The Oligosaccharides of Serum Lipoproteins: A Brief Review and Localization of an Apoprotein Sialyl Transferase in Golgi Apparatus and Partial Purification of the Enzyme[1]

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Abstract

Many of the apoprotein subunits of the serum lipoproteins of humans and other species contain covalently-bound carbohydrates in the form of oligosaccharides attached to specific amino acid residues. Glycosylation of the apoproteins occurs in specific cell organelles organized into two linear routes of synthesis and secretion. Early glycosylation may determine which route is followed or glycosylation may vary depending on which route is followed. Since bound oligosaccharides appear to be involved in recognition by both hepatic and extrahepatic receptors, the synthesis-secretion route may ultimately control the behavior of lipoproteins in blood. Both abnormal recognition and abnormal glycosylation of serum lipoproteins are encountered with atherosclerotic and/or dislipoproteinemic patients. An apoprotein sialyltransferase which terminally glycosylates circulating lipoproteins is localized in Golgi apparatus, where it is strongly bound to the membranes. This activity of the Golgi apparatus has been solubilized using detergents and purified 150-fold.

Serum lipoproteins may be divided into three classes based on flotation density in the ultracentrifuge (3). These are very low density lipoproteins (VLDL) ($d<1.006$), low density lipoproteins (LDL) ($d = 1.006$-1.063) and high density lipoproteins (HDL) ($d = 1.063$-1.210). Lipoproteins of all species studied, including man, have great similarity in the protein subunits (apoproteins) of these circulating lipoproteins. VLDL contains apoproteins B, C-1, C-2, and C-3 in addition to an arginine-rich protein. LDL contains primarily apoprotein B and sometimes the arginine-rich protein. HDL contains A-1, A-2 and the arginine-rich and C peptides. Many of these peptides contain covalently-bound carbohydrate (Table 1) so that these circulating lipoproteins are actually glycolipoproteins.

Oligosaccharides of glycoproteins in general have been implicated as controlling factors in their secretion. Eylar (4) observed that all of a group of secreted proteins were glycoproteins and that most intracellular proteins were not. He interpreted this as evidence for a glycosylation-dependency of glycoprotein secretion. Knopf et al. (10) found that addition of fucose to light chains of immunoglobulin secreted by murine myeloma cells only occurs immediately before their secretion as if the terminal fucose of the oligosaccharides allowed the last step in secretion to occur. Finally, Pottenger et al. (18) demonstrated that apoproteins which accumulate in rough endoplasmic reticulum vesicles of orotic acid-induced fatty livers were deficient in N-acetylglucosamine, galactose, and sialic acid.

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TABLE 1. *Comparison of the carbohydrate composition of human and rat apolipoproteins.*

Apoprotein	Carbohydrate Composition			
	Human	Ref.	Rat	Ref.
B	Man, GalNAc, Gal, NAN	26		
A-I	Hexose, hexosamine	20		
C-3	GalNAc, Gal, NAN	1	GalNAc, NAN	5
Arg-rich	Binds to ConA-sepharose	24	Man, Gal, Glu, NAN N-acetylhexosamine	18

Abbreviations: Gal = Galactose; GalNAc = N–acetylgalactosamine; Glu = Glucose; Man = Mannose; NAN = N-acetylneuraminic acid (sialic acid).

Contrary to Eylar's (4) expectations, more recent studies have shown that human serum, bovine milk, and bovine pancreatic juice, all secreted fluids, contain from 40 to 95% non-glycosylated proteins; non-glycosylated collagen is also secreted in certain disease states (23). Pottenger et al. (18) prefer the interpretation that lipoproteins were abnormally glycosylated because they had not passed through Golgi apparatus where the terminal glycosyl transferases are concentrated. A reasonable model of synthesis-secretion of glycoproteins has been constructed which may explain these seemingly disparate observations (16). Lipoproteins may exit the cell via secretory vesicles derived from either the Golgi apparatus or, more directly, via vesicles derived from smooth endoplasmic reticulum (SER). Presumably, proteins secreted by SER vesicles are less glycosylated than those secreted by Golgi apparatus-derived vesicles, paralleling the subcellular distribution of the glycosyl transferases that catalyze sugar additions. Sugars are added to glycoproteins in specific sequence, the product of one reaction becoming the substrate of the next transferase in the pathway (8). Also certain glycosylations near the core region of the glycoprotein oligosaccharide can preclude the possibility of more terminal glycosylations (8). Non-glycosylated collagen and other non-glycosylated serum proteins might be secreted by the SER route, thus bypassing certain terminal glycosylation steps. Glycosyl transferases catalyzing additions of mannose and internal N-acetylglucosamines are found in endoplasmic reticulum as well as Golgi apparatus (22). The observed increased levels of internal sugars such as mannose in lipoproteins of orotic acid-induced fatty livers may reflect such activities. That alterations of normal intracellular flow of lipoproteins can occur has been demonstrated by Werderitsh et al. (27). Hyperlipoproteinemic rabbits had disoriented Golgi apparatus with large accumulations of lipid in smooth endoplasmic reticulum in the apical cytoplasm.

The potential importance of terminal sialic acids in determining the clearance of glycoproteins from blood has been shown by Morrell et al. (14) in that removal of sialic acid from a variety of glycoproteins caused their rapid accumulation in the liver through interactions with a specific receptor (7). Although the role of sialic acid in determining lipoprotein clearance is not certain, oligosaccharides in general are involved in a wide range of effector-receptor interactions (2). There-

fore it is to be expected that the secretory route will profoundly influence the behavior of secreted lipoproteins in the circulation. The abnormal occurrence of lipoproteins in arterial intima during atherogenesis (25) and some forms of hyperlipoproteinemia, may be a reflection of altered lipoprotein-receptor interactions.

