a cause of the difference in their Fc-binding activities. This paper will describe structural studies on the asparagine-linked sugar chains of bovine Clq and their location in the glycoprotein molecule.

EXPERIMENTAL PROCEDURES

RESULTS

Liberation of the Sugar Chains of Bovine C1q as Oligosaccharides—In order to determine the number of asparagine-linked sugar chains in 1 molecule, a time course study of the liberation of oligosaccharide by hydrazinolysis was performed by using 1 mg of bovine Clq and 6′-sialyllactose as an internal standard as described in detail previously (9). Based on the radioactivities of tritium incorporated into 6′-sialyllactose and the oligosaccharide mixture, and on M, = 407,000 of bovine Clq (4), the molar amount of the sugar chains liberated from 1 mol of the glycoprotein was calculated for each incubation time. The amount of sugar chains liberated reached a plateau at 10 h of hydrazinolysis and the total number of liberated oligosaccharides at this stage was 6 mol/mol of bovine Clq.

Based on this result, 30 mg of bovine Clq were subjected to 10 h of hydrazinolysis. One-fifth of the oligosaccharide fraction was reduced with NaBH₄ to obtain tritium-labeled oligosaccharides, and the remaining four-fifths was reduced with NaBH₃/H₂O to obtain deuterium-labeled samples for methylation analysis. The yield of tritium-labeled oligosaccharides was 2.1 × 10⁶ cpm. In order to facilitate the detection of the deuterium-labeled oligosaccharides in further purification steps, 1.0 × 10⁶ cpm of the tritium-labeled oligosaccharides was added.

Separation of Oligosaccharides by Paper Electrophoresis—Both radioactive and deuterium-labeled samples were

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The abbreviations used are: GlcNAc, N-acetylglucosamine; NeuG1, N-glycolylneuraminic acid; A and N, acidic and neutral radioactive fractions, respectively; A-N, neutral oligosaccharide obtained by sialidase treatment of A fraction; N-I, major component of N fraction. All sugars mentioned in this paper were of D-configuration except for fucose which has an L-configuration. Subscript OT is used to indicate NaB[3H₄]-reduced oligosaccharides.

2 Portions of this paper (including “Experimental Procedures,” Table I, and Figs. 1–5 and 7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1628, cite authors, and include a check or money order for $5.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
subjected to paper electrophoresis at pH 5.4. As shown in Fig. 1, acidic and neutral radioactive fractions were detected in the ratio of approximately 1:1. The yields of N and A fractions were $5.4 \times 10^5$ and $4.6 \times 10^5$ cpm, respectively.

When an aliquot of a fraction was incubated with sialidase, it was completely converted to neutral oligosaccharide, A-N. By mild acid hydrolysis (0.01 M HCl at 100 °C for 3 min), after which part of the original A fraction still remained, fraction A gave only a neutral component as a degradation product (data not shown). This result indicated that the acidic oligosaccharide was a monosialyl derivative. The sialic acid released from bovine C1q by sialidase digestion was identified as N-glycolylneuraminic acid by paper chromatography using butanol-1/acetic acid/water (12:3:5) as a solvent and no N-acetylgalactosaminic acid was detected.

**Paper Chromatography of N and A-N Fractions—** Upon paper chromatography, fraction A-N gave a single radioactive peak (Fig. 2B), while fraction N was separated into a minor peak which has the same mobility as A-N and a slower migrating major peak (Fig. 2A). The molar ratio of the major and the minor peaks based on their radioactivities was approximately 9:1. Preliminary experiments indicated that A-N and the minor component of fraction N have the same structure. Therefore, the structure of the major component of N fraction, N-1, was studied in detail together with that of A-N.

