Biochimica et Biophysica Acta, 445 (1976) 67—73

BBA 67871

THE SERUM SIALYLTRANSFERASE ACTIVITY IN α_1-ANTITRYPSIN DEFICIENCY

STEN ERIKSSON and CHRISTER LARSSON

Departments of Internal Medicine and Clinical Chemistry, University of Lund, Malmö General Hospital, Malmö (Sweden)

(Received January 26th, 1976)

Summary

α_1-Antitrypsin phenotypes Pi M and Z, purified by the thiol-disulfide exchange procedure, were desialylated by treatment with neuraminidase covalently coupled to Sepharose and used as acceptors of sialic acid in an assay system for serum sialic acid transferase (CMP-*N*-acetylneuraminate:D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1) activity. Both asialoantitrypsins were equally effective as acceptors in contrast to native Pi Z antitrypsin which did not accept any sialic acid.

Serum sialyltransferase activity was determined in 38 adult α_1-antitrypsin deficient individuals (Pi Z, MZ, FZ, SZ) with normal liver function and was found to be of the same magnitude as the activity in normal individuals (Pi M). Equal activities were also found in 5 Pi Z patients with cirrhosis of the liver.

The results strongly argue against the concept that sialyltransferase deficiency provides the molecular basis for α_1-antitrypsin deficiency.

Introduction

A typical finding in homozygous α_1-antitrypsin deficiency (phenotype Pi Z) is an accumulation of periodic acid/Schiff-(PAS)-positive inclusion bodies in the hepatocytes, especially in the periportal areas [1—3]. Electron micrographs indicate that the material is located in the rough endoplasmic reticulum [1,3, 4]. The inclusion bodies have been isolated, purified and shown to consist of aggregated asialoantitrypsin [5]. Recently Kuhlenschmidt et al. [6] reported a young patient with homozygous α_1-antitrypsin deficiency (Pi Z) and advanced liver cirrhosis who displayed a severe deficiency in serum CMP-*N*-acetylneura-

Address correspondence to: Sten Eriksson, Department of Internal Medicine, Malmö General Hospital, S-214 01 Malmö, Sweden.

minic acid:asialoglycoprotein sialic acid transferase (CMP-*N*-acetylneuraminate: D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1) activity having been assayed by means of asialoantitrypsin and asialotransferrin as acceptor proteins. This deficiency was suggested to provide the molecular basis for the accumulation of α_1-antitrypsin within liver parenchymal cells. In a preliminary communication we reported the serum sialyltransferase activity to be normal or elevated in 17 α_1-antitrypsin deficient newborns, with one single exception [7]. In the present report we have studied the serum sialyltransferase activity in 43 adults with different Pi Z-phenotypes with or without cirrhosis of the liver and in 38 controls (Pi M). Asialo-α_1-antitrypsin of the ordinary phenotype (Pi M) was used as acceptor protein but in addition variant α_1-antitrypsin of phenotype Pi Z was tested as acceptor.

Materials and Methods

Blood samples were obtained from 76 individuals with normal liver function and Pi phenotypes, M, MZ, Z, SZ, FZ and from 5 patients with liver cirrhosis and Pi phenotype Z. They were allowed to clot at room temperature and sera were obtained by centrifugation at 1500 × *g* for 15 min. The sera were stored at −20°C and assayed within 1—2 weeks, except for the sera from 4 of the cirrhotic patients which had been stored for approximately 2 years. The clinical data of the patients with cirrhosis have been given elsewhere [8]. In addition to cirrhosis all these patients had a malignant hepatoma.

CNBr-activated Sepharose 4 B was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden; neuraminidase (EC 3.2.1.18) (*Vibrio cholerae)* from Behringwerke AG and CMP-*N*-[^{14}C]acetylneuraminic acid, specific activity $1.1 \cdot 10^7$ cpm/μmol, from New England Nuclear. Sulphosalicylic acid was obtained from BDH Chemicals; Permablend III and Soluene 350 from Packard. Agarose gel electrophoresis was performed according to the method of Johansson [9] and protein determination according to the method of Hartree [10].

