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Human Plasma *trans*-Sialidase Donor and Acceptor Specificity

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Abstract—Earlier we have isolated from human plasma desialylated low density lipoproteins (dLDL) and showed that, first, dLDL induce cholesterol esters accumulation—the main process accompanying atherosclerosis development. Second, the process of lipoprotein desialylation took place in plasma, and, finally, sialic acids removed from LDL are transferred to other serum glycoconjugates. In this study we have isolated from human plasma an enzyme transferring sialic acid residues (*trans*-sialidase) by affinity chromatography and studied its donor and acceptor specificity. Isolated enzyme in the presence of saccharide-acceptor can remove sialic acids from different lipoproteins, glycoproteins (fetuin, transferrin), and gangliosides (GM3, GD3, GM1, GD1a, GD1b). Plasma enzyme translocates α2-6, α2-3 and to a lower extent α2-8 bonded sialic acids. Sialoglycoconjugates of human serum erythrocytes, serum lipoproteins, glycoproteins, and gangliosides can serve as donors of sialic acid for *trans*-sialidase. Desialylated lipoproteins, especially dLDL, are more preferable sialic acid acceptors. Transferred sialic acid is found to be α2-6, α2-3, and α2-8 connected.

Key words: atherosclerosis, human plasma, *trans*-sialidase, lipoproteins, gangliosides, glycoconjugates, sialic acid

Several years ago we found and isolated from human blood plasma low density lipoproteins (LDL) that had a lower content of sialic acid [1, 2]. Desialylated LDL also had a low content of neutral carbohydrates [3], neutral lipids, and phospholipids [4]. Desialylated LDL (dLDL) were characterized by a higher density, a smaller size, and an increased electronegative charge [4]. Changes in the tertiary structure of apolipoprotein B (apoB) and modification of lysine residues could explain the decreased interaction of dLDL with the B,E-receptor [5]. In contrast to native LDL, dLDL can bind the scavenger-receptor, asialoglycoprotein-receptor, and cellular surface proteoglycans. An increased uptake and decreased degradation of dLDL can cause the accumulation of lipids in cells cultured from human aortic intima [5]. We have found a strong negative correlation between LDL sialic acid level and LDL capacity to induce accumulation of intracellular cholesterol [6]. Also, the removal of sialic acid from LDL by neuraminidase treatment leads to the appearance of LDL capacity to increase cholesterol content in smooth muscle cells and macrophages [1, 2].

Recently, we have shown that LDL desialylation occurs in human blood plasma [7]. Moreover, it was found that the sialic acid removed from LDL was observed in the trichloroacetic acid (TCA)-insoluble (protein-bound) fraction, but not in free form. Heat treatment and influence of heavy metals ions inhibit sialic acid transferring [8]. Thus, the enzyme removing sialic acid from LDL transfers it to another plasma component; hence, the enzyme is a *trans*-sialidase. Homogenous *trans*-sialidase was isolated from human plasma by affinity chromatography [8]. In this work we studied donor and acceptor specificity of human plasma *trans*-sialidase.

Abbreviations: HDL) high density lipoproteins; IDL) intermediate density lipoproteins; LDL) low density lipoproteins; VLDL) very low density lipoproteins; apoB) apolipoprotein B; PBS) phosphate buffered saline; BSA) bovine serum albumin; RCA_{120}) *Ricinus communis* agglutinin; PAA) polyacrylamide.

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MATERIALS AND METHODS

Lipoprotein isolation. Lipoproteins and lipoprotein deficient plasma were isolated from human plasma by methods described earlier [7]. Lectin-chromatography on *Ricinus communis* agglutinin (RCA_{120}; Boehringer Mannheim GmbH, Germany) immobilized on agarose was used to separate the sialylated and desialylated lipoproteins [9].