Upon Bio-Gel P-4 column chromatography, the radioactive oligosaccharides N-1 and A-N thus obtained behaved as single components eluting at the positions of 16.2 and 15.3 glucose units, respectively (Fig. 3, A and B). When aliquots ($5 \times 10^4$ cpm) of the radioactive oligosaccharides N-1 and A-N were hydrolyzed in 4 N HCl at 100 °C for 2 h and subjected to paper electrophoresis using borate buffer, pH 9.5, after N-acetylation, only N-acetylgalactosaminol was detected as a radioactive component in both cases. This result indicated that the reducing termini of both oligosaccharides are N-acetylgalactosamine.

**Sequential Exoglycosidase Digestion of Oligosaccharides N-1 and A-N—** In order to determine the anomeric configuration and sequence of each monosaccharide in N-1 and A-N, the radioactive N-1 and A-N were subjected to sequential exoglycosidase digestion and analyzed by Bio-Gel P-4 column chromatography. The original oligosaccharides were not susceptible to the action of β-N-acetylgalactosaminidase, α-mannosidase, and β-mannosidase (data not shown). However, when aliquots ($4 \times 10^4$ cpm each) of the radioactive N-1 and A-N were incubated with jack bean α-mannosidase, a mannose residue was released from R-Manα1→6(R-Manα1→3)Manα1→-4GlcNAc→ (Fig. 5C). In the case of N-1, structure I in Fig. 4 was confirmed by the sequential digestion by jack bean β-N-acetylgalactosaminidase, and the minor component of fraction N have the same structure.

In order to assign the galactose residue substituted by sialic acid, the following experiments were performed. Detection of 3 mol of 3,6-di-O-methylgalactitol was detected. In the case of A-N, 2 mol of 2,3,4,6-tetra-O-methylgalactositol and 1 mol of 2,4,6-tri-O-methylgalactositol were detected. Detection of 3 mol of 3,6-di-O-methyl-2-N-methylacetamido-2-deoxyglucitol indicated that the N-acetylgalactosamine residues at the reducing termini of both oligosaccharides are substituted at C-4 and C-6 positions by other sugars. After inactivation of the enzyme by α-L-fucosidase digestion, further confirming that the reducing termini of both oligosaccharides are N-acetylgalactosamine. From these results, the structures of N-1 and A-N can be written as (Galβ1→4Galβ1→GlcNAcβ1→Manα1→3 Manβ1→GlcNAcβ1→(Fucα1→GlcNAc)2) and Galβ1→(Galβ1→GlcNAcβ1→Manα1→3 Manβ1→GlcNAcβ1→(Fucα1→GlcNAc)2), respectively.

**Methylation Analysis of Oligosaccharides N-1 and A-N—** In order to determine the location of each glycosidic linkage, deuterium-labeled oligosaccharides N-1 and A-N were subjected to methylation analysis. As summarized in Table I, both oligosaccharides gave the same partially O-methylated alditois and N-methyleneaminoalditols in almost the same ratio except for the amount of 2,4,6-tri-O-methylgalactositol. Detection of 1 mol of 1,3,5-tri-O-methyl-2-N-methylacetamido-2-deoxyglucitol indicated that the N-acetylgalactosamine residues at the reducing termini of both oligosaccharides are substituted at C-4 and C-6 positions by other sugars. After inactivation of the enzyme by α-L-fucosidase digestion, further confirming that the reducing termini of both oligosaccharides are N-acetylgalactosamine. From these results, the structures of N-1 and A-N can be written as (Galβ1→4Galβ1→GlcNAcβ1→Manα1→3 Manβ1→GlcNAcβ1→(Fucα1→GlcNAc)2) and Galβ1→(Galβ1→GlcNAcβ1→Manα1→3 Manβ1→GlcNAcβ1→(Fucα1→GlcNAc)2), respectively.
heating at 100 °C for 3 min, the pH of the reaction mixture was adjusted to 5.0, and then the mixture was digested with sialidase. The neutral oligosaccharide thus obtained was smaller than A-N by 2 glucose units indicating that two galactose and a sialic acid residue were removed from oligosaccharide A (Fig. 5D). The radioactive peak in Fig. 5D released one N-acetylglucosamine residue upon incubation with jack bean β-N-acetylmuraminidase (Fig. 5E). These results indicated that the sialic acid residue of oligosaccharide A is exclusively linked to the Galβ1→4GlcNAc outer chain of A-N. Based on the results so far described, the structures of the all asparagine-linked sugar chains of bovine Clq are proposed as shown in Fig. 6.

