Biochemistry (Moscow), Vol. 66, No. 4, 2001, pp. 397-401. Translated from Biokhimiya, Vol. 66, No. 4, 2001, pp. 489-494.

Sialyltransferase Activity in Normal and Atherosclerotic Human Aorta Intima

E. V. Gracheva*, N. N. Samovilova, N. K. Golovanova, O. P. Il'inskaya, E. M. Tararak, and N. V. Prokazova

Institute of Experimental Cardiology, Cardiology Research Center, Russian Ministry of Health, 3-ya Cherepkovskaya ul. 15a, Moscow, 121552 Russia; fax: (095) 415-2962; E-mail: gracheva@cardio.ru

Received June 26, 2000
Revision received October 16, 2000

Abstract—Sialyltransferase activity has been determined in Golgi membrane fractions isolated from atherosclerotic and normal intima of human aorta by measuring the transfer of N-acetylneuraminic acid (NeuAc) from CMP-NeuAc to asialofetuin. The asialofetuin-sialyltransferase activity was found to be twofold higher in the atherosclerotic intima than in the normal intima. The mean value of the apparent Michaelis constant (K_m) for the sialylating enzyme in both tissues did not differ and was 57 μM. In contrast, the maximal velocity (V_{max}) was 2-fold higher for the atherosclerotic intima than for the normal intima. These results suggest that expression of asialofetuin-sialyltransferases of the aortal intima may be increased in atherosclerosis.

Key words: sialyltransferase activity, human aorta intima, Golgi membrane fractions, atherosclerosis

Sialic acids are found at the terminal position of cell surface oligosaccharide chains of glycoproteins and glycolipids [1]. Termination of carbohydrate chain synthesis significantly depends on the activity and expression of sialyltransferases transferring sialic acid residues from the donor, CMP-sialic acid, to the synthesized carbohydrate chain. The level of these enzymes is tissue- and cell-specific [2] and changes during cell differentiation, embryogenesis, and carcinogenesis [3].

The sialic acid residue determines the ability of integral glycoproteins and glycolipids to integrate into the plasma membrane. The specific arrangement of glycoproteins and glycolipids on the cell surface, in turn, determines receptor activity, adhesion functions, and formation of cell–cell interactions [1]. Posttranslational modification of secretory glycoproteins by glycosyltransferases results in the attachment of sialic acid residues at the terminal position. This enables secretion of these molecules into the extracellular matrix and protects them from proteases and endocytosis by hepatocytes and macrophages [4]. The integral glycoproteins of smooth muscle cells of vascular wall have not been studied intensively. Interest has been mainly focused on secretory proteins such as collagen, elastin, etc. [5]. No data on glycosyltransferases of vascular smooth muscle cells are available in the literature.

Much evidence has now accumulated indicating that the composition of glycoconjugates such as gangliosides in the intima undergoes significant qualitative and quantitative changes in atherosclerosis [6-8]. In a recent paper, we reported a significant increase in soluble sialyltransferases in blood of atherosclerotic patients in comparison with healthy subjects [9]. In the present study, we investigated sialyltransferase activity in normal and atherosclerotic intima of human aorta.

MATERIALS AND METHODS

Cytidine 5′-monophospho[^{14}C]-N-acetylneuraminic acid (CMP-[^{14}C]NeuAc), ammonium salt (specific activity 287 mCi/mmol) was purchased from Amersham (UK); cytidine 5′-monophospho-N-acetylneuraminic acid (CMP-NeuAc), sodium salt, and asialofetuin were purchased from Sigma (USA); sodium cacodylate was purchased from Ferak (Germany).

Aortas from 25-65-year-old men and women were obtained aseptically within 12 h after death due to accidents, frozen in liquid nitrogen, and stored at –70°C until use. On the day of an experiment, the tissues were thawed,

* To whom correspondence should be addressed.