Preparation of radiolabeled lipoproteins, glycoproteins, and glycoconjugates. Sialylated glycoconjugates of lipoproteins (VLDL, IDL, LDL, HDL), glycoproteins (fetuin and transferrin; Sigma, USA), gangliosides GM3, GD3, GM1, GD1a, GD1b, GT1b, GQ1b, polyacrylamide (PAA)-bound saccharides and erythrocytes were ^{3}H-labeled in the C-8 position of sialic acid as described by Veh et al. [10]. The gangliosides isolated from bovine brain and liver were kindly provided by N. V. Prokazova (Cardiology Research Center, Moscow, Russia) and Dr. I. Mikhalev (Institute of Bioorganic Chemistry, Moscow, Russia). Soluble 30-40 kD polyacrylamide-bound saccharides (Sug-PAA) and biotinylated probes (Sug-PAA-biot) [11] were obtained from Syntesome GmbH (Germany). Erythrocytes were isolated according to Beutler et al. [12]. The specific radioactivity was 0.18-10.2 mCi/mmol bound sialic acid.

Isolation of *trans*-sialidase by affinity chromatography. A 0.7-ml sample of lipoprotein-deficient serum was applied to a column containing 2 ml Neu5Acα2-8-Neu5Ac-Sepharose FF-6 (Syntesome GmbH). The column was washed with 20 ml of 50 mM Tris-HCl (pH 7.0). *trans*-Sialidase was eluted with 5 ml of 5 mM sialic acid in 50 mM Tris-HCl buffer and was dialyzed three times against 2000 volumes of 50 mM Tris-HCl (pH 7.0). Enzyme was concentrated by ultrafiltration (1000 MW cut off; Amicon, USA) and stored at −70°C. Enzyme homogeneity was established by SDS-PAGE (4-15%) [13] and mass-spectrometry [14]. Protein concentration was determined according to Lowry et al. [15].

Assay of sialic acid content. Sialic acid content in lipoproteins, glycoproteins, and gangliosides was measured by method of Warren [17] with modification [16].

Assay of *trans*-sialidase activity. For the detection of *trans*-sialidase activity, ^{3}H-labeled lipoprotein or glycoprotein sialic acid donors were covalently bound to CNBr-activated Sepharose [18]. The assay mixtures containing, in a final volume of 0.2 ml, 10-20 μg of donor bound to Sepharose, 10-50 μg of the acceptor, 50 mM Tris-HCl (pH 7.0), 1 mM DTT, 2 mM $CaCl_2$, and 5-25 μg human plasma *trans*-sialidase preparation were incubated for the indicated time at 37°C with gentle shaking. A control mixture contained no enzyme preparation. After incubation, 0.3 ml ice-cold water was added to samples that were then centrifuged (5000*g*, 5 min) at 4°C. For radioactivity determination, 0.2-ml samples of supernatant were used.

To determine the *trans*-sialidase activity using gangliosides as sialic acid donors, the assay mixture containing 3.6 nmol of ^{3}H-labeled ganglioside, 0.5 mg/ml asialofetuin, 50 mM Tris-HCl (pH 7.0), 1 mM DTT, and 2 mM $CaCl_2$ was incubated with 10 μg human plasma *trans*-sialidase preparation for 8 h at 37°C. Incubation was stopped by addition of 1 ml 10% TCA. After centrifugation (10,000*g*, 10 min) the pellet was delipidated by sequential treatment with 2 ml of 2-propanol, chloroform–methanol mixture (1 : 1 v/v), and diethyl ester. The delipidated pellet was dissolved in 0.1 N NaOH and its radioactivity was determined.

Identification of formed glycoside linkage type. Ninety six-well plates (Nunc, Roskilde, Denmark) were coated with 100 μl of lactose covalently bound with polyacrylamide (PAA) at a concentration of 10 μg/ml in PBS, pH 7.2, at 37°C for 18 h. The wells were washed four times with PBS containing 0.05% Tween 20. Bovine serum albumin (BSA) (2%, 100 μl) in PBS was added to wells and incubated for 1 h at 20°C. Then the wells were washed with PBS containing 0.2% BSA (PBS/BSA) and 50 μl of fetuin (100 μg/ml) before 50 μl of *trans*-sialidase (68 μg/ml) was added. After a 4-h incubation at 37°C, wells were washed and 100 μl biotin-labeled lectin (10 μg/ml) isolated from *Maackia amurensis* seeds (MAL II) or elderberry (*Sambucus nigra*) bark lectin (EBL) (1 μg/ml; Vector Laboratories, Burlingame, USA) was added to wells and incubated for 1 h. After additional washing (four times) with PBS/BSA, 100 μl streptavidin-peroxidase was added and incubated for 60 min at 37°C. After washing with PBS/BSA, 100 μl of substrate mixture (0.1 mg/ml *o*-phenylenediamine in sodium citrate, pH 4.5, with 0.003% H_2O_2) was added and incubated for 30 min at 37°C. The reaction was stopped with 20 μl 50% sulfuric acid and the absorbance was read on a Multiscan Bichromatic microplate reader (Labsystems OY, Finland) at 492 nm.

