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The Monosaccharide Composition and Sequence of the Carbohydrate Moiety of Human Serum Low Density Lipoproteins†

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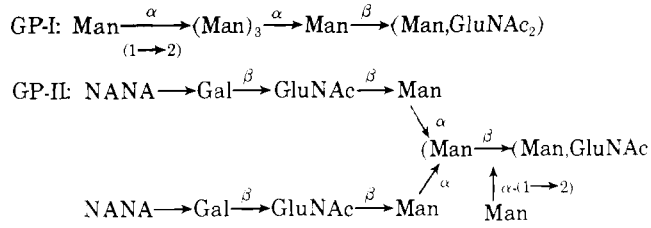
ABSTRACT: Human serum low density lipoprotein (d = 1.027–1.045) was delipidated with organic solvents and the apoprotein digested with thermolysin. The digest was fractionated by gel filtration and DEAE-cellulose chromatography. Two glycopeptides were obtained. One of the glycopeptides (GP-I) contained 2 residues of *N*-acetylglucosamine and 6 residues of mannose per mole of the glycopeptide, while the other contained 2 sialic acid, 5 mannose, 2 galactose, and 3 *N*-acetylglucosamine residues per mole of glycopeptide. The results of sequential enzymatic digestion with purified glycosidases, periodate oxidation, and partial acid hydrolysis lead us to propose the following structures for the two glycopeptides:



These glycopeptides represent at least 50% of the carbohydrate moiety of LDL.

The protein moiety of low density lipoproteins has been shown to contain 5–9% carbohydrate consisting of galactose, mannose, glucosamine, and sialic acid (Schultze and Heide, 1960; Ayrault-Jarrier, 1961; Marshall and Kummerow, 1962; Swaminathan and Aladjem, 1974; Kwiterovich et al., 1974). Shore and Shore (1973) demonstrated that serum VLDL[1] interacts with concanavalin-A and McConathy and Alaupovic (1974) showed that LDL interacts with concanavalin-A while HDL does not, both studies indicating the presence of terminal glucopyranosyl, mannopyranosyl, or sterically related residues in LDL. Margolis and Langdon (1966) found that sialic acid residues could be removed from native LDL with sialidase treatment without affecting lipid binding. The present paper describes the results of our studies to define the number, nature, and the monosaccharide sequence of the carbohydrate units in LDL. We have prepared glycopeptides from LDL and succinylated LDL using a specific protease, thermolysin. The monosaccharide sequence was deduced from the combined application of acid hydrolysis, periodate oxidation, and digestion with glycosidases of demonstrated specificity.

Materials and Methods

Low density lipoprotein (d = 1.027–0.045 gm/cc) was isolated from pooled normal human serum containing 0.1% EDTA as previously reported (Aladjem et al., 1957). Approximately 100 mg of protein was obtained from 1 l. of serum. The purity of preparation was tested by immunochemical methods using specific antisera. Within the detectability of these methods, no contamination of HDL, VLDL, or of other serum proteins was found.

Delipidation was performed using ether–ethanol mixtures. To a preparation of native LDL (or succinylated native LDL) dialyzed against 0.15 M NaCl overnight and diluted to a protein concentration of 3 to 4 mg/ml, 20 volumes of a mixture of ethanol–ether (3:1 v/v) was added dropwise with continuous stirring. The solution was stirred for 4 to 6 h and centrifuged at 1500g for 20 min. The precipitated protein was extracted with ethanol–ether (3:2 v/v) and centrifuged. The white precipitate was washed with ether and then dried in a current of N_2.

Succinylation was performed on native LDL as described by Scanu et al. (1968) followed by delipidation as described above. Completion of the succinylation reaction was monitored by ninhydrin reaction (Frankel-Conrat, 1957).

Preparation of Glycopeptides from LDL. Thermolysin

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[1] Abbreviations used: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; S-LDL, succinylated low density lipoproteins; Gal, galactose; Man, mannose; GluNAc, *N*-acetylglucosamine; NANA, *N*-acetylneuraminic acid.

was employed for the preparation of glycopeptides since the amino acid composition of LDL shows a high content of hydrophobic amino acids. To a suspension of delipidated LDL (approximately 600 mg of protein; 35 mg of carbohydrate) in 20 ml of 0.1 M ammonium bicarbonate buffer, pH 8.0, 2% by weight of thermolysin (Calbiochem) was added, and the mixture was incubated at 37 °C. At the end of 24 h another equal portion of thermolysin was added and the incubation continued to 48 h. The digest was centrifuged; the clear supernatant (containing about 18 mg of carbohydrate) was fractionated on a Sephadex G-50 column (2.4 × 40 cm) equilibrated with water. The effluent was monitored for absorption at 280 nm and the carbohydrate content was determined by the phenol-H_2SO_4 reaction (DuBois et al., 1956). The fractions showing positive carbohydrate reaction were pooled and concentrated by ultrafiltration using UM-2 membranes (Amicon). The filtrate was checked for the presence of carbohydrate. No carbohydrate was found in the filtrate.