Sialic acid was determined according to the method of Warren [11] with thiobarbituric acid after hydrolysis in 0.05 M sulfuric acid and was performed by Dr. J.-O. Jeppsson, of the Department of Clinical Chemistry. α_1-Antitrypsin was determined by electroimmunoassay according to the method of Laurell [12]. Phenotyping was performed at the Department of Clinical Chemistry, using the method of Laurell and Persson [13]. α_1-Antitrypsins Pi M and Z were available at the laboratory and had been prepared from plasma by thiol-disulfide interchange according to the method of Laurell et al. [14].

Coupling of neuraminidase to CNBr-activated Sepharose 4 B

4500 units neuraminidase were dialyzed against coupling buffer (0.1 M $NaHCO_3$ with 0.05 M NaCl) and coupled to 3 g CNBr-activated Sepharose 4 B according to manufacturers instructions. Final gel volume was 10.5 ml.

Desialylation of α_1-antitrypsin

After dialysis against 0.1 M acetate buffer (containing 0.009 M $CaCl_2$, pH 5.4) 15 ml α_1-antitrypsin (concentration 4 g/l) was circulated for 24 h through

a 10 ml column containing 5 ml Sepharose-neuraminidase gel at a flow rate of 15 ml/h. In agarose gel electrophoresis the neuraminidase-treated antitrypsin migrated as an α_2-globulin. The treated antitrypsin contained no detectable sialic acid.

Sialyltransferase assay

Asialoantitrypsin was dialyzed against a 0.1 M acetate buffer, pH 5.9 containing 0.003 M sodium azide. The incubation mixture contained 2 nmol asialoantitrypsin, 11 nmol CMP-*N*-[^{14}C]acetyl-neuraminic acid and 5—30 μl serum in a final volume of 260 μl. Incubation was carried out at 37°C for 45 min with constant agitation. The reaction was stopped by the addition of 3 ml 5% sulphosalicylic acid. The reaction mixture was then sonicated for 20 s with a 150 W MSE ultrasonic disintegrator operating at an amplitude of 12 μm and centrifuged at 1700 × *g* for 10 min. The supernatant was discarded. The pellet was resuspended in 3 ml 5% sulphosalicylic acid, sonicated for 20 s and centrifuged. This procedure was repeated twice. The resulting pellet was dissolved overnight in 0.4 ml Soluene. 10 ml scintillator fluid (9.2 g Permablend III dissolved in toluene to 1 l) was then added and radioactivity measured in a Packard liquid scintillation counter. The counting efficiency was 65%. Blanks containing ordinary α_1-antitrypsin were prepared as described above and always contained less than 10% of sample activity.

Results

Under the assay conditions described the incorporation of sialic acid into acceptor protein was invariably linear with serum amounts up to 30 μl. Addition of more serum resulted in a progressive retardation of activity and a plateau was reached (Fig. 1). The same pattern was obtained whether asialoantitrypsin Pi M or Z was used as acceptors in contrast to native antitrypsin Z which did not accept any measurable amount of sialic acid (Fig. 2). Under standard assay conditions (20 μl serum incubated for 45 min) approximately 1% of added ^{14}C-labelled sialic acid was incorporated into the acceptor protein, corresponding to an occupation of 1% of the theoretical number of available acceptor sites. The formation of sialylated α_1-antitrypsin was linear with time, and approximately 3% of added sialic acid was incorporated after 2 h incubation of 20 μl serum. Addition of increasing amounts of non-desialylated α_1-antitrypsin to the reaction mixture in the routine assay (20 μl serum, 45 min) resulted in a progressive decrease in transferase activity. The percentage decrease was 10% after addition of 0.1 mg α_1-antitrypsin and 36% after 0.4 mg.

Transferase activity in normal (Pi M) individuals under different conditions are summarized in Table I. The enzyme activity was significantly lower in 0.006 M EDTA-plasma than in serum, indicating requirement for metal ions. No increase in activity was noted in the presence of 0.005 M $CaCl_2$. Therefore routine determinations were always performed on serum. Fasting sera were regularly used although postprandial samples had the same transferase activity. No differences were found between sexes. Enzyme activity was not stimulated by the presence of 1% Triton X-100 in the incubation medium.