Statistical analysis. The significance of differences between group mean values was evaluated by multiple *t*-test of one-way analysis of variance using the BMDP statistical program package [19].

RESULTS

Donor specificity of the human serum *trans*-sialidase. In investigation of human plasma *trans*-sialidase donor specificity, we started with subfractions of the human blood lipoproteins. For this purpose we isolated all main classes of lipoproteins circulating in serum (VLDL, IDL, LDL, and HDL). Using lectin-chromatography on *Ricinus communis* agglutinin (RCA_{120}) bound to agarose, sialylated and desialylated lipoproteins were subfractionated from total lipoprotein fractions. Table 1 shows that the most appropriate substrate (donor) for the *trans*-sialidase is sialylated LDL. So the rate of sialic acid transfer from sialylated LDL was 17-fold higher than from desialylated LDL. Sialic acid

Table 1. Rate of sialic acid transfer from the sialylated and desialylated lipoproteins on asialofetuin

Donor	Rate of sialic acid transfer, fmol sialic acid/mg protein per min
Sialylated VLDL-Sepharose	82 ± 12
Desialylated VLDL-Sepharose	< 2
Sialylated IDL-Sepharose	312 ± 34
Desialylated IDL-Sepharose	21 ± 2
Sialylated LDL-Sepharose	4110 ± 410
Desialylated LDL-Sepharose	240 ± 6
Sialylated HDL-Sepharose	9 ± 1
Desialylated HDL-Sepharose	< 2

transfer from sialylated IDL occurred 13-fold slower than from native IDL. The rate of sialic acid transfer in the presence of VLDL and HDL was significantly lower than for LDL and IDL. Thus, activity of *trans*-sialidase to transfer sialic acid from the other sialylated lipoproteins was as follows: IDL > VLDL > HDL. All the examined desialylated lipoproteins were less effective substrates for the *trans*-sialidase than sialylated LDL (Table 1). In this experiment sialylated and desialylated lipoproteins were used at the same concentrations of sialic acid.

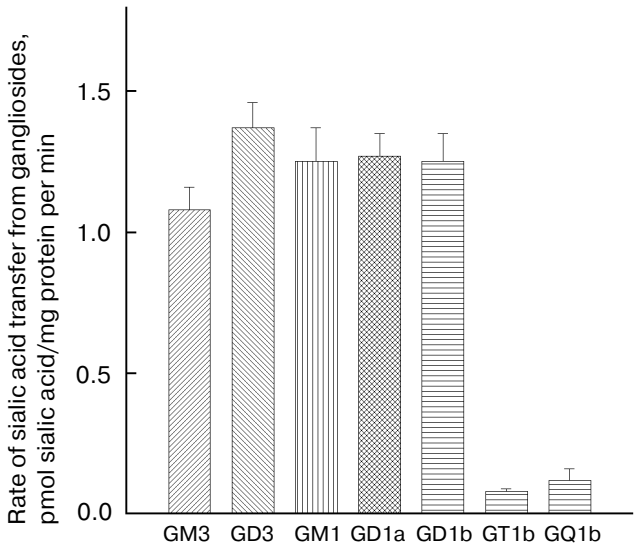

Fig. 1. Sialic acid transfer from gangliosides on asialofetuin (*trans*-sialidase activity was measured as described in "Materials and Methods"; data represent mean of three determinations ± SEM).