Further purification of glycopeptide was carried out by DEAE-cellulose chromatography. The concentrated glycopeptide was loaded on a column of DEAE-cellulose (1 × 40 cm) equilibrated with 0.05 M sodium phosphate buffer, pH 6.8, and washed with the same buffer. The peptides were eluted with a gradient between 0.05 M sodium phosphate buffer, pH 6.8, and 0.2 M NaCl in the same buffer.

Further purification of glycopeptides was achieved by preparative paper chromatography on Whatman 3MM paper. The purity of glycopeptides was checked by paper and thin-layer chromatography and high-voltage electrophoresis. Paper and thin-layer chromatography were performed using 1-butanol–pyridine–acetic acid–water, 15:10:3:12 v/v (Margoliash, 1962). High-voltage electrophoresis was performed in pyridine–acetic acid–water, pH 1.9 (Hielmann et al., 1957).

Carbohydrate Determinations. Total carbohydrate was estimated by the phenol–sulfuric acid method (DuBois et al., 1956) and also by the anthrone method (Trevelyan and Harrison, 1952). The neutral sugars were released from glycopeptides and also from LDL following digestion with 1 N H_2SO_4 at 100 °C for 2–4 h in sealed tubes. The acid was neutralized with Dowex-1 HCO_3 and the supernatant was deionized with mixed-bed resin MB-3 (Mallinkrodt). Small aliquots were dried in small tubes and the neutral sugars were determined by gas–liquid chromatography as trimethylsilyl ethers (Sweeley et al., 1963) on OV-1 columns. In some experiments capillary columns were employed for determination of small quantities (less than 200 mg) of carbohydrate. The details of this method will be published elsewhere (Swaminathan, Apon, and Aladjem, unpublished).

Hexosamines were released from glycopeptides with 4 N HCl at 100 °C for 4 h in sealed tubes and estimated after purification by Dowex-50 H^+ chromatography according to the method of Boas (1953). Glucosamine and galactosamine were also determined in the amino acid analyzer using fluorescamine system. Both sugars react with fluorescamine and were well resolved. The details of this method will be published elsewhere (Swaminathan and Aladjem, unpublished).

Sialic acid was determined by the thiobarbituric acid procedure of Warren (1959) and also by the resorcinol method (Svennerholm, 1958).

Isolation of Glycosidases. β-D-Galactosidase, β-D-*N*-acetylglucosaminidase, and β-D-mannosidase were prepared from *Aspergillus niger* (*A. niger*) by the method of Matta and Bahl (1972). α-D-(1→2)Mannosidase was purified from *A. niger* as previously described by Swaminathan et al. (1972). α-D-Mannosidase was prepared from jack bean meal as described by Snaith and Levvy (1968).

Digestion with Glycosidases. The glycopeptides (0.02–0.05 μmol) were incubated with 5–10 units of the appropriate glycosidases in 5 mM sodium citrate buffer, pH 4.7. Incubations with α-mannosidase (jack bean) were done in 5 mM acetate buffer containing 0.1 mM $ZnSO_4$. The incubation lasted from 24 to 48 h with further additions of enzyme for complete hydrolysis. Aliquots of the reaction mixture were removed at various time intervals and analyzed for neutral sugars by the reducing sugar procedure of Somogyi and Nelson (Spiro, 1966). *N*-Acetylglucosamine was analyzed by the method of Reissig et al. (1955). Quantitation of the neutral sugars was done by gas–liquid chromatography. In some instances, for isolation of the digested core, the hydrolyzate was passed through a small column of Sephadex G-25 (1 × 15 cm) equilibrated and developed with water. The fractions containing the residual glycopeptide were pooled, lyophilized, and used for further analysis.

Amino Acid Analysis. Amino acid analyses were performed after hydrolysis with 6 N HCl containing 1% thioglycolic acid for 24 h at 110 °C in sealed tubes in vacuo, using the fluorescamine method (Stein et al., 1973).

