Heterogeneity of N-linked Sugar Chains of Apolipoprotein B-100 in Watanabe Heritable Hyperlipidemic and Fasting Rabbits

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We have elucidated the structures of N-linked sugar chains of human apolipoprotein (apo) B-100 (Arch Biochem Biophys 1989; 273:197-205). To investigate the role of the carbohydrate moieties of apolipoprotein B-100, we determined the structures of the N-linked sugar chains of apo B-100 purified from low density lipoprotein (LDL) of a Watanabe heritable hyperlipidemic (WHHL) rabbit and compared them with those of fasting Japanese White rabbits. The N-linked oligosaccharides of apo B-100 were liberated by hydrazinolysis, followed by NaB₃H₄ reduction, and were fractionated by paper electrophoresis and Bio-Gel P-4 column chromatography. These consisted of one neutral fraction (N) and two acidic fractions (A₁ and A₂) in both WHHL and fasting rabbits. N contained high mannose type oligosaccharides consisting of Man₆GlcNAc₂ to Man₉GlcNAc₂, A₁ and A₂ contained mono- and disialylated biantennary complex type oligosaccharides, respectively. The molar ratios of N, A₁, and A₂ were 5:2:2 in the WHHL rabbit and 4:2:5 in fasting rabbits. The content of sialic acid residues in the WHHL rabbit was calculated to be 0.64 by taking the value of that in fasting rabbits as 1.0. These results show the heterogeneity of N-linked sugar chains of apo B-100 in WHHL and fasting rabbits and suggest the possibility that the characteristics of LDL in WHHL rabbits may be altered by making the surface charge more positive than in fasting rabbits. (Arteriosclerosis 10:386-393, May/June 1990)
dase, which cleaves only the Manα1-2Man linkage, purified from Aspergillus saitoi,20 was a generous gift of Akira Kobata (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Glycosidase digestion of the radioactive oligosaccharides was performed according to the method of Mizuochi et al.21

Oligosaccharides

(Manα1-2)6Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNACβ1-4GlcNACotβ1-4GlcNACot][Manα4-GlcNAC-GlcNACot], Galβ1-4GlcNACβ1-2Manα1-6(Galβ1-4GlcNACβ1-2Manα1-3)Manβ1-4GlcNACβ1-4GlcNACot(Galβ1-GlcNAC2-Manα2-GlcNAC-GlcNACot), GalNACβ1-2Manα1-6(GlcNACβ1-2Manα1-3)Manβ1-4GlcNACβ1-4GlcNACot (GlcNAC2-Manα2-GlcNAC-GlcNACot), Manα1-6(Manα1-3)Manβ1-4GlcNACβ1-4GlcNACot (Manα1-GlcNAC-GlcNACot), Manβ1-4GlcNACβ1-4GlcNACot (Manα1-GlcNAC-GlcNACot), GlcNACβ1-4GlcNACot (GlcNAC-GlcNACot), GlcNACot, and Manα1-3Manα1-6(Manα1-3)Manβ1-4GlcNACβ1-4GlcNACot (Manα1-GlcNAC-GlcNACot), were obtained from human apo B-100.9 (Subscript OT is used to indicate NaB^-reduced oligosaccharides. All sugars mentioned in this article are in the β-configuration.)

Preparation of Low Density Lipoprotein from Rabbits

Plasma was prepared with 0.1% (wt/vol) ethylenediaminetetraacetic acid disodium salt (EDTA-Na2) and 0.02% (wt/vol) NaCl from blood obtained from a WHHL rabbit fasted for 12 hours and from three fasting rabbits. Because sufficient LDL could not be obtained from a single fasting rabbit for an analysis of oligosaccharide structures, the plasma from three rabbits were pooled for analysis. LDL was prepared according to the method of Stein and Stein22 with a slight modification. The LDL fraction (d = 1.025 to 1.050) was dialyzed against 0.15 M NaCl, 0.01% (wt/vol) EDTA-Na2, and 0.02% (wt/vol) NaCl2, at pH 7.2.