Sialic acid is a regular component of lipid- and protein-bound glycoconjugates in all lipoprotein classes. Thus, we decided to check whether gangliosides can serve as donors for human *trans*-sialidase. The data in Fig. 1 show that gangliosides having one or two sialic acids are donors for *trans*-sialidase. *trans*-Sialidase activity determined for gangliosides containing three or four sialic acids was 2-4-fold lower than that for less sialylated gangliosides (Fig. 1).

We also examined whether sialic acid transfers from glycoconjugates of human blood erythrocytes. It was demonstrated that the rate of labeled sialic acid transfer from the glycoconjugates of erythrocyte membranes onto desialylated fetuin was 16.6 ± 0.6 pmol/mg protein per min.

To answer the question from what kind of carbohydrate chains does *trans*-sialidase remove sialic acid, we used oligosaccharides covalently bound to polyacrylamide carrying terminal α2-3, α2-6, and α2-8 bound sialic acid. Data in Table 2 demonstrate that *trans*-sialidase transfers the sialic acid bound by α2-6 linkage with galactose residue (as Neu5Acα2-6Galβ1-4Glc-PAA-Sepharose). The enzyme is less effective in the case of sialic acid bound by α2-3 linkage with galactose in both Neu5Acα2-3Galβ1-4Glc-PAA-Sepharose and Neu5 Acα2-3Galβ1-3GalNAcα-PAA-Sepharose glycoconjugates. Suitable substrates for *trans*-sialidase were fucosylated oligosaccharides $SiaLe^x$ and $SiaLe^a$ (α2-3 linkage with galactose residues). In this case *trans*-sialidase activity was closer to α2-6 than α2-3 unfucosylated derivatives. The rate of sialic acid transfer from Neu5Acα2-3Galβ1-3GalNAcα-PAA-Sepharose (a fragment of carbohydrate O-chains) was 2.5-fold higher than with Neu5Acα2-3Galβ1-4Glc-PAA-Sepharose.

The *trans*-sialidase activity with glycoconjugates containing two α2-8 bound sialic acids as donors was dozens of times lower than with glycoconjugates containing α2-6 bound sialic acids. It is important to note that the human serum *trans*-sialidase could not use cytidine-5′-monophospho-[^{14}C]sialic acid as a substrate (Table 2).

Acceptor specificity of the human serum *trans*-sialidase. As mentioned above, sialic acid can be transferred to different components of blood serum *in vitro*. Using the isolated enzyme, we examined its acceptor specificity in detail.

The rate of sialic acid removal from LDL was very low in the absence of acceptor (Fig. 2). Addition of native (sialylated) fetuin to the incubation medium had practically no affect on the rate of hydrolysis and transfer. In contrast, asialofetuin markedly stimulated sialic acid removal from LDL (Fig. 2).

Desialylated lipoproteins were more preferable acceptors for *trans*-sialidase. At the same time serum *trans*-sialidase was able to transfer sialic acid to sialylated LDL > IDL > VLDL > HDL (in order of decreasing transfer rate) (Table 3). Effectiveness of such process was lower than with desialylated lipoproteins. The maximal sialic acid transfer

Table 2. Rate of sialic acid transfer from sialylated oligosaccharides bound to polyacrylamide

Glycoconjugate-donor	Rate of sialic acid transfer, pmol/mg protein per min
Neu5Acα2-6Galβ1-4Glc-PAA-Sepharose	42.2 ± 3.3
Neu5Acα2-3Galβ1-4Glc-PAA-Sepharose	12.4 ± 1.0
Neu5Acα2-3Galβ1-3GalNAcα-PAA-Sepharose	30.6 ± 2.0
$(Neu5Ac\alpha2\text{-}8)_2$-PAA-Sepharose	1.6 ± 0.4
$SiaLe^x$-PAA-Sepharose	25.3 ± 2.2
$SiaLe^a$-PAA-Sepharose	36.7 ± 2.1
Cytidine-5′-monophospho-[^{14}C]sialic acid	< 0.2

Table 3. Rate of sialic acid transfer from ^{3}H-labeled fetuin on the sialylated and desialylated lipoproteins

Acceptor	*trans*-Sialidase activity, pmol sialic acid/mg protein per min
Control	0.24 ± 0.02
Sialylated VLDL	0.58 ± 0.03
Desialylated VLDL	0.77 ± 0.06
Sialylated IDL	0.79 ± 0.08
Desialylated IDL	1.36 ± 0.05
Sialylated LDL	1.09 ± 0.06
Desialylated LDL	2.48 ± 0.14
Sialylated HDL	0.44 ± 0.04
Desialylated HDL	0.83 ± 0.07

rate was on LDL, it was 1.5-fold higher than on IDL and 2-3-fold higher than on VLDL and HDL (Table 3).