Protein Determination. Protein determinations were done by the Lowry method (Lowry et al., 1951). Human serum albumin served as standard. Protein concentrations were also obtained from amino acid analyses.

Periodate Oxidation. Periodate oxidation was done as described by Baenziger and Kornfeld (1974). The glycopeptide (0.02–0.05 μmol) was treated with 50 μl of 0.4 M sodium metaperiodate in 0.005 M acetate buffer, pH 4.6, at 4 °C for 24 h. Excess periodate was destroyed by adding 3–5 μl of ethylene glycol. Aliquots of the sample were hydrolyzed for determination of neutral sugars and hexosamines which were determined as described above.

Molecular Weight Determination. Molecular weights of glycopeptides were determined by gel filtration on Sephadex G-50 as described by Bahl (1969). Minimal molecular weights were calculated from chemical composition according to Mahowald et al. (1962).

Results

Carbohydrate Composition. The carbohydrate moiety of LDL is composed of galactose, mannose, glucosamine, and sialic acid. Literature values and our results of the average carbohydrate composition of eight LDL preparations are shown in Table I. Sialic acid values showed marked variation. The values for sialic acid ranged from 6 to 17 μg per mg of protein. The hexosamine values and the values for galactose and mannose were fairly constant. The values reported in the literature are included for comparison.

Partial Acid Hydrolysis. In order to establish optimal hydrolysis conditions, the effect of acid hydrolysis on LDL was investigated.

Hydrolysis of delipidated LDL with 0.1 N H_2SO_4 at 80 °C for 1 h released all the bound sialic acid. Hydrolysis at 100 °C with 0.1 N H_2SO_4 for 3 h released 34% of the total galactose and about 5% of the total mannose.

The rate of release of monosaccharides from LDL during hydrolysis with 1 N H_2SO_4 at 100 °C was different for each sugar (Figure 1). Thus galactose was completely released in about 2 h and mannose in about 4 h. The initial rates of release decreased in the following order: galactose,

Table I: Carbohydrate Content of LDL (Percent Dry Weight).

	Reference				
	a	*b*	*c*	*d*	*e*
Sialic acid	1.5	1.3	0.35	0.6	1.73 ± 0.71
Glucosamine	2.0	1.2	1.20	0.9	0.94 ± 0.14
Galactose	2.7		3.23	1.8	2.13 ± 0.26
Mannose	2.7			3.7	4.88 ± 0.64

[a] Shultze and Heide (1960). [b] Ayrault-Jarrier (1961). [c] Marshall and Kummerow (1962). [d] Kwiterovich et al. (1974). [e] (Swaminathan and Aladjem, this study); the results are expressed as percent of protein content determined by amino acid analysis. The results of eight independent analyses are given as mean ± standard deviation.

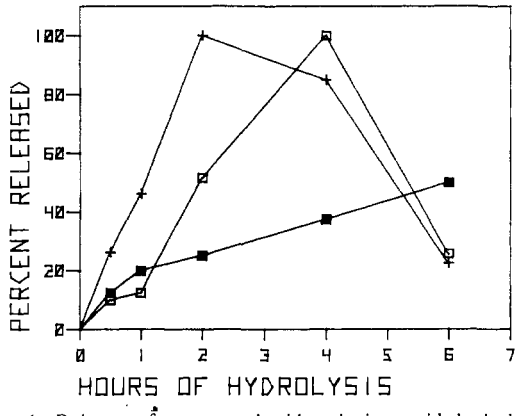


FIGURE 1: Release of monosaccharides during acid hydrolysis of LDL. LDL (6.8 mg of protein) was hydrolyzed with 1 N H_2SO_4 at 100 °C for various time periods. Neutral sugars and glucosamine were determined as described under methods. (X) Galactose; (■) glucosamine; (□) mannose. The amounts of galactose, glucosamine, and mannose at their maximal levels correspond to 137, 31.3, and 299 μg, respectively.