Preparation of Apolipoprotein B-100

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), LDL was delipidated by extraction five times with 10 vol of isopropanol/distilled water, 1:1 (vol/vol)23 and four times with 10 vol of ethanol/diethyl ether, 3:1 (vol/vol).24 Protein was dissolved in 62.5 mM Tris buffer containing SDS (10 mg/ml protein) and 1% (vol/vol) 2-mercaptoethanol. The initial electrophoretic separation was carried out in 4% (wt/vol) polyacrylamide gels by the method of Laemmli.25 For localization of the bands, representative gels were fixed in ethanol/ether, 3M (vol/vol).24 Protein was dissolved in 62.5 mM EDTA-Na2 and 0.02% (wt/vol) NaCl2, at pH 7.2.

SDS-PAGE according to the method of Kane et al.24 Periodic acid-Schiff (PAS) staining was performed by the method of Segrest and Jackson.26

Liberation of the Asparagine-linked Sugar Chains of Apolipoprotein B-100 from WHHL and Fasting Rabbits

Apo B-100 from WHHL and fasting rabbits was suspended in anhydrous hydrazine and was heated in sealed tubes at 100°C for 8 hours as reported previously.27 The reaction mixtures were then evaporated to dryness under reduced pressure over concentrated H2SO4. The residues were freed from hydrazine by repeated evaporation with toluene and were dissolved in saturated aqueous NaHCO3 solution, and all free amino groups were acetylated completely with acetic anhydride. The reaction mixtures were passed through columns of Dowex 50W (H+), and the columns were washed with five bed volumes of distilled water. The eluates and washings were combined and evaporated to dryness under reduced pressure. The residues were then subjected to paper chromatography for 2 days to remove the degradation products derived from the peptide moiety. The area corresponding to 0 to 5 cm from the origin, where the oligosaccharides larger than lactose were included, was cut out, and the oligosaccharides were recovered by elution with distilled water. The oligosaccharide fractions obtained from apo B-100 from both WHHL and fasting rabbits were reduced with 166.6 μCl of NaB^3H4 in 100 μl of 0.05 M NaOH at 30°C for 4 hours.

Analytical Methods

High-voltage paper electrophoresis and Bio-Gel P-4 column chromatography were carried out according to the procedures described by Mizuochi et al.28 and Yamashita et al.29

Results

SDS-PAGE of Apolipoproteins of Low Density Lipoproteins from WHHL and Fasting Rabbits

The apolipoprotein components of LDL from WHHL and fasting rabbits after electrophoresis on 4% (wt/vol) gels are shown in Figure 1. In both WHHL and fasting rabbits, apo B-100 represents the major component of apo B. A minor component corresponding to apo B-95 was observed in WHHL and fasting rabbits, and another minor component with the mobility of apo B-48 was detected in the WHHL rabbit (Figures 1A and 1D). These results in the WHHL rabbit are identical to those in a previous report.30 To identify carbohydrates in the components of apo B of the WHHL rabbit, the gels were stained by means of PAS reduction. All three components were stained (Figure 1B). When we checked the samples after elution from gels by re-electrophoresis, only apo B-100 was detected in both WHHL and fasting rabbits (Figures 1C and 1E).

Fractionation of Oligosaccharides by Paper Electrophoresis

The radioactive oligosaccharide mixtures obtained from apo B-100 were subjected to paper electrophoresis...
Figure 1. Sodium dodecyl sulfate-polyacrylamide gel (4%) electrophoretograms of apolipoproteins of low density lipoprotein (LDL) and apolipoprotein B-100 from a Watanabe heritable hyperlipidemic (WHHL) rabbit and fasting Japanese White rabbits stained with Coomassie brilliant blue R-250 (A, C, D, and E) and periodic acid-Schiff (PAS) (B). A. Apolipoproteins of LDL from the WHHL rabbit. B. Apolipoproteins of LDL from the WHHL rabbit with PAS reaction. C. Apolipoprotein B-100 of WHHL rabbit. D. Apolipoproteins of LDL from fasting Japanese White rabbits. E. Apolipoprotein B-100 of fasting Japanese White rabbits. The scale represents molecular mass of standards (kDa).