0006-2979/01/6604-0397$25.00 ©2001 MAIK "Nauka/Interperiodica"

and further procedures were carried out at 4°C. The aortas were cleaned free of fat and connective tissue and cut lengthwise. The occurrence and the type of atherosclerotic lesions were defined according to the classification proposed by Smith [10]. Two types of aortas were defined: the first had atherosclerotic lesions (presumably atherosclerotic plaques without necrosis, occupying about half the total inner surface of the aortas) and the second without visible indications of lesions. For each specimen, the intima was mechanically separated from the media and the adventitia along the secondary internal limiting membrane with the use of pincers as described earlier [11]. Thus, the intimas prepared from the first type of specimens are further referred to as atherosclerotic intima; the intimas prepared from the second type are further referred as control intima. Two aorta specimens from different subjects were separated into atherosclerotic plaque area and adjacent uninvolved area. The intimas isolated from these areas were used for comparative study on sialyltransferase activity. The separated layer of the intima was blotted with filter paper, weighed, and cut into small pieces. The intima pieces were frozen in liquid nitrogen and ground to powder which was then suspended in 37.5 mM Tris-maleate buffer, pH 6.75, containing 0.25 M sucrose (1 g tissue in 20 ml buffer). The tissue suspension was homogenized in a Potter homogenizer (glass–Teflon) and centrifuged at 1,000*g* for 10 min. The resulting supernatant was centrifuged at 9,000*g* for 20 min. After removing the pellet, the supernatant was further centrifuged at 100,000*g* for 1 h. The final pellet, enriched in Golgi membranes, was resuspended using a Potter homogenizer in 0.1 M cacodylate buffer, pH 6.8, containing 0.3% Triton X-100. Membrane protein was determined by a modified Lowry method [12] using bovine serum albumin (Reanal, Hungary) for the calibration curve. The isolated Golgi fractions were stored at –70°C.

Sialyltransferase activity was determined by a modification of the method of Senn et al. [13]. The intimal Golgi membranes (160-200 μg protein) in cacodylate buffer were incubated with 0.05 mM CMP[^{14}C]NeuAc (total activity 100 nCi) and 0.5 mg asialofetuin in total volume 0.2 ml for 1 h at 37°C. Sialyltransferase activity in the atherosclerotic and the uninvolved intima isolated from the same aorta specimen was determined in a similar way with one exception: the concentration of CMP[^{14}C]NeuAc was 2 times lower (0.025 mM) with the same total activity (100 nCi). The reaction was stopped by addition of 1% phosphotungstic acid (PTA) in 5% trichloroacetic acid (TCA) on ice and left overnight in a refrigerator. The precipitates were filtered through GF/C filters on 3 MM Whatman paper (presoaked in PTA–TCA mixture) and washed with 10 ml of this mixture. Ethanol–ether mixture (2 : 1) was used for the last washing. The pellets bound to GF/C filters were air-dried overnight. Radioactivity was measured in a 1215 Rackbeta II scintillation counter (LKB, Sweden). Two samples were used as controls (without plasma or asialofetuin) in each experiment. All experiments were conducted in duplicate with accuracy of ±5%. Sialyltransferase activity was expressed as pmol sialic acid transferred to 0.5 mg asialofetuin in 1 h by 1 mg protein of the Golgi membranes.

Two atherosclerotic and two control intima specimens were used to study the relationship between sialyltransferase activity and Golgi membrane protein or CMP-NeuAc concentration.

Apparent K_m and V_{max} were calculated by nonlinear regression (RADLIG 4.0, Biosoft, Great Britain). Statistical analysis was performed using InStat, version 1.12a software (GraphPad, USA).

RESULTS

Our experiments showed that sialyltransferase activity was too low to be determined in total homogenate of intima as it was performed in related studies for liver and brain [13, 14]. However, it was possible to measure sialyltransferase activity in fractions of partially purified Golgi membranes. The yield of the Golgi membranes from the atherosclerotic and control intimas did not differ significantly and was 1.19 ± 0.05 (n = 5) and 1.65 ± 0.47 mg (n = 4) protein per g wet tissue, respectively.