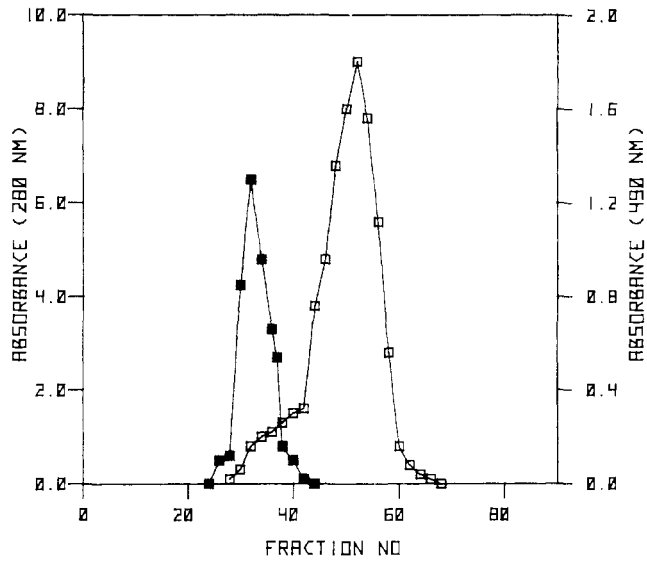


FIGURE 2: Separation of glycopeptides from the thermolysin digest of delipidated LDL on Sephadex G-50. Approximately 18 mg of carbohydrate was applied in a volume of 10 ml to the Sephadex column (2.5 × 50 cm) and developed with water. Fractions (3 ml) were collected. (□) Absorbance at 280 nm; (■) absorbance at 490 nm.

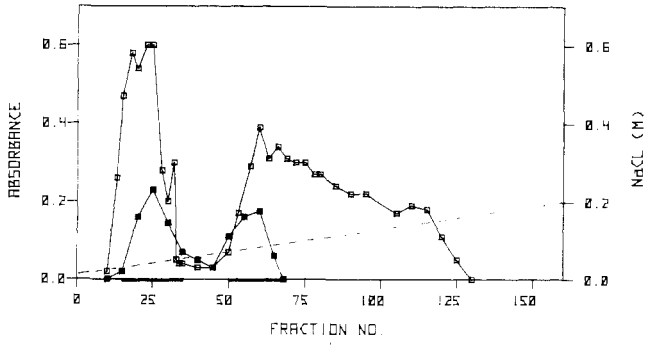


FIGURE 3: Separation of glycopeptides from LDL on DEAE-cellulose. (□) Absorbance at 280 nm; (■) absorbance at 490 nm.

glucosamine, mannose. Only about 50% of total glucosamine was released in 6 h of hydrolysis with 1 N H_2SO_4. However, hydrolysis with 4 N HCl at 100 °C for 4 h was found to give maximal release of hexosamines.

Isolation of Glycopeptides from LDL. Glycopeptides were prepared from delipidated LDL by digestion with thermolysin followed by fractionation on Sephadex G-50 (Figure 2) and DEAE-cellulose chromatography (Figure 3). Two glycopeptides, GP-I and GP-II, were obtained. These glycopeptides were found by thin-layer chromatography to contain contaminating noncarbohydrate peptides and, therefore, were purified by preparative paper chromatography. The glycopeptides stayed close to the origin and were eluted with dilute acetic acid.

Amino acid analysis of GP-I and GP-II revealed that these peptides contained about 30 amino acid residues. Molecular weights were estimated by gel filtration to be about 4200 and 5100 daltons, respectively. A preparation of succinyl LDL was digested with thermolysin and fractionated by gel filtration on Sephadex G-25 and DEAE-cellulose chromatography (Figure 4). Three glycopeptides s-GP-I, s-GP-II, and s-GP-III were obtained. s-GP-I and s-GP-II showed the presence of peptide contaminants and were further purified by preparative paper chromatography. The molecular weights of s-GP-I, s-GP-II, and s-GP-III were found to be 5800, 5500, and 5500, respectively.

The carbohydrate compositions of the glycopeptides from apo-LDL and succinyl-LDL are given in Table II. On comparison, it may be seen that the carbohydrate compositions of GP-I and s-GP-I are very similar and that they contain only mannose and glucosamine. The compositions of GP-II and s-GP-III are very similar and contain galactose, sialic acid, mannose, and glucosamine. The composition of s-GP-II, however, showed fewer sialic acid and mannose residues compared with those of s-GP-III and GP-II. Studies of carbohydrate sequence showed that the general carbohydrate sequence was similar to those of s-GP-III and GP-II (see further).