The error of the method calculated on the basis of 24 double determinations

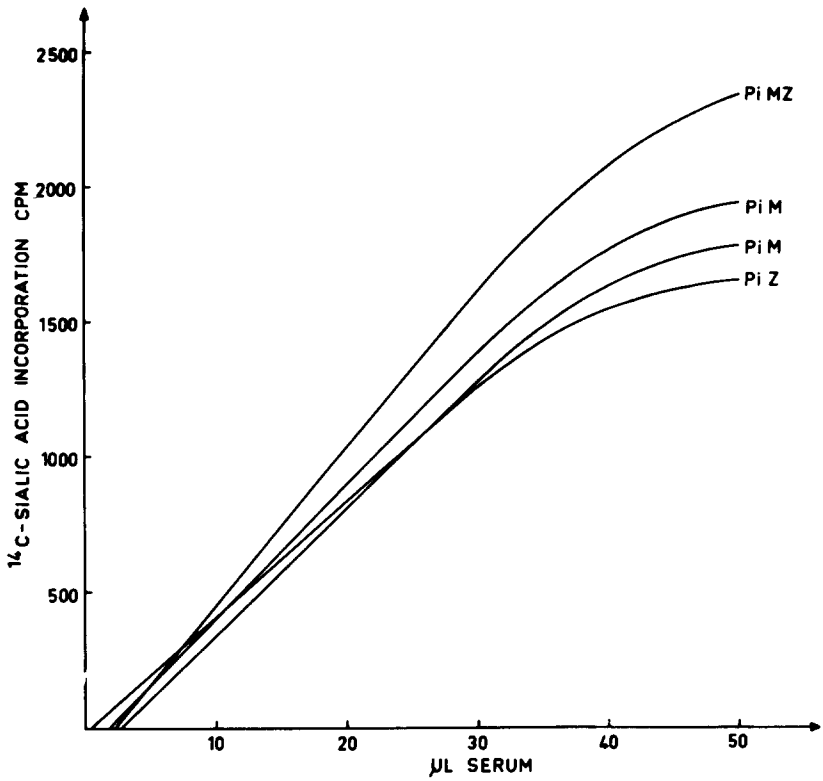

Fig. 1. Effect of increasing amounts of serum from various phenotypes on the incorporation of ^{14}C-labelled sialic acid in asialo-α_1-antitrypsin (Pi M). Standard assay conditions (see text).

(range 732—1751 cpm) was found to be 50 cpm corresponding to a variation coefficient of 4.4%.

The level of transferase activity in different Pi Z phenotype sera is summarized in Table II. All sera were freshly drawn except for the sera of 4 Pi Z indi-

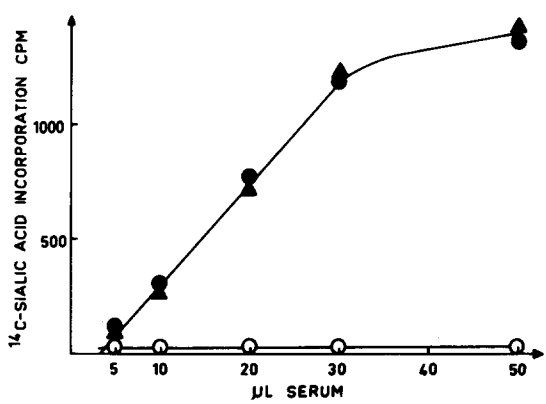

Fig. 2. Effect of increasing amounts of normal serum on the incorporation of ^{14}C-labelled sialic acid in asialo-α_1-antitrypsin Pi M (●), asialo-α_1-antitrypsin (Pi Z) (▲) and non-desialylated α_1-antitrypsin Pi Z (○). Incubation time 30 min.

TABLE I

SIALYLTRANSFERASE ACTIVITY IN Pi M-INDIVIDUALS. COMPARISON BETWEEN SERUM AND PLASMA, MALES AND FEMALES AND FASTING AND NON-FASTING SERUM

Category	Number	Sialyltransferase activity (cpm/20 μl/45 min, mean ± S.D.)	Significance of difference
Fasting serum	13	1157 ± 213	$P < 0.001$
Fasting EDTA-plasma	13	800 ± 164	
Fasting serum	5	1222 ± 135	Not significant
Non-fasting serum	5	1147 ± 193	
Fasting serum, males	17	1031 ± 169	Not significant
Fasting serum, females	21	1106 ± 203	

viduals with advanced liver cirrhosis, which had been stored at −20°C for approximately 2 years. The transferase activity in the latter was about 50% lower than in controls. A freshly drawn serum from another Z individual with liver cirrhosis had normal sialyltransferase activity. The enzyme activities of sera from individuals with normal liver function and phenotype Pi MZ, Z, SZ and FZ did not differ from normal Pi M levels.