The determination of total sialic acid in aorta intima showed that its content in the atherosclerotic and control tissues was similar: 204.5 ± 52.9 (n = 3) and 210.0 ± 46.4 μg (n = 3) sialic acid per g wet tissue, respectively. The mean value for asialofetuin-sialyltransferase activity in five atherosclerotic intimas was 2 times higher (p = 0.003) than in five control intimas (Table 1). For both types of intima, the velocity of asialofetuin-sialylating (pmol NeuAc/h

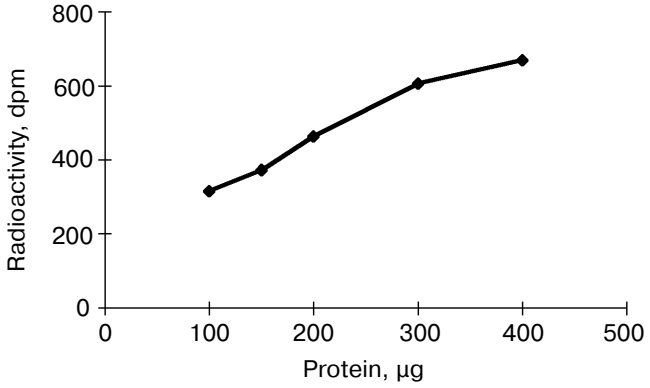

Fig. 1. Relationship between sialyltransferase activity (dpm) of human intima and Golgi membrane protein. The reaction mixture contained 100-400 μg membrane protein of the atherosclerotic intima, 0.05 mM CMP-NeuAc with total activity of 100 nCi, and 0.5 mg asialofetuin.

Table 1. Sialyltransferase activity in atherosclerotic and normal intima of human aorta

Atherosclerotic intima			Normal intima		
No.	pmol NeuAc/h per mg membrane protein	pmol NeuAc/h per g wet tissue*	No.	pmol NeuAc/h per mg membrane protein	pmol NeuAc/h per g wet tissue*
1	143.7	119.2	1	49.5	—
2	166.3	143.1	2	91.0	67.9
3	125.1	104.2	3	100.5	38.7
4	87.0	70.0	4	70.1	58.4
5	119.3	109.3	5	42.5	28.9
Mean ± S.D.	128.1 ± 21.3**	109.2 ± 17.7**	Mean ± S.D.	70.7 ± 20.0	48.2 ± 14.6

Note: The reaction mixture contained 0.05 mM CMP[^{14}C]NeuAc with total activity of 100 nCi, 0.5 mg asialofetuin, and 180-200 μg Golgi membrane protein. Values are means for duplicate measurements.
* Calculated data.
** Significantly different compared to normal intima, $p = 0.003$.

per mg Golgi membrane protein) increased linearly with Golgi membrane protein (in the range 100-300 μg) in the reaction mixture (Fig. 1). The time for sialyltransferase activity to reach the maximum averaged 1 h for both types of intima and stayed constant for at least 3 h. Two atherosclerotic intimas and two control intimas were used in kinetic experiments to study the relationship between sialyltransferase activity and CMP-NeuAc concentration in the reaction mixture. These experiments showed that despite varying K_m (up to 50%) from specimen to specimen, mean values of K_m for the atherosclerotic and control intima were the same, 56.5 μM, whereas V_{max} for the atherosclerotic intima was ~2 times higher than that for the control intima (Table 2, Fig. 2).

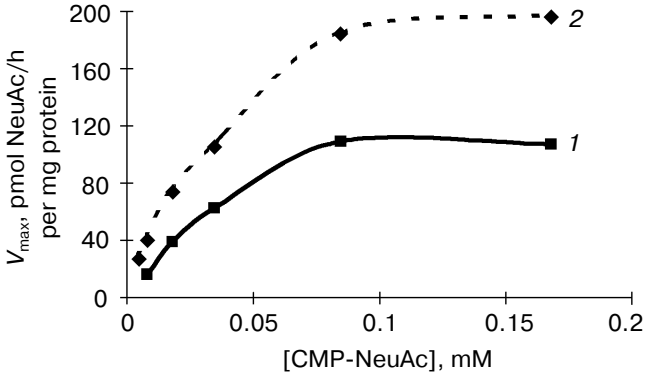

Fig. 2. Relationship between sialyltransferase activity in normal (*1*) and atherosclerotic (*2*) intima of human aorta and CMP-NeuAc concentration. The reaction mixture contained 200 μg Golgi membrane protein isolated from either normal or atherosclerotic intima, 0.005-0.2 mM CMP-NeuAc with total activity of 100 nCi, and 0.5 mg asialofetuin. One of three typical experiments is presented.