Figure 2. Paper electrophoretogram of the radioactive oligosaccharides. The radioactive oligosaccharide mixture was subjected to paper electrophoresis at pH 5.4. The arrows indicate the positions of the authentic oligosaccharide standard and the dye front. 1. Lactitol. 2. Bromophenol blue. A. Watanabe heritable hyperlipidemic rabbit. B. Fasting Japanese White rabbits. At pH 5.4. As shown in Figure 2, one neutral fraction (N) and two acidic fractions (A1 and A2) were obtained from WHHL and fasting rabbits, respectively. Both A1 and A2 were converted to neutral oligosaccharides by sialidase digestion. Mild acid hydrolysis (0.01 N HCl at 100°C for 3 minutes) of A2, in which part of the original acidic component still remained, gave another acidic component with the same mobility as A1, together with a neutral component on paper electrophoresis. In contrast, fraction A1 treated under similar conditions gave only a neutral product (data not shown). The results indicate that A1 and A2 contain one and two sialic acid residues, respectively. The percent molar ratios of neutral, monosialylated, and disialylated oligosaccharide fractions were 54.3:24.3:21.4 in the WHHL rabbit and 38.4:18.0:43.6 in the fasting rabbits.

Structures of the Neutral Oligosaccharide Fraction N

The neutral oligosaccharide fraction N obtained from apo B-100 of WHHL rabbits was analyzed by Bio-Gel P-4 column chromatography and gave the fractionation pattern shown in Figure 3A. To determine the monosaccharide sequence, the radioactive component N was subjected to sequential exoglycosidase digestion, and the products thus obtained were analyzed by Bio-Gel P-4 column chromatography. Upon incubation with Aspergillus saitoi α-mannosidase, most of the radioactive component N (indicated by the bar) was converted to the oligosaccharide with the same mobility as authentic Man5-GlcNAc2 (Figure 3B). A trace amount of fraction N, whose elution position was 13.5 glucose unit, was resistant to this enzymatic digestion. This minor component gave the same results as oligosaccharide a in the A1N fraction, as described below. When the radioactive oligosaccharide indicated by the bar in Figure 3B was subjected to exoglycosidase digestion with jack bean α-mannosidase, the radioactive product was detected at the same elution position as that of authentic Man-GlcNAc-GlcNAc2, releasing four mannose residues (Figure 3C). Upon incubation with snail β-mannosidase, this radioactive product was converted to the oligosaccharide with the same mobility as authentic GlcNAc-GlcNAc2, releasing one mannose residue (Figure 3D). The radioactive product in Figure 3D was converted to the oligosaccharide with the same mobility as authentic GlcNAc2 by jack bean β-N-acetylhexosaminidase digestion, releasing one N-acetylgalactosamine residue (Figure 3E). When most of the radioactive component N (indicated by the bar in Figure 3A) was incubated with Streptomyces griseus endo-β-N-acetylhexosaminidase H, which specifically cleaves high mannose type oligosaccharides and hybrid type oligosaccharides,31,32 the radioactive product was detected at the same elution position as that of authentic GlcNAc2 (data not shown). These results indicate that most of oligosaccharide N is a high mannose type oligosaccharide, and 0 to 4 mannose residues attached to Man5-GlcNAc2 with an α1-2 linkage. Since the structure of Manα1-6(Manα1-3)Manα1-6(Manα1-3) Manβ1-4GlcNAcβ1-4GlcNAc2 based on these results and the knowledge of assembly and processing of the N-linked oligosaccharide33 the structure of the fraction N should be considered (Manα1-2)Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc2. The elution pattern of the neutral oligosaccharide fraction obtained from apo B-100 of fasting rabbits was similar to that of the