Effect of Glycosidases on GP-II. The carbohydrate composition of GP-II showed the presence of 2 sialic acid, 2 galactose, 5 mannose, and 3 glucosamine residues. Effect of glycosidase on GP-II is described in Table III (experiments 1-3). Treatment of GP-II with β-galactosidase, or β-*N*-acetylglycosaminidase did not release any sugar (experiment 1, Table III). On the other hand, jack bean meal α-D-mannosidase, which hydrolyzes α-(1→2), α-(1→4), or α-(1→6) linked mannose residues (Li, 1967) as well as *A. niger* α-D-(1→2)-mannosidase (specific for α-D-(1→2) linked mannose only) both released 1 residue of mannose (experiments 1 and 2, Table III). On mixed incubations with both mannosidases, still only 1 mol of mannose was re-

Table II: Carbohydrate Composition of Glycopeptides from LDL and S-LDL.

	Residues/Mole of Glycopeptide				
	Sialic Acid	Galactose	Mannose	*N*-Acetylglucosamine	Mol Wt
From LDL					
GP-I			6.0	1.7	4200,[a] 4050[b]
GP-II	2.0	2.0	4.8	2.8	5100,[a] 4900[b]
From S-LDL					
s-GP-I			6.0	1.9	5800[a]
s-GP-II	0.8	2.1	4.1	2.6	5500[a]
s-GP-III	2.0	2.1	5.2	2.9	5500[a]

[a] From gel filtration. [b] From chemical composition.

Table III: Effect of Glycosidases on GP-II and s-GP-II.

	Residues Released per Mole			
Experiment	Sialic Acid	Galactose	Mannose	*N*-Acetylglucosamine
1. GP-II + β-galactosidase then β-*N*-acetylglucosaminidase then α-D-mannosidase (jack bean)			1.0	
2. GP-II + α-(1→2)-mannosidase (*A. niger*) then α-D-mannosidase (jack bean)			1.0 0.1	
3. GP-II + neuraminidase (*V. cholera*) then β-galactosidase then β-*N*-acetylglucosaminidase then α-D-mannosidase (jack bean) then β-*D*-mannosidase (*A. niger*)	2.0	1.9	2.6 1.2	1.8
4. s-GP-II + β-*D*-galactosidase then β-D-*N*-acetylglucosaminidase then α-D-mannosidase (jack bean) then β-D-mannosidase (*A. niger*)		1.0	0.9	1.0
5. s-GP-II + α-(1→2)-mannosidase (*A. niger*) then α-mannosidase (jack bean)				
6. s-GP-II + neuraminidase (*V. cholera*) then β-D-galactosidase then β-D-*N*-acetylglucosaminidase then α-D-mannosidase (jack bean) then β-D-mannosidase (*A. niger*)	0.8	2.0	2.0 1.4	2.0

Table IV: Effect of Glycosidases on GP-I.

Experiment	Residues of Mannose Released
1. GP-I + α-(1→2)-mannosidase (*A. niger*)	1.0
+ α-D-mannosidase	2.9
2. GP-I + α-D-mannosidase (jack bean)	4.1
then β-D-*N*-acetylglucosaminidase then β-D-mannosidase (*A. niger*)	0.4

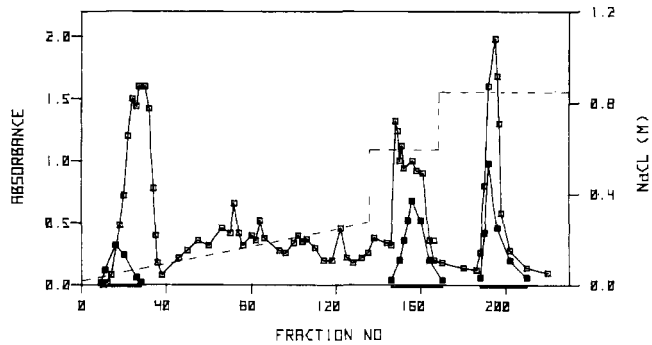


FIGURE 4: Separation of glycopeptides from succinyl-LDL on DEAE-cellulose. (□) Absorbance at 280 nm; (■) absorbance at 490 nm.

leased, showing the presence of an α-D-(1→2) linked mannose and that mannose is a nonreducing terminal.

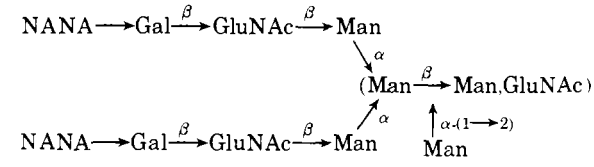
Treatment with sialidase (*Vibrios cholera*) removed 2 residues of sialic acid (experiment 3, Table III). Two residues of galactose were released with β-galactosidase (*A. niger*) from desialyzed GP-II. This suggests that the 2 residues of galactose occupy penultimate positions to the sialic acid residues.