The data shown in Fig. 3 demonstrate the rate of labeled sialic acid transfer from fetuin to lactosyl-ceramide and individual gangliosides. The highest rate of sialic acid transfer was found for lactosyl-ceramide, the precursor of gangliosides GM3 and GD3. The rate of sialic acid transfer was similar using gangliosides GM1, GD1a, and GD1b as acceptors (Fig. 3). For GT1b, the rate of sialic acid transfer was approximately 2-fold lower than that with gangliosides containing one or two sialic acids (Fig. 3). Ganglioside GQ1b did not significantly change *trans*-sialidase activity.

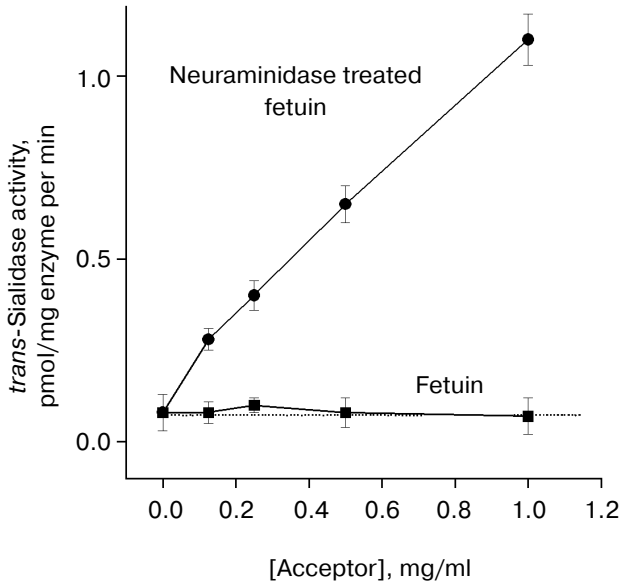

Fig. 2. Rate of sialic acid transfer on native and asialofetuin. The assay mixtures contained in a final volume of 0.2 ml, 0.01 ml of ^{3}H-labeled LDL-Sepharose (10 μg protein, 105,000 dpm), the indicated concentration of native and asialofetuin, 50 mM Tris-HCl (pH 7.0), 1 mM DTT, 2 mM $CaCl_2$, and 10 μg human plasma *trans*-sialidase preparation and were incubated for 8 h at 37°C. Data represent a mean of three determinations ± SEM.

We have also examined the possibility of sialic acid transfer on glycoconjugates of human blood erythrocytes. It was demonstrated that the rate of labeled sialic acid transfer from fetuin to erythrocyte glycoconjugates partially desialylated by neuraminidase was 0.49 ± 0.04 pmol/mg protein per min.

To reveal what kind of carbohydrate chains can accept sialic acid, we used synthetic oligosaccharides covalently bound to polyacrylamide. From the data demonstrated in Table 4, lactose, N-acetyllactosamine, and N-acetylgalactosamine bound to polyacrylamide cause a 3.5- to 4-fold increase in the rate of sialic acid transfer from fetuin. It should be noted that lactose, sialyllactose, N-acetylgalactosamine, and trisialic acid nonbound to polymer did not increase *trans*-sialidase activity. On the other hand, the rate of sialic acid transfer rose significantly when polyacrylamide-bound glycoconjugates containing oligosialic acids were used as an acceptor.

Trisaccharides Le^x and Le^a bound to polyacrylamide stimulated sialic acid transfer by 4.5- and 6-fold, respectively (Table 4). The stimulation of *trans*-sialidase activity was also observed for Galβ1-3GalNAcα-PAA, a fragment of glycoprotein O-chains.