Figure 3. Sequential exoglycosidase digestion of the radioactive oligosaccharides N from a Watanabe heritable hyperlipidemic (WHHL) rabbit and fasting Japanese White rabbits. The radioactive oligosaccharides were subjected to Bio-Gel P-4 column chromatography, and the radioactivity of an aliquot in each tube (2.2 ml/tube) was measured by liquid scintillation spectrometry. The arrows indicate the elution positions of glucose oligomers (numbers indicate the glucose units) added as internal standards, and the arrowheads indicate the elution positions of authentic oligosaccharides. I. Man5GlcNAc2GlcNAc10. II. Man5GlcNAc2GlcNAc10. III. Man5GlcNAc2GlcNAc10. IV. Man5GlcNAc2GlcNAc10. V. GlcNAc5GlcNAc10. VI. GlcNAc5GlcNAc10. VII. GlcNAc5GlcNAc10. VIII. GlcNAc5GlcNAc10. A. The oligosaccharide fraction N from the WHHL rabbit. B. The radioactive peaks in A after Aspergillus saitoi α-mannosidase digestion. C. The radioactive peak indicated by the bar in B after jack bean α-mannosidase digestion. D. The radioactive peak in C after snail β-mannosidase digestion. E. The radioactive peak in D after jack bean β-N-acetylhexosaminidase digestion. F. The radioactive peak in E after jack bean α-glucosidase digestion. G. The radioactive peak in F after β-glucosidase digestion. H. The radioactive peak in G after jack bean α-mannosidase digestion. I. The radioactive peak in H after jack bean β-glucosidase digestion. J. The radioactive peak in I after jack bean α-galactosidase digestion. K. The radioactive peak in J after jack bean β-galactosidase digestion. L. The radioactive peak in K after jack bean α-mannosidase digestion. M. The radioactive peak in L after jack bean β-glucosidase digestion. N. The radioactive peak in M after jack bean α-galactosidase digestion. O. The radioactive peak in N after jack bean β-galactosidase digestion. P. The radioactive peak in O after jack bean α-mannosidase digestion. Q. The radioactive peak in P after jack bean β-glucosidase digestion. R. The radioactive peak in Q after jack bean α-galactosidase digestion. S. The radioactive peak in R after jack bean β-galactosidase digestion. T. The radioactive peak in S after jack bean α-mannosidase digestion. U. The radioactive peak in T after jack bean β-glucosidase digestion. V. The radioactive peak in U after jack bean α-galactosidase digestion. W. The radioactive peak in V after jack bean β-galactosidase digestion. X. The radioactive peak in W after jack bean α-mannosidase digestion. Y. The radioactive peak in X after jack bean β-glucosidase digestion. Z. The radioactive peak in Y after jack bean α-galactosidase digestion. AA. The radioactive peak in Z after jack bean β-galactosidase digestion. BB. The radioactive peak in AA after jack bean α-mannosidase digestion. CC. The radioactive peak in BB after jack bean β-glucosidase digestion. DD. The radioactive peak in CC after jack bean α-galactosidase digestion. EE. The radioactive peak in DD after jack bean β-galactosidase digestion. FF. The radioactive peak in EE after jack bean α-mannosidase digestion. GG. The radioactive peak in FF after jack bean β-glucosidase digestion. HH. The radioactive peak in GG after jack bean α-galactosidase digestion. II. The radioactive peak in HH after jack bean β-galactosidase digestion. JJ. The radioactive peak in II after jack bean α-mannosidase digestion. KK. The radioactive peak in JJ after jack bean β-glucosidase digestion. LL. The radioactive peak in KK after jack bean α-galactosidase digestion. MM. The radioactive peak in LL after jack bean β-galactosidase digestion. NN. The radioactive peak in MM after jack bean α-mannosidase digestion. OO. The radioactive peak in NN after jack bean β-glucosidase digestion. PP. The radioactive peak in OO after jack bean α-galactosidase digestion. QQ. The radioactive peak in PP after jack bean β-galactosidase digestion. RR. The radioactive peak in QQ after jack bean α-mannosidase digestion. SS. The radioactive peak in RR after jack bean β-glucosidase digestion. TT. The radioactive peak in SS after jack bean α-galactosidase digestion. UU. The radioactive peak in TT after jack bean β-galactosidase digestion. VV. The radioactive peak in UU after jack bean α-mannosidase digestion. WW. The radioactive peak in VV after jack bean β-glucosidase digestion. XX. The radioactive peak in WW after jack bean α-galactosidase digestion. YY. The radioactive peak in XX after jack bean β-galactosidase digestion. ZZ. The radioactive peak in YY after jack bean α-mannosidase digestion. AAA. The