After removal of sialic acid and galactose, β-*N*-acetylglucosaminidase (*A. niger*) removed 2 residues of *N*-acetylglucosamine showing a sialic acid, galactose, *N*-acetylglucosamine sequence. Further digestion with α-mannosidase (jack bean) removed approximately 3 residues of mannose. When the above sequence of digestions was carried out using the glycopeptide GP-II from which 1 residue of mannose has been removed by digestion with α-(1→2)-mannosidase (*A. niger*), 2 mol of mannose were released with α-mannosidase after removal of *N*-acetylglucosamine (not shown in table).

On periodate oxidation of glycopeptide GP-II only 2 residues of mannose and 3 residues of *N*-acetylglucosamine are left intact (Table IV).

The glycopeptide obtained after digestion of GP-II with sialidase, β-galactosidase, β-*N*-acetylglucosaminidase, and α-mannosidase showed a glucosamine to mannose ratio of 0.9:1.7. Treatment of this core with β-mannosidase (*A. niger*) released 1.2 residue of mannose.

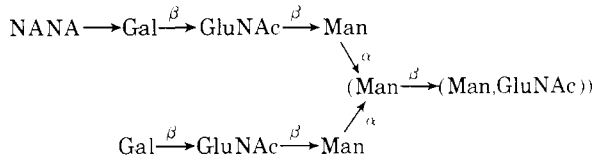
The above experiments point to the following sequence for the carbohydrate unit of GP-II:



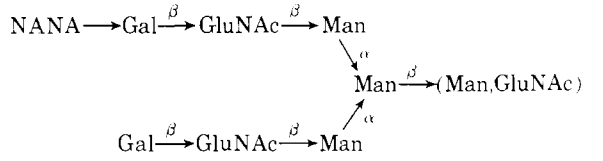
Effect of Glycosidases on s-GP-II. The sequential digestion of s-GP-II with various glycosidases and the results are summarized in Table III (experiments 4–6). The structure of s-GP-II is very similar to that of GP-II except that s-GP-II lacks 1 residue of sialic acid and 1 residue of α-(1→2) linked mannose.

Treatment of s-GP-II with β-*N*-acetylglucosaminidase or α-mannosidase did not release any sugar (experiment 5, Table III). Digestion with β-galactosidase released 1 residue of galactose (experiment 4, Table III). Further digestion with β-*N*-acetylglucosaminidase released 1 residue of *N*-acetylglucosamine. α-Mannosidase removed 0.9 residue of mannose. Further digestion with β-mannosidase did not release any mannose. The results of these experiments suggested the following sequence: Gal→GluNAc→Man→ with galactose at the nonreducing end.

On treatment with neuraminidase (*Vibrios cholera*), 0.8 residue of sialic acid was released from s-GP-II (experiment 6, Table III). On further treatment with β-galactosidase 2 residues of galactose were released. On digestion with β-*N*-acetylglucosaminidase and α-mannosidase, 2 residues of acetylglucosamine and 2 residues of mannose were released, respectively. Further digestion with β-mannosidase released 1.4 residues of mannose. These experiments point to the following structure:

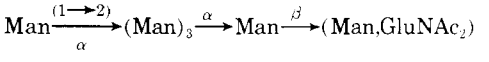


Periodate oxidation of the glycopeptide s-GP-II left 1 residue of mannose and 3 residues of glucosamine intact (Table V). The mannose that survives the periodic oxidation is likely to be the branch point and the following structure is proposed:



Effect of Glycosidases on GP-I. The composition of GP-I showed the presence of mannose and glucosamine in the ratio of 3:1. Six residues of mannose and 2 residues of *N*-acetylglucosamine are present per mole of glycopeptide.

Incubation of GP-I with β-*N*-acetylglucosaminidase did not release any glucosamine from the peptide. Incubation with α-D-(1→2)-mannosidase (*A. niger*) removed 1 residue of mannose (Table IV), showing a terminal α-(1→2) linked mannose. Further incubation with jack bean meal α-mannosidase released 3 mol of mannose. Incubation with β-mannosidase released 0.4 residue of mannose. No hexosamine was removed on incubation with β-*N*-acetylglucosaminidase. The following structure is proposed:



On periodate oxidation, 2 residues of mannose and the 2 glucosamine residues are left intact (Table V). These mannose residues are likely to be the branch points.