Distribution of the Asparagine-linked Sugar Chains in the Bovine Clq Molecule—Like human Clq, bovine Clq is composed of noncollagenous and collagenous regions (4). In order to determine the distribution of the asparagine-linked sugar chains in the Clq molecule, collagenous and noncollagenous regions isolated from bovine Clq were subjected to hydrazinolysis and oligosaccharides thus liberated were analyzed by paper electrophoresis after NaB[3H] reduction. The radiopaper electrophoretograms are shown in Fig. 7. Because of the limited amount of samples available, the electrophoretograms were obtained by measuring the radioactivities in 1-cm paper pieces by liquid scintillation spectrometry. The noncollagenous region gave almost the same electrophoresis pattern as intact Clq shown in Fig. 1, while no radioactive oligosaccharide was recovered from the collagenous region. These results indicate that the asparagine-linked sugar chains of bovine Clq are exclusively distributed in the noncollagenous region, probably forming the globular head of the bovine Clq molecule as in the case of human Clq.

**DISCUSSION**

It was confirmed in this study that bovine Clq contains six asparagine-linked sugar chains in one molecule. This number agrees well with the previous data of monosaccharide composition by Yonemasu et al. (4) that 1 mol of bovine Clq contains 18 mannosyl residues, because all sugar chains have three mannosyl residues as their core structure.

As a whole molecule of glycoprotein, bovine Clq seems to have structural similarities in many respects to human Clq. Both glycoproteins are composed of collagenous and noncollagenous regions, and their asparagine-linked sugar chains are exclusively included in the noncollagenous region. Although the three-dimensional vision of bovine Clq has not been presented, the occurrence of six asparagine-linked sugar chains in this glycoprotein suggests that it all has the structure of “bouquet of six tulips,” as noted in the case of human Clq (12). In human Clq, an asparagine-linked sugar chain is supposed to be linked to each globular region composed of noncollagenous region (3).

In spite of the structural similarity described above, the carbohydrate portions of bovine Clq are not the same as those of human Clq. As reported previously (3), human Clq has a series of mono- and disialyl biantennary complex type asparagine-linked sugar chains and the structure of their outer chain moieties is either NeuAcα2→6Galβ1→4GlcNAcb1→ or Galβ1→4GlcNAcβ1→. The all asparagine-linked sugar chains of bovine Clq have the same Manα1→6(Manα1→3) Manβ1→4GlcNAcβ1→(4Fucα1→6)GlcNAc core as those of human Clq. However, their outer chain moieties are either Galβ1→3Galβ1→4GlcNAcβ1→ or NeuGlcα2→6Galβ1→4GlcNAcβ1→ and the latter outer chain is located only on Manα1→3 side. As the result, no disialyl sugar chains is included in bovine Clq. Whether the structural difference of the carbohydrate moieties of both glycoprotein is the cause of their different behavior toward latex particles coated with human IgG (4) is an interesting subject for future study.

The Galβ1→3Galβ1→4GlcNAcβ1→ outer chain was originally found in the asparagine-linked sugar chains of plasma membrane glycoproteins of calf thymocytes (10, 13, 14), and recently found in the sugar chains of rat erythrocyte membrane glycoproteins (15, 16). That no sialic acid is linked to this trisaccharide outer chain further supports our previous suggestion that the trisaccharide cannot be an acceptor for sialyl transferase (10).

A special comment is necessary for the fact that only N-glycolyneuraminic acid is found as a sialic acid residue of bovine Clq. This is in strict contrast to the sugar chain of bovine blood coagulation factor X. This glycoprotein contains a mucin-type sugar chain on Thr189. We have previously reported that the sugar chain shows heterogeneity, including nine different structures, and both N-acetyl- and N-glycolyneuraminic acids are distributed in these sugar chains in random fashion (17). The blood coagulation factor X used in the previous study on the mucin-type sugar chain was purified from pooled bovine blood sample, while the Clq used in this study was purified from a single cow. Therefore, individual or strain differences may occur in the sialic acid moiety of glycoproteins produced in cattle.