radioactive peak in ZZ after jack bean β-glucosidase digestion. BBB. The radioactive peak in AAA after jack bean α-galactosidase digestion. CCC. The radioactive peak in BBB after jack bean β-galactosidase digestion. DDD. The radioactive peak in CCC after jack bean α-mannosidase digestion. EEE. The radioactive peak in DDD after jack bean β-glucosidase digestion. FFF. The radioactive peak in EEE after jack bean α-galactosidase digestion. GGG. The radioactive peak in FFF after jack bean β-galactosidase digestion. HHH. The radioactive peak in GGG after jack bean α-mannosidase digestion. IJJ. The radioactive peak in HHH after jack bean β-glucosidase digestion. KKK. The radioactive peak in IJJ after jack bean α-galactosidase digestion. LLL. The radioactive peak in KKK after jack bean β-galactosidase digestion. MMM. The radioactive peak in LLL after jack bean α-mannosidase digestion. NNN. The radioactive peak in MMM after jack bean β-glucosidase digestion. OOO. The radioactive peak in NNN after jack bean α-galactosidase digestion. PPP. The radioactive peak in OOO after jack bean β-galactosidase digestion. QQQ. The radioactive peak in PPP after jack bean α-mannosidase digestion. RRR. The radioactive peak in QQQ after jack bean β-glucosidase digestion. SSS. The radioactive peak in RRR after jack bean α-galactosidase digestion. TTT. The radioactive peak in SSS after jack bean β-galactosidase digestion. UUU. The radioactive peak in TTT after jack bean α-mannosidase digestion. VVV. The radioactive peak in UUU after jack bean β-glucosidase digestion. WWW. The radioactive peak in VVV after jack bean α-galactosidase digestion. XXX. The radioactive peak in WWW after jack bean β-galactosidase digestion.
were analyzed by Bio-Gel P-4 column chromatography. Upon incubation with jack bean β-galactosidase, the radioactive oligosaccharides a and b moved to the same elution position as authentic GlcNAc-Mα1-2Man-GlcNAc-GlcNAcOT, releasing two and one galactose residues, respectively (solid line in Figure 4B). This product was converted to the oligosaccharide with the same mobility as authentic Man3-β1-2Man-GlcNAc-GlcNAcOT, by jack bean β-N-acetylgalactosaminidase digestion, releasing two N-acetylgalactosamine residues (dotted line in Figure 4B). Subsequently, upon incubation with jack bean α-mannosidase, this radioactive product was converted to the oligosaccharide with the same elution position as authentic Man-GlcNAc-GlcNAcOT, releasing two mannose residues (Figure 4C). When the radioactive product in Figure 4C was sequentially digested with small β-mannosidase and jack bean β-N-acetylgalactosaminidase, the results were the same as those of fraction N (data not shown). The same radioactive product as shown by the dotted line in Figure 4B was obtained from oligosaccharides a and b by incubation with a mixture of diplococcal β-galactosidase and diplococcal β-N-acetylgalactosaminidase (data not shown). Therefore, these results indicate that oligosaccharide a has two Galβ1-4GlcNAcβ1-2Man groups, and oligosaccharide b has one each of Galβ1-4GlcNAcβ1-2Man and GlcNAcβ1-2Man groups linked to the trimannosyl core of Man3-β1-4GlcNAc-GlcNAcOT, since diplococcal β-galactosidase cleaves the Galβ1-4GlcNAc linkage but not the Galβ1-3GlcNAc or the Galβ1-6GlcNAc linkages,34 and diplococcal β-N-acetylgalactosaminidase cleaves the GlcNAcβ1-2Man but not the GlcNAcβ1-4Man or the GlcNAcβ1-6Man linkages35; oligosaccharide b released one N-acetylgalactosamine residue and moved to the same elution position as oligosaccharide d, while oligosaccharide a was resistant to this enzymatic digestion upon incubation with jack bean β-N-acetylgalactosaminidase and γ-galactosidase (Figure 4D). Since the structure of the trimannosyl core should be considered Manα1-6(Manα1-3)Manβ1-4GlcNAc-GlcNAcOT, based on the results of sequential exoglycosidase digestion and the knowledge of assembly and processing of the N-linked oligosaccharides,30-32 oligosaccharide a was shown to have the structure of Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcOT, and oligosaccharide b lacks one galactose residue of this oligosaccharide (see Figure 6).