Discussion

The results of this study suggest the presence of two structurally distinct types of carbohydrate units in low density lipoproteins. One type contains mannose and glucosamine while the other type contains mannose, galactose, sialic acid, and glucosamine. Two forms of the latter glycopeptide have been characterized and shown to have essentially similar monosaccharide sequences with minor differences.

Preliminary studies with acid hydrolysis for different time intervals suggested the likely arrangement of sugar residues in the order: sialic acid, galactose, glucosamine, mannose. Incomplete release of glucosamine under these conditions suggested its location in more interior positions. Considering the presence of another structural type containing mannose and glucosamine, higher initial rates of mannose release during acid hydrolysis might be expected. The absence of such a release suggests that fewer units only are present relative to the other complex type.

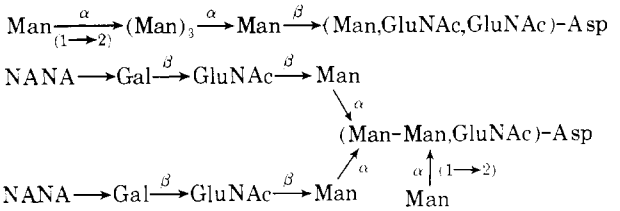
Digestion with glycosidases has been very useful in elucidating the monosaccharide sequence in this study. Use of specific glycosidases of *A. niger* has helped in predicting the linkage type in some cases in addition to the anomeric configuration of the linkage. However, assignments of the intersugar linkages and exact branch points have to come from methylation studies.

The linkage of the carbohydrate units to protein is presumably through asparagine residue to glucosamine after extensive digestion of GP-II and GP-I with pronase followed by gel filtration on Sephadex G-25; two glycopeptides were obtained that contained only aspartic acid as the major amino acid (Swaminathan and Aladjem, unpublished).

Based on the structural studies reported above, the following structures are proposed for the two major glycopeptides.

type I

(GP-I and s-GP-I)



type II

(GP-II and s-GP-III)

The presence of similar units has been shown in other glycoproteins. The type I carbohydrate unit, for instance, is similar to that which is present in ovalbumin (Johansen et al., 1961), thyroglobulin (Spiro, 1965), ribonuclease B (Plummer and Hirs, 1964), deoxyribonuclease (Catley et al., 1969), and immunoglobulins (Baenziger and Kornfeld, 1974). Carbohydrate units with terminal sequences similar to that of type II have been shown to be present in human chorionic gonadotropin (Bahl, 1969), α_1-acid glycoproteins (Eylar and Jeanloz, 1962; Wagh et al., 1969), thyroglobulin (Spiro, 1965), and immunoglobulins (Baenziger and Kornfeld, 1974). However, the type II carbohydrate units of LDL seem to differ from similar units of other glycoproteins in two respects. One is the presence of an α-(1→2) linked mannose residue in the core of the carbohydrate unit; the other is that the type II structure contains only 3 glu-

Table V: Effect of Periodate Oxidation on Glycopeptides.

Experiment	Residues per Mole of Glycopeptide			
	Sialic Acid	Galactose	Mannose	*N*-Acetylglucosamine
1. Intact GP-I			6.0	1.7
+ $NaIO_4$			2.0	1.7
2. Intact GP-II	2.0	2.0	4.8	2.8
+ $NaIO_4$			2.0	2.8
3. Intact s-GP-II	0.8	2.1	4.2	2.6
+ $NaIO_4$			0.9	2.6

cosamine residues per mole, 2 of which were accounted for in two chains penultimate to galactose and 1 residue at the core. This would then suggest the absence of a chitobiose core unit attached to the protein. The mannose residues which are attached to the core glucosamine were not completely removed.