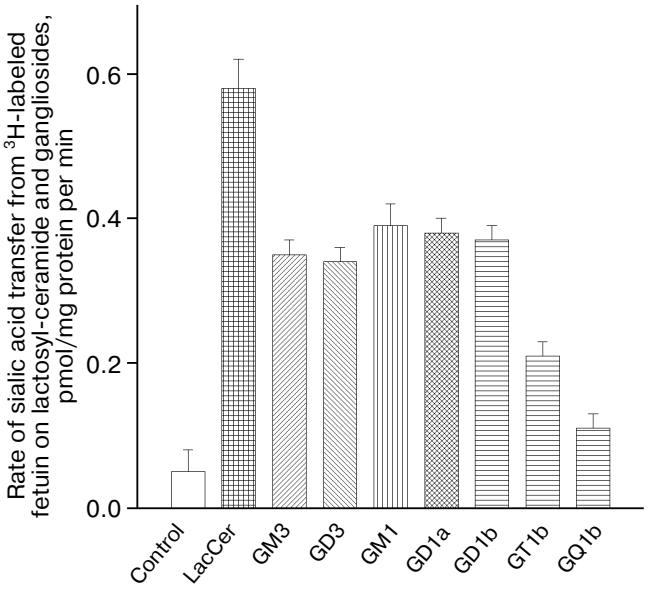

Fig. 3. Rate of sialic acid transfer from ^{3}H-labeled fetuin on lactosyl-ceramide and gangliosides. The assay mixtures contained in a final volume of 0.2 ml, 0.01 ml of ^{3}H-labeled fetuin-Sepharose (5 μg protein, 145,000 dpm), 100 nmol of lactosyl-ceramide or gangliosides as the acceptors, 50 mM Tris-HCl (pH 7.0), 1 mM DTT, 2 mM $CaCl_2$, and 5 μg human plasma *trans*-sialidase preparation and were incubated for 8 h at 37°C. After incubation, gangliosides from the reaction mixture were extracted and separated by thin-layer chromatography as described in "Materials and Methods". Data represent a mean of three determinations ± SEM.

Determination of sialic acid linkage type in newly formed glycoconjugates. To determine the type of linkage formed by the sialic acid with glycoconjugates, we used biotin-labeled lectins: elderberry (*Sambucus nigra*) bark lectin (EBL), binding with the terminal disaccharide fragment Neu5Acα2-6Gal and with *Maackia amurensis* seeds lectin (MAL II), which is specific for the Neu5Acα2-3Gal fragment. As follows from Table 4, both lectins bound with lactose-PAA incubated with fetuin and *trans*-sialidase, thus proving the formation of both α2-6 and α2-3 linkages as a result of the *trans*-sialidase reaction.

DISCUSSION

We have determined that sialylated LDL is a more preferable substrate for plasma *trans*-sialidase compared to VLDL, IDL, and especially HDL (Table 1). It is known that the main apoprotein of VLDL, IDL, and LDL is apoB-100, and the contents and ratios of gangliosides in VLDL and LDL are similar [20]. It should be noted that a decrease in the sialic acid transferring rate from apoB-containing lipoproteins corresponds to the increase of lipoprotein particle size. Thus, the structure of sialylated glycoproteins exposed on the surface of lipoprotein particles may play role in sialic acid transfer by the *trans*-sialidase.

Unfortunately, almost nothing is known about structure of glycoconjugates of apoA-1 and apoA-2, the main apoproteins of HDL. The ganglioside composition of HDL is similar to those of VLDL and LDL [21]; therefore, the real reasons for the low rate of sialic acid transfer from these lipoproteins are not clear.

Mono- and disialylated gangliosides, containing α2-3 and α2-8 glycoside linkage sialic acid as a terminal and branch fragment, were efficient donors for *trans*-sialidase (Fig. 1). So, the type of linkage and sialic acid position in the carbohydrate moiety of gangliosides may not be decisive for interaction with the *trans*-sialidase. GT1b and the GQ1b demonstrated a very low activity as donors of sialic acid (Fig. 1). Hence, all gangliosides, having one or two sialic acids appear to be the most preferable substrate for human serum *trans*-sialidase.