The radioactive oligosaccharides c and d released 1 mol each of galactose and N-acetylgalactosamine residues by sequential digestion with jack bean β-galactosidase (solid and dotted lines in Figure 4E, respectively) and jack bean β-N-acetylgalactosaminidase (solid and dotted lines in Figure 4F, respectively). The elution position of the radioactive products in Figure 4F were the same as those of authentic Man3-β1-GlcNAc-GlcNAcOT and Man3-β1-GlcNAc-GlcNAcOT. Upon incubation of these radioactive products in Figure 4F with jack bean α-mannosidase, three and two mannose residues were released, respectively, and the elution position was the same as that of authentic Man-GlcNAc-GlcNAcOT. The two radioactive components in Figure 4F were obtained from oligosaccharides c and d by incubation with a mixture of diplococcal β-galactosidase and diplococcal β-N-acetylgalactosaminidase (data not shown). These results indicate that oligosaccharides c and d have a Galβ1-4GlcNAcβ1-2Man group in their oligomannosyl cores. To determine the location of the Galβ1-4GlcNAc group, oligosaccharides c and d and both radioactive peaks in Figures 4E and 4F were subjected to endo-β-N-acetylgalactosaminidase D digestion. Both radioactive peaks in Figure 4F were converted to GlcNAcOT, but those in Figure 4E were not, by this enzymatic digestion. Since endo-β-N-acetylgalactosaminidase D requires the presence of an unsubstituted α-mannosyl residue linked at the C-3 position of the β-mannosyl residue for its substrate,30-32 these results indicate that the structures of oligosaccharides c and d should be Manα1-3(6)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcOT, and Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcOT, respectively. The elution pattern of oligosaccharide A1N of fasting rabbits was similar to that of the WHHL rabbit (Figure 4G). The results of sequential exoglycosidase digestion and Bio-Gel P-4 column chromatography were the same as those of the WHHL rabbit (data not shown). The molar ratio of the one major and three minor components was 45:26:14:15.