Discussion

The assay procedure used for serum sialyltransferase activity is simple and well reproducible. As a source of acceptor protein we used α_1-antitrypsin prepared according to the thiol-disulfide exchange procedure of Laurell et al. [14], known to preserve the microheterogeneity of the glycoprotein better than ordinary ion-exchange procedures. Furthermore the mild desialylation procedure on Sepharose-neuraminidase columns should give little damage to asialoantitrypsin in contrast to treatment with dilute sulfuric acid [6]. The incorporation of sialic acid calculated from available acceptor sites was 1% (assuming 6 sialic acid residues in Pi M α_1-antitrypsin and a molecular weight of 50 000) in the present method. Kuhlenschmidt et al. [6] found 0.02% incorporation in their

TABLE II

SIALYLTRANSFERASE ACTIVITY IN FASTING SERA FROM INDIVIDUALS WITH DIFFERENT Pi TYPES

Pi type	Number	Sialyltransferase activity (Cpm/20 μl/45 min, mean ± S.D.)	Significance of difference
M	38	1072 ± 190	
MZ	13	1164 ± 123	Not significant
Z (normal liver function)	14	1154 ± 257	Not significant
SZ	9	1207 ± 306	Not significant
FZ	2	1124 ± 280	Not significant
Z * liver cirrhosis	1	910	—
Z ** liver cirrhosis	4	537 ± 186	—

* Freshly drawn serum.

** These samples were stored at −20°C for 2 years; this has resulted in a 50% loss in sialyltransferase activity when compared with freshly drawn serum.

assay using 0.15 mg asialoantitrypsin. Mookerjea et al. [15] reported less than 3% incorporation in their assay procedure using asialoorosomucoid as acceptor protein and 2 h incubation.

Whether asialoantitrypsin Pi M or Pi Z is used as acceptor a plateau is reached when more than 30 μl of serum is added to the reaction mixture (Figs. 1 and 2). Obviously this phenomenon is not due to saturation of available binding sites, as prolonged incubation (20 μl serum, 2 h) results in a linear increase of transferred sialic acid. More probably, the inhibition of transferase activity obtained with larger amounts of serum is due to the inhibiting effect of increasing amounts of ordinary sialoglycoproteins present in the serum added. Sialyltransferase transfers sialic acid residues not only to α_1-antitrypsin but to a large number of glycoproteins i.e. orosomucoid [15], transferrin [6,7] and ceruloplasmin [16]. An analogous inhibition could also be obtained by adding increasing amounts of fully sialylated antitrypsin to the reaction mixture. In the routine assay we have therefore used 20 μl serum, which invariably fall on the linear part of the curve. It is also obvious from Fig. 2 that antitrypsin Pi Z, untreated with neuraminidase, cannot incorporate any sialic acid, This is of interest in view of reports from several laboratories that serum Pi Z antitrypsin lacks several terminal sialic acid residues [17—19]. The result indicates a steric hindrance to incorporation of the missing sialic acid into the Pi Z molecule.

It is essential to use serum in the assay as EDTA exerts an inhibiting effect on the transferase activity (Table I). Addition of Ca^{2+} ions did not restore the activity. It is possible that the sialyltransferase requires Mn^{2+} ions for optimal activity analogous to serum *N*-acetyl gluc›samine transferase [20] but this possibility has not been tested. Although fasting and non-fasting serum samples (Table I) did not differ in transferase activity, we have preferred to use fasting samples to avoid variations in long chain fatty acid levels. Such acids are known to inhibit transferase activity [16].