**Acknowledgment**—We would like to express our gratitude to Ikuko Ueda for her skillful secretarial assistance.

**REFERENCES**

Asparagine-linked Sugar Chains of Bovine C1q

Asparagine-linked Sugar Chains of Bovine Clq

Supplemental Materials to

STRUCTURES OF THE ASPARAGINE-LINKED SUGAR CHAINS OF THE FIRST COMPONENT OF BOVINE COMPLEMENT

By

Teigo Miwauchi, Tatsuhiko Taniguchi, Junichi Fujii-Miyakawa
Yasuhisa Tagawa, Tatsuro Tanaka, and Atsuo Kusama

The details of "Experimental Procedures" Table and Figures 1 to 5 and 7 are presented.

**Experimental Procedures**

Bovine Clq and its Oligosaccharides --- Bovine Clq was purified from bovine serum by the method described previously (1). Oligosaccharides and non-oligosaccharides region of Clq were prepared respectively by protein digestion and collagenase digestion of purified bovine Clq as reported in the work of Haas et al. (10).

Oligosaccharides --- Hexose2 → Gal/GalNAc → Man → GlcNAc → GlcNAc → GlcNAc → GlcNAc → GlcNAc
Hexose3 → Gal/GalNAc → Man → GlcNAc → GlcNAc → GlcNAc → GlcNAc → GlcNAc
Hexose4 → Gal/GalNAc → Man → GlcNAc → GlcNAc → GlcNAc → GlcNAc → GlcNAc

The radioactive sugars were subjected to Bio-Gel P-4 column chromatography and the radioactivity in each tube (1 ml/tube) was measured by liquid scintillation spectrometry. The small arrows indicate the eluting positions of glucose oligomers (numbers indicate the glucose units) added as internal standards and the big arrows indicate those of authentic oligosaccharides.

Fig. 1. Paper electrophoresis of oligosaccharides liberated from bovine Clq by hydrazinolysis. After reduction with NaBH4, the oligosaccharide mixture was subjected to paper electrophoresis at pH 3.6. Arrows indicate the positions where authentic sugars migrated. A, intact oligosaccharide N-1; B, the radioactive peak in (D) after incubation with jack bean α-mannosidase; C, the radioactive peak in (D) after jack bean α-N-acetylhexosaminidase digestion; D, the radioactive peak in (E) after 8-mannosidase treatment; E, the radioactive peak in (F) after jack bean α-N-acetylhexosaminidase digestion.
Asparagine-linked Sugar Chains of Bovine C1q

Table 1. Molar ratio of alditol acetates obtained from hydrolysates of permutated oligosaccharides.

<table>
<thead>
<tr>
<th>Methylated sugar</th>
<th>Molar ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>( \alpha )</th>
<th>( A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactitol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3,4,6-Tetra-O-methyl 1,5-di-O-acetyl</td>
<td>1.9</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>1,4,5-Tri-O-methyl 1,3,6-tri-O-acetyl</td>
<td>2.0</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>2,3,4-Tri-O-methyl 1,3,6-tri-O-acetyl</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Mannitur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4,6-Tri-O-methyl 1,3,5-tri-O-acetyl</td>
<td>2.1</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl 1,3,5-tri-O-acetyl</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Fucitol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-Tri-O-methyl 1,3,5-tri-O-acetyl</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>1,4-Di-O-acetamido-2-acetamido-2-fucosylitol</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1,3,6,4-di-O-methyl 1,5-di-O-acetyl</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methyl 5,3,6-dii-O-acetyl</td>
<td>2.6</td>
<td>3.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers were calculated by making the values of 2,4-di-O-acetyl-mannitol as 1.0.