To determine what type of sialic acid linkages and what kind of sialylated carbohydrate chains can be hydrolyzed by the *trans*-sialidase, we used synthetic sialylated glycoconjugates (Table 2). It was found that the *trans*-sialidase hydrolyzes and transfers the sialic acid bound to galactose residues by α2-6 linkage more effectively than sialic acid bound in a α2-3 position. Appropriate donors for the *trans*-sialidase were also fucose-containing $SiaLe^x$ and $SiaLe^a$, glycoconjugates with the sialic acid bound by the α2-3 linkage. The lowest

Table 4. Rate of sialic acid transfer from ^{3}H-labeled fetuin bound to Sepharose on glycoconjugates

Glycoconjugate-acceptor	Rate of sialic acid transfer, % of control
Control	100 ± 8
Lac-PAA	426 ± 30
LacNAc-PAA	418 ± 46
GalNAc-PAA	354 ± 20
Le^x-PAA	450 ± 34
Le^a-PAA	581 ± 46
Galβ1-3GalNAcα-PAA	489 ± 33
$(Neu5Ac\alpha2\text{-}8)_2$-PAA	224 ± 30
$(Neu5Ac\alpha2\text{-}8)_5$-PAA	205 ± 15
$(Neu5Ac\alpha2\text{-}8)_3$	95 ± 10
Lac	98 ± 8
PAA	103 ± 7

activity of *trans*-sialidase was found for oligosialic acid–polyacrylamide as a substrate (an analog of the gangliosides GD3, GD1b, and GT1b). Hence, using the synthetic glycoconjugates the possibility of enzymatic hydrolysis of sialic acid bound by α2-8 linkage was confirmed.

Thus plasma *trans*-sialidase is able to transfer sialic acid from the main sialylated glycoconjugates of glycoproteins and gangliosides of the plasma and blood cells. Additionally, the highest rate of *in vitro* sialic acid transfer was observed for the α2-6 linkage, and the lowest rate for the α2-8 linkage (Table 2).

As shown above, sialic acid released by *trans*-sialidase is bound to carbohydrate chains of other plasma components. We have found that serum glycoproteins, lipoproteins, and gangliosides can serve as acceptors for released sialic acid. Also, sialic acid may be transferred onto glycoconjugates of blood erythrocytes.

The capacity of apoB-containing lipoproteins to accept sialic acid decreases in the following order: LDL > IDL > VLDL (Table 3). The lowest rate of the sialic acid transfer was observed for sialylated HDL. Desialylated lipoproteins containing terminal galactose residues were more effective acceptors of sialic acid than sialylated ones.

Among examined sphingolipids, the best sialic acid acceptor was lactosyl-ceramide, a neutral lipid containing terminal galactose (Fig. 3). Gangliosides with one or two sialic acids also were appropriate acceptors of the transferred sialic acid. Thus, gangliosides with a high negative charge (GT1b and GQ1b) demonstrate decreased acceptor properties.

From study of sialic acid transfer from donors to oligosaccharides covalently bound to polyacrylamide, it was shown that lactose, N-acetyllactosamine, and N-acetylgalactosamine can be acceptors for sialic acid (Table 4). Polyacrylamide-bound Le^x and Le^a also bound the sialic acid effectively. It was observed that sialic acid could be transferred onto the typical fragment of O-chains of glycoproteins—Galβ1-3GalNAcα-PAA. Polyacrylamide-bound oligomers of the sialic acid ($n = 2$ and $n = 5$) were weak acceptors. On the other hand, free trisialic acid, not bound with polyacrylamide or ceramide, does not function as an acceptor (Table 4). The same results have been obtained for free lactose non-bound to polyacrylamide. Thus, the acceptor should be a rather high molecular weight or polyvalent molecule.

To determine the type of sialic acid linkage that was formed by *trans*-sialidase, we used bond-specific lectins: MAL II (α2-3 linkage) and EBL (mainly α2-6 linkage). It was demonstrated that sialic acid may form both α2-3 and α2-6 linkage with galactose residues. Hence, *trans*-sialidase is able to attach the sialic acid residue to an acceptor by α2-3, α2-6, and α2-8 linkages, at least *in vitro* (see above).