Table 2. Kinetic properties of sialyltransferases of human aorta intima for CMP-NeuAc

Golgi membranes	Specimen number	K_m, μM*	V_{max}*
Atherosclerotic intima	1	67.7	384
	2	45.0	254
	mean	56.3	319
Normal intima	1	43.1	153
	2	70.1	174
	mean	56.6	164

* K_m and V_{max} were calculated by graphical nonlinear regression (Program RADLIG 4.0). Values are means for duplicate measurements.

As shown in Table 3, sialyltransferase activities in the uninvolved and atherosclerotic areas of intimas isolated from the same aorta specimen were similar.

DISCUSSION

The attachment of sialic acid to the non-reducing terminal position of carbohydrate chains of glycoproteins and

Table 3. Sialyltransferase activity of atherosclerotic and adjacent uninvolved areas of intimas isolated from the same aorta specimen (pmol NeuAc/h per mg Golgi membrane protein)*

Golgi membranes	Specimen 1	Specimen 2
Atherosclerotic intima	86.5	55.1
Uninvolved intima	97.5	49.7

* The reaction mixture contained 0.025 μM CMP[^{14}C]NeuAc with total activity of 100 nCi, 0.5 mg asialofetuin, and 200 μg Golgi membrane protein. Values are means for duplicate measurements.

glycolipids is catalyzed by sialyltransferases, enzymes of the Golgi apparatus and the endoplasmatic reticulum. Sialic acid is glycosidically linked to either the 3- or 6-hydroxyl groups of the galactose residue or to the 6-hydroxyl group of the N-acetylgalactosamine residue and can form polysialic chains via their 8-hydroxyl group. At present 19 sialyltransferases are known. Sialyltransferases are specific for the acceptor oligosaccharide chain of glycoproteins and are defined into three major types: 1) α2,6-sialyltransferases, which attach a sialic acid residue to the 6-hydroxyl group of either the galactose or galactosamine and glucosamine residue of oligosaccharide chains; 2) α2,3-sialyltransferases, which attach a sialic acid residue to the 3-hydroxyl group of galactose residues; 3) α2,8-sialyltransferases, which form disialic sequences. Also, some enzymes of these types display specificity to respective monosaccharide residues in oligosaccharide chains which are bound to the protein by N- or O-glycosyl linkages [15]. To investigate a particular enzyme, it is necessary to use specific acceptors. At the same time, to investigate total sialyltransferase activity, a universal acceptor of sialic acid is required. However, although specific acceptors are difficult to find, no universal acceptors are known. Thus, when choosing the acceptor of sialic acid for estimation of total sialyltransferase activity in human aorta intima, we considered the most frequently occurring terminal sialylated sequences of glycoproteins in animal tissues [16, 17]. The level of sialyltransferases in animal tissues is low; therefore, its estimation requires large amounts of the acceptor glycoprotein. Asialofetuin, a desialylated protein of embryonic bovine plasma, contains N- and O-linked glycosidic chains as well and can be sialylated by α2,6- and α2,3-types of sialyltransferases [17].

Specimens of the atherosclerotic and control intimas were obtained from aortas of separate subjects. This allowed us to avoid possible error in comparing the normal and pathological states because influence of atherosclerotic lesions occurring in aorta on the metabolic processes in adjacent uninvolved areas cannot be excluded [7, 18]. For sialyltransferase activity, this supposition was confirmed when we compared atherosclerotic and adjacent uninvolved intima isolated from the same atherosclerotic aorta (Table 3).

The results of this study show that asialofetuin-sialyltransferase activity in human aorta intima increased on average 2-fold in vessels with atherosclerotic lesions. Similar mean K_m values for the atherosclerotic and normal intimas and a 2-fold increase in V_{max} for the atherosclerotic intima are evidence of increased expression in atherosclerosis of the same asialofetuin-sialylating enzymes in human aorta intima. These results are in good accordance with data on increased levels of soluble forms of asialofetuin-sialyltransferases in blood serum of patients with aortal intima thickening revealed by angiography [19]. No difference in sialyltransferase activity was observed for the atherosclerotic and adjacent uninvolved intima isolated from the same vessel. This fact suggests that in atherosclerosis, despite the absence of atherosclerotic lesions in the uninvolved aorta areas, the biochemical changes can occur. The increased levels of sialyltransferases can be related to certain processes in the intima during formation of atherosclerotic lesions: the change in phenotype of smooth muscle cells from contractile to secretory as well as the increase in synthesis of proteins and their secretion into the extracellular matrix [20].