An understanding of the characteristics of the glycosyl transferases plus clarification of the mechanisms of their incorporation into a specific biosynthesis-secretory pathway will contribute much toward completing the conceptual framework of lipoprotein synthesis and assembly. Munro et al. (17) have shown an apolipoprotein sialyltransferase to be concentrated 45-fold in rat liver Golgi apparatus. Yet, no work has been directed toward isolation, purification and detailed characterization of these enzymes.

As part of a study to isolate and characterize the glycosyl transferases of lipoprotein glycosylation, Golgi apparatus were isolated from rat liver as described (15). The isolated Golgi apparatus were treated with ultrasound for 1 minute (Branson sonifier, micro tip, amplitude setting of 1) in 0.05 M KCl containing 0.1 M Tris-maleate, pH 6.6 and 14 mM 2-mercaptoethanol. Membranes were collected by centrifugation (85,000 X g for 1 hr), resuspended in the same buffer and Triton X-100 was added to 0.6% at a protein concentration of about 1 mg/ml of final solution. This solution was treated with ultrasound for 1 min, amplitude setting of 1. Following a 3.5 hr incubation at 4°, insoluble residue was removed by centrifugation (1 hr, 100,000 X g). This treatment completely solubilized the sialyltransferase in a form satisfactory for further purification and characterization. The recovery was 68% of that in the original Golgi apparatus.

To assay the sialyltransferase, VLDL were isolated from whole rat blood by the method of Koga et al. (9), delipidated according to Scanu et al. (21) and desialated using neuraminidase from *Clostridium perifringens* (Sigma Chemical Co., St. Louis, 0.14 enzyme units/mg protein) (28). Incubations were for 10-20 min at 37°. The reaction mixture contained, in a final volume of 0.05 ml, the following: 0.15 M sodium cacodylate, 0.5% Triton X-100, 0.5% Triton CF-54, 10 mM $MgCl_2$, 14 mM 2-mercaptoethanol, 18 nmoles ^{14}C-CMP-sialic acid (6.6 mCi/mmole), 7-10 μg asialoVLDL, and 15-60 μg enzyme protein, final pH 6.4. The reaction was terminated by addition of chloroform-methanol (2:1, by volume) to precipitate protein and extract lipids. The precipitated protein was washed twice each with acetone-water (1:1, by volume) and water, and radioactivity was determined by liquid scintillation counting. Enzyme specific activities were calculated from the counts per min of radioactive sialic acid transferred to the desialylated VLDL. Proteins were estimated by the Lowry et al. (13) method following extraction to remove excess Triton X-100 (7).

The sialyltransferase of VLDL sialylation was concentrated 79-fold in Golgi apparatus relative to the starting homogenate (Table 2). The enzyme was tightly bound to the membrane; treatment with KCl to release secretory and extrinsic membrane proteins resulted in a further purification of the sialyltransferase of 1.5. The final purification of the

TABLE 2. *Purification of CMP-NAN: desialylated apoVLDL sialyl transferase from Golgi apparatus of rat liver.*

Enzyme Source	Specific Activity (nanomoles sialic acid/hr/mg protein)	Relative Specific Activity	Total Activity (nanomoles sialic acid/hr/10 g rat liver)
Total Homogenate	0.32	1	390
Golgi Apparatus[a]	25	79	150
Golgi Apparatus Membranes			
Low Salt Extracted[b]	38	121	105
Triton X-100 Extract[c]	47	150	100

[a] Recovery of Golgi apparatus determined from other criteria (15) was about 40%. This means that (150 X 100/40)/390 = 96% of the activity was probably Golgi apparatus-associated in the original homogenate.

[b] Low salt extraction solubilized 150 − 105/150 X 100 = 30% of the activity. The remainder was associated with the Golgi apparatus membranes.

[c] Triton X-100 solubilized 100/105 X 100 = 96% of the activity remaining with the low salt extracted membranes.

Triton X-100 solubulized enzyme was 150-fold. This partially purified sialyltransferase had an absolute requirement for detergent or lipids.

Recent findings from several laboratories implicate a disfunction in sialyltransferase in atherogenesis. Lee and Breckenridge (11) report lower quantities of both mannose and sialic acid in apoproteins from type II hyperlipoproteinemic subjects while galactose and N-acetylglucosamine were unchanged. In a second paper, these authors could find no differences in carbohydrate chains of individually isolated glycopeptides of LDL, suggesting that the lesion was the result of change in proportion of sialylated glycopeptides, i.e., due to decreased amount of glycopeptides of similar composition to those found in normal subjects (12). This contrasts with studies of the abnormal lipoproteins in abetalipoproteinemia, where an oversialylated form of HDL predominates (19). A recent report (6) provides evidence that a structurally defective LDL may not bind properly to surface receptors. Cholesterol synthesis in leukocytes from the affected subjects was not suppressed by the subject's own LDLs but was suppressed by LDLs from control subjects. Conversely, LDLs from the diseased subjects failed to suppress cholesterol synthesis in control leukocytes. Thus the complex carbohydrate array of the oligosaccharide chains of circulating lipoproteins may provide surface characteristics that are important to recognition processes. Knowledge of how sialyltransferases of the Golgi apparatus and the operation of alternate secretory routes interact to generate specific carbohydrate sequences may be important to understanding and treatment of various forms of hyperlipoproteinemia and atherogenesis.

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