When fraction A2N of the WHHL rabbit was analyzed by Bio-Gel P-4 column chromatography, the single radioactive peak eluted at the same position as authentic Galβ2-4GlcNAcβ2-Man3-β1-GlcNAc-GlcNAcOT (Figure 5A). The results of exoglycosidase digestion were the same as those of oligosaccharide a of A1N (Figures 5B and 5C). Upon incubation with a mixture of diplococcal β-galactosidase and diplococcal β-N-acetylgalactosaminidase and analysis by Bio-Gel P-4 column chromatography, the radioactive product was eluted at the same position as authentic Man3-β1-GlcNAc-GlcNAcOT (data not shown). Based upon these results, oligosaccharide A2N has two Galβ1-4GlcNAcβ1-2Man groups attached to the trimannosyl core of Man3-β1-GlcNAc-GlcNAcOT. For A2N of fasting rabbits, the elution pattern was similar to that of the WHHL rabbit (Figure 5D), and the results of sequential exoglycosidase digestion were the same (data not shown). The structures of the N-linked oligosaccharides of apo B-100 in WHHL and fasting rabbits and the percent molar ratio of each are proposed in Figure 6.

Discussion

In this study, we have elucidated the structures of the N-linked sugar chains of apo B-100 obtained from a WHHL rabbit and compared them with those of fasting rabbits. These consisted of high mannose type and biantennary complex type oligosaccharides, which contained one or two sialic acid residues. As a minor component of A1, hybrid type oligosaccharides existed. The structural components of rabbit apo B-100 were the same as those of human apo B-100. In normolipidemic human subjects, the carbohydrate structures of apo B-100 and the ratios of each oligosaccharide fraction were constant among individuals.30-32 Although unique structures in WHHL...
W-glycosylation sites of human apo B-100 were directly sequenced, and it was shown that 16 were found to be thought to play several roles, such as signaling for protein cellular differentiation and interaction, and protection from proteolytic degradation. Recently, all 19 potential N-glycosylation sites of human apo B-100 were directly sequenced, and it was shown that 16 were found to be glycosylated and three were not. Of the 16 glycosylated peptides, seven were found in the domain from amino acid residues 3071 to 4100. This domain appears to include a multifunctional region of apo B-100, LDL receptor binding domain(s), some of heparin-binding sites, and some of the segments that interact with arterial proteoglycan. These results suggest that the carbohydrate moieties of apo B-100 are important in LDL metabolism.

The content of sialic acid residues in the WHHL rabbit was calculated to be 0.64 by taking the value of that in fasting rabbits as 1.0, because the acidic oligosaccharides had one or two sialic acid residues. Therefore, the sialic acid content of apo B-100 of the WHHL rabbit was considered to be lower than that of fasting rabbits. It has been recognized that sialic acids are involved in the regulation of a great variety of biological phenomena. This function of sialic acids appears to be due mainly to their peripheral position in glycoconjugates and their strongly negative charge. The physiological significance of sialic acids in LDL has been investigated. Camejo et al. investigated the interaction of LDL with arterial proteoglycan, which has been thought to play an important role in atherosclerotic development. They showed that the sialic acid content in LDL was inversely correlated with the insoluble complexes formed between LDL and the arterial proteoglycan and that LDL and arterial proteoglycan complexes increased endocytosis of the lipoprotein and stimulation of lipid synthesis in macrophages. Recently, the segments mediating the interaction of LDL with arterial proteoglycans were elucidated. In some of these segments, N-linked sugar chains were clustered. The decrease in sialic acid content of apo B-100 could alter the surface charge of LDL to make it more positive, and might contribute to the progressive development of atherosclerosis by increasing the binding capacity for arterial proteoglycans in WHHL rabbits. Orekhov et al. showed that atherogenic patients' LDL had a two- to fivefold lower level of sialic acid as compared with the nonatherogenic LDL of healthy donors. It is suggested that the decrease of sialic acid level in LDL may result from the heterogeneity of the N-linked sugar chain of apo B-100.

The mechanism for the heterogeneity of the N-glycosylation of apo B-100 has yet to be clarified. A difference in the oligosaccharide processing might exist between WHHL and fasting rabbits. A key factor in determining the synthesis of N-linked oligosaccharides is the level of expression of the various glycosyltransferases. The same series of glycosyltransferases are considered to act in both rabbits because no different structure of oligosaccharides existed between them. Two other factors that influence the N-glycosylation are the conformation of the polypeptide chain and the cellular metabolic state.

In humans, the heterogeneity of apo B-100 has been shown by immunological methods, the restriction fragment length polymorphism method, and direct DNA sequence. To the best of our knowledge, however, no genetic or biochemical evidence of mutation of amino acid sequence of apo B-100 has been reported in WHHL rabbits. Further investigation of the amino acid sequence of apo B-100 in WHHL rabbits is necessary.

The synthesis and the secretion of apo B are closely related to intrahepatic lipid metabolism.
rabbits, cholesterol synthesis is suppressed in the liver, and this altered lipid metabolism might affect the N-glycosylation of apo B-100 during apo B-100 synthesis in the WHHL rabbit.

We have shown the heterogeneity of the N-linked oligosaccharides of apo B-100 between WHHL and fasting rabbits and discussed the possibility of its biological significance in atherosclerosis with respect to sialic acid residues. Further study will be required to determine the biological roles of N-linked oligosaccharide of apo B-100 and to resolve the mechanism for this heterogeneity.

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Figure 6. The proposed structures of the oligosaccharides in N, A1N, and A2N fractions and the molar ratio of each in the Watanabe heritable hyperlipidemic rabbit and fasting Japanese White rabbits.
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Index Terms: carbohydrate structures • N-linked (asparagine-linked) sugar chains • apolipoprotein B-100 • glycoproteins • familial hypercholesterolemia • Watanabe heritable hyperlipidemic rabbits
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