It should be noted that, according to our results, total sialic acid in the control and atherosclerotic intimas was similar, though the expression of sialyltransferases in the atherosclerotic intima was higher than in the normal intima. Similar discrepancies were revealed in studies between sialyltransferase activity and level of sialylated tissue proteins and were considered to be due to simultaneous activation of sialidases [14]. As the atherosclerotic lesions in aorta generally resemble an infiltration focus [21], the activation of catabolic enzymes there, in particular sialidases, cannot be excluded.

This work was supported by the Russian Foundation for Basic Research (grant No. 99-04-48382) and Swiss State Research Foundation.

REFERENCES

1. Traving, C., and Schauer, R. (1998) *Cell Mol. Life Sci.*, **54**, 1330-1349.
2. Paulson, J. C., and Weinstein, J. (1989) *J. Biol. Chem.*, **264**, 10931-10934.
3. Paulson, J. (1989) *TIBS*, **14**, 272-276.
4. Schauer, R. (1985) *TIBS*, **10**, 357-360.
5. Wight, T. N. (1989) *Arteriosclerosis*, **9**, 1-20.
6. Bobryshev, Yu. V., Lord, R. S. A., Golovanova, N. K., Gracheva, E. V., Zvezdina, N. D., Sadovskaya, V. L., and Prokazova, N. V. (1997) *Biochim. Biophys. Acta*, **1361**, 287-294.
7. Wen, F-Q., Jabbar, A. A., Patel, D. A., Kazarian, T., and Valentino, L. A. (1999) *Arterioscler. Thromb. Vasc. Biol.*, **19**, 519-524.

8. Mukhin, D. N., Chao, F. F., and Kruth, H. S. (1995) *Arterioscler. Thromb. Vasc. Biol.*, **15**, 1607-1615.
9. Gracheva, E. V., Golovanova, N. K., Ezhov, M. V., Malyshev, P. P., Kukharchuk, V. V., and Prokazova, N. V. (1999) *Biochemistry (Moscow)*, **64**, 1315-1319.
10. Smith, E. B., and Smith, R. H. (1976) in *Atherosclerosis Rev.* (Paoletti, R., and Gotto, A. M., eds.) Vol. 1, Raven Press, New York, pp. 119-136.
11. Mukhin, D. N., and Prokazova, N. V. (1992) *Atherosclerosis*, **93**, 173-177.
12. Peterson, G. L. (1977) *Analyt. Biochem.*, **83**, 346-356.
13. Senn, H.-J., Manke, C., Dieter, P., Tran-Thi, T.-A., Fitzke, E., Gerok,W., and Decker, K. (1990) *Arch. Biochem. Biophys.*, **278**, 161-167.
14. Coughlan, C. M., Seckl, J. R., Fox, D. J., Unsworth, R., and Breen, K. C. (1996) *Glycobiology*, **6**, 15-22.
15. Harduin-Lepers, A., Recchi, M.-A., and Delannoy, P. (1995) *Glycobiology*, **5**, 741-758.
16. Corfield, A. P., and Schauer, R. (1982) in *Sialic Acids: Chemistry, Metabolism and Function. Cell Biology Monographs* (Schauer, R., ed.) Vol. 10, Springer Verlag, Wien-New York, pp. 195-261.
17. Kornfeld, R., and Kornfeld, S. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Kornfeld, R., and Kornfeld, S., eds.) Plenum Press, N. Y., pp. 6-34.
18. Oda-Tamai, S., Kato, S., and Akamatsu, N. (1991) *Biochem. J.*, **280**, 179-185.
19. Gracheva, E. V., Golovanova, N. K., Kononova, O. I., Kozlov, S. G., Prokazova, N. V., and Lyakishev, A. A. (1995) *Biochemistry (Moscow)*, **60**, 718-722 (Russ.).
20. Shanahan, C. M., and Weissberg, P. L. (1999) *Curr. Opin. Lipidol.*, **10**, 507-513.
21. Ross, R. (1999) *New Engl. J. Med.*, **340**, 115-126.