The serum transferase activity in sera representing both homozygous (Z) and different heterozygous (MZ, SZ, FZ) deficiency states with normal liver function did not differ significantly from that in normal serum (Table II). In the present series comprising only adult patients we did not discover any case with a reduction of transferase activity to the 10% level. In view of the present results and those obtained in our earlier series of 17 newborns [7], we thus conclude that serum sialyltransferase deficiency is not implicated in the pathogenesis of the ordinary case of α_1-antitrypsin deficiency. The Pi Z patient with sialyltransferase deficiency described by Kuhlenschmidt et al. [6] had an advanced cirrhosis of the liver but the case found by us [7] had normal liver function. This fact argues against the possibility that sialyltransferase deficiency is especially linked, as a primary or secondary phenomenon, to the development of liver disease in α_1-antitrypsin deficiency. To exclude this possibility definitely we studied the transferase activity in sera from 5 adult Pi Z patients with advanced liver disease. Only one fresh serum was available with normal transferase activity (Table II). Sera from the other 4 cases had been stored for approximately 2 years resulting in a considerable loss of activity. This long storage time makes the results difficult to interpret but none the less it is evident that in no case was the activity as low (about 10% of normal) as found in earlier cases with sialyltransferase deficiency [6,7].

There is no firm evidence that the sialyltransferase activity determined in serum reflects the level in the hepatic Golgi apparatus [15,21,22]. The possibility that locally subnormal transferase levels are responsible for the defect sialylation of α_1-antitrypsin seems remote, however, in view of recent data [23] on a more extensive glycosylation defect in hepatic α_1-antitrypsin resulting in a concomitant deficiency of free galactosyl residues.

Acknowledgements

We are indebted to Mr. Ragnar Alm for skilful technical assistance. This work was supported by grants from the National Swedish Association against Heart and Chest Diseases and from the Medical Faculty, University of Lund.

References

1 Sharp, H.L. (1971) Hosp. Pract. 6, 83—96
2 Berg, N.O. and Eriksson, S. (1972) N. Engl. J. Med. 287, 1264—1267
3 Lieberman, J., Mittman, C. and Gordon, H.W. (1972) Science 175, 63—65
4 Feldman, G., Bignon, J. and Chahinian, P. (1973) Lancet 1, 1515—1515
5 Eriksson, S. and Larsson, C. (1975) N. Engl. J. Med. 292, 176—180
6 Kuhlenschmidt, M.S., Yunis, E.J., Jammarino, R.M., Turco, S.J., Peters, S.P. and Glew, R.H. (1974) Lab. Invest. 31, 413—419
7 Eriksson, S. and Larsson, C. (1975) N. Engl. J. Med. 292, 925—926
8 Eriksson, S. and Hägerstrand, I. (1974) Acta Med. Scand. 195, 451—458
9 Johansson, B.G. (1972) Scand. J. Clin. Lab. Invest. 29, Suppl. 124, 7—19
10 Hartree, E.F. (1972) Anal. Biochem. 48, 422—427
11 Warren, L. (1959) J. Biol. Chem. 234, 1971—1975
12 Laurell, C.-B. (1972) Scand. J. Clin. Lab. Invest. 29, Suppl. 124, 21—37
13 Laurell, C.-B. and Persson, U. (1973) Biochim. Biophys. Acta 310, 500—507
14 Laurell, C.-B., Pierce, J., Persson, U. and Thulin, E. (1975) Eur. J. Biochem. 57, 107—113
15 Mookerjea, S., Michaels, M.A., Hudgin, R.L., Moscarello, M.A., Chow, A. and Schachter, H. (1972) Can. J. Biochem. 50, 738—740
16 Hickman, J., Ashwell, G., Morell, A.G., van den Hamer, C.J.A. and Scheinberg, I.H. (1970) J. Biol. Chem. 245, 759—766
17 Cox, D.W. (1973) Lancet 2, 844—845
18 Chan, S.K. and Rees, D.C. (1975) Nature 255, 240—241
19 Jeppsson, J.-O. and Laurell, C.-B. (1974) in Proteases and Biological Control II, Cold Spring Harbor, in the press
20 Mookerjea, S., Chow, A. and Hudgin, R.L. (1971) Can. J. Biochem. 49, 297—299
21 Schachter, H., Jabbal, I., Hudgin, R.L., Pinteric, L., McGuire, E.J. and Roseman, S. (1970) J. Biol. Chem. 245, 1090—1100
22 Hudgin, R.L. and Schachter, H. (1971) Can. J. Biochem. 49, 829—837
23 Jeppsson, J.-O., Larsson, C. and Eriksson, S. (1975) N. Engl. J. Med. 293, 576—579