At the present time, we do not know the physiological role of the human plasma *trans*-sialidase. We propose that the enzyme may affect numerous processes that depend on the sialylation/desialylation state of carbohydrate chains of different cellular and non-cellular components. The *trans*-sialidase may modulate the activities of plasma enzymes and carriers of metal ions, change the life-time of glycoproteins, lipoproteins and cells in the circulation, modify cell-to-cell interaction including adherence of blood cells with vascular endothelium [22], etc.

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REFERENCES

1. Orekhov, A. N., Tertov, V. V., Mukhin, D. N., and Mikhailenko, I. A. (1989) *Biochem. Biophys. Res. Commun.*, **162**, 206-211.
2. Orekhov, A. N., Tertov, V. V., and Mukhin, D. N. (1991) *Atherosclerosis*, **86**, 153-161.
3. Tertov, V. V., Orekhov, A. N., Sobenin, I. A., Morrisett, J. D., Gotto, A. M., Jr., and Guevara, J. G., Jr. (1993) *J. Lipid Res.*, **34**, 365-375.
4. Tertov, V. V., Sobenin, I. A., Gabbasov, Z. A., Popov, E. G., Jaakkola, O., Solakivi, T., Nikkari, T., and Orekhov, A. N. (1992) *Lab. Invest.*, **67**, 665-675.
5. Tertov, V. V., and Orekhov, A. N. (1997) *Exp. Mol. Pathol.*, **64**, 127-145.
6. Orekhov, A. N., Tertov, V. V., Sobenin, I. A., Smirnov, V. N., Via, D. P., Guevara, J., Gotto, A. M., Jr., and Morrisett, J. D. (1992) *J. Lipid Res.*, **33**, 805-807.
7. Tertov, V. V., Kaplun, V. V., Sobenin, I. A., and Orekhov, A. N. (1998) *Atherosclerosis*, **138**, 183-195.
8. Tertov, V. V., Kaplun, V. V., Sobenin, I. A., Boytsova, E. Yu., Bovin, N. V., and Orekhov, A. N. (2001) *Atherosclerosis*, **159** (1), 103-115.
9. Tertov, V. V., Sobenin, I. A., Tonevitsky, A. G., Orekhov, A. N., and Smirnov, V. N. (1990) *Biochem. Biophys. Res. Commun.*, **167**, 1122-1127.
10. Veh, R. W., Corfield, A. P., Sander, M., and Schauer, R. (1977) *Biochim. Biophys. Acta*, **486**, 145-160.
11. Bovin, N. V. (1998) *Glycocon. J.*, **15**, 431-446.
12. Beutler, E., Blume, K. G., Kaplan, J. C., Lhor, J. W., Ramot, B., and Valentine, W. N. (1977) *Br. J. Haematol.*, **35**, 331-340.
13. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
14. Karas, M., Ingendoh, A., Bahr, U., and Hillenkamp, F. (1989) *Biomed. Environ. Mass Spectrom.*, **18**, 841-845.
15. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
16. Sobenin, I. A., Tertov, V. V., and Orekhov, A. N. (1998) *J. Lipid Res.*, **39**, 293-299.
17. Warren, L. (1959) *J. Biol. Chem.*, **234**, 1971-1975.
18. Ohkuma, S., and Poole, B. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 3327-3331.
19. Dixon, W. J., and Brown, M. B. (1977) *Biomedical Computer Programs. P-Series*, University of California Press, Berkeley, pp. 185-203.
20. Senn, H.-J., Orth, M., Fitzke, E., Wieland, H., and Gerok, W. (1989) *Eur. J. Biochem.*, **191**, 657-662.
21. Chatterjee, S., and Kwiterowich, P. O., Jr. (1984) *Can. J. Biochem. Cell. Biol.*, **62**, 385-397.
22. Schauer, R. (1982) *Adv. Carbohydr. Chem. Biochem.*, **40**, 131-234.