The average molecular weight of the carbohydrate moiety of type I is about 1500 and for the type II is about 2400. Assuming a molecular weight of 2.5×10^6 for the native LDL consisting of 20% protein, one can calculate that 15–20 units of these carbohydrate moieties might be present in 1 mol of LDL. The higher content of galactose suggests that type II units predominate over type I. However, presence of carbohydrate units in what may be different degrees of completion, such as may be the case with glycopeptide s-GP-II, should not be ignored. It has not yet been determined whether the glycopeptides S-GP-II and GP-II occur on the same molecule or whether there exists a subpopulation of LDL each with different types of glycopeptides. It is also worth looking at the following: the galactose to glucosamine weight ratio of delipidated LDL was found to be 2; the ratio for glycopeptide GP-II was 0.7. Glycopeptide GP-I does not contain galactose. The glycopeptides which we have isolated and characterized, therefore, do not account for all the galactose of LDL. It is likely, therefore, that apo-LDL contains additional glycopeptides which are of different composition and structure and that these glycopeptides were present in the core fraction of apo-LDL which was not digestible with thermolysin. We do not know whether all LDL molecules contain similar carbohydrates, or whether the carbohydrate moiety differs in different molecules thus giving rise to subpopulations. In the latter case, one might expect antigenic heterogeneity due to carbohydrate differences. As far as we know, this possibility has not yet been investigated.

The function of the carbohydrate moiety in LDL is not clear at present. Some possible functions of carbohydrate in glycoproteins have been proposed in the past, such as in the secretion from the cell (Eylar, 1965) or in the regulation of their catabolism (Morell et al., 1971). Carbohydrate units without any apparent function may also be added to a protein possessing the requisite structural sequence obtained by way of mutation (Marshall, 1972). In this connection, it is of interest to note that the secretion of LDL is inhibited by orotic acid administration (Windmueller and Levy, 1967) and orotic acid might affect glycosylation of lipoproteins in the liver (Pottinger et al., 1973). Morell et al. (1971) have shown that the removal of sialic acid from glycoproteins decreases their biological half-life. Similar studies using native and desialyzed LDL labeled with ^{125}I did not show significant differences in their respective rates of disappearance (Swaminathan and Aladjem, 1974).

Interaction of LDL with Sepharose-bound concanavalin-A has been observed (McConathy and Alaupovic, 1974). Our finding of carbohydrate units with terminal mannose units would explain this interaction. Since similar interaction has been observed with VLDL also, it is possible that VLDL has similar carbohydrate units and/or that the reactions are only due to the LDL component which is present in most VLDL subpopulations (Pearlstein and Aladjem, 1972). The VLDL apoprotein has been shown to contain another type of carbohydrate unit which contains galactose, sialic acid, and galactosamine in glycosidic linkage with a threonine residue (Brewer et al., 1974).

The presence of LDL in atherosclerotic plaques (Woolf and Pilkington, 1965; Smith and Slater, 1970) has been demonstrated, and uptake of LDL by cultured fibroblasts has been reported (Brown and Goldstein, 1974). These processes might involve the interaction of the carbohydrate moiety of LDL with cell membranes. In view of the fact that human LDL has been demonstrated to be taken up specifically and degraded by human aortic smooth muscle cells (Stein and Stein, 1975) and cultured fibroblasts (Goldstein and Brown, 1974b) and that LDL has been shown to regulate the content of free and esterified cholesterol in human fibroblasts (Goldstein and Brown, 1974a), it is conceivable that the relative "atherogenicity" of LDL may depend upon the carbohydrate moiety.

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Solubilization of an α-Bungarotoxin-Binding Component from Rat Brain†

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ABSTRACT: Binding of [^{125}I]-α-bungarotoxin to rat brain was investigated. Picomole quantities of specific toxin binding sites per gram of fresh tissue were found in particulate preparations as well as detergent extracts of whole brain. The toxin-binding macromolecules can be solubilized in low concentrations of Triton X-100. Specific binding occurs to a single class of sites with a dissociation constant of 5.6×10^{-11} M. The association rate constant in 10 mM sodium phosphate, pH 7.4, was determined to be 6.8×10^5 M^{-1} s^{-1}; the half-life of the complex was found to be 5.1 h, corresponding to a dissociation rate constant of 3.8×10^{-5} s^{-1}. The binding macromolecules resemble peripheral nicotinic acetylcholine receptors in toxin binding kinetics, solubility, isoelectric point, and hydrodynamic properties.

Considerable information on the biochemistry of nicotinic acetylcholine receptors from peripheral tissues is available at present. Much of the progress in this field has been due to the use of α-bungarotoxin (αBgt[1]) and related neurotoxins which bind with high affinity and selectivity to cholinergic receptors in skeletal muscle and electric organ (Hall, 1972; Changeux, 1975). In contrast, little is known concerning αBgt-binding components of the central nervous system and their relationship with nicotinic receptors.

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[1] Abbreviations used: αBgt, α-bungarotoxin: [^{125}I]-αBgt, [^{125}I]-α-bungarotoxin; CM-cellulose, carboxymethyl-cellulose.