Cleavage of oligosaccharides by rat kidney sialidase

Influence of substrate structure

L. Cohen-Forterre, G. Mozère, J. André and M. Sternberg

Groupe de Recherches sur la Biochimie et la Pharmacologie des Vaisseaux et du Rein, Département de Biochimie, Faculté de Médecine Broussais-Hôtel-Dieu, 45, rue des Saints-Pères, 75270 Paris Cédex 06, France

Received 15 May 1984

The specificity of the sialidase activity present in rat kidney cortex (12 000 × *g* pellet) was studied with various tritiated oligosaccharidic substrates: (i) αNeuAc2→3βGal1→4Glc-itol[^{3}H], αNeuAc2→6βGal1→4Glc-itol[^{3}H] and αNeuAc2→8αNeuAc2→3βGal1→4Glc-itol[^{3}H] from bovine colostrum; (ii) αNeuAc2→6βGal1→4βGlcNAc-itol[^{3}H], αNeuAc2→3βGal1→4βGlcNAcl→2αMan1→3βMan1→4GlcNAc-itol[^{3}H]. αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3(βGal1→4GlcNAc1→2αMan1→6)βMan1→4GlcNAc-itol[^{3}H]et αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3(αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→6)βMan1 4GlNAc-itol[^{3}H] isolated from the urine of a patient with mucolipidosis I. The enzyme cleaves α2→3 and α2→8 linkages at a greater rate than the α2→6 bonds. Its activity decreases with the length of the oligosaccharidic chain. Substitution of a glucose moiety by Nacetylglucosamine results in diminished activity. The specificity of rat kidney sialidase differs from that reported for other mammalian of viral sialidases.

Rat kidney Sialidase specificity Oligosaccharidic substrate

1. INTRODUCTION

Kidney cortex lysosomal sialidase (EC 3.2.1.18) [1–5] may play a major role in the regulation of the negative charges present on the glomerular capillary wall and responsible for the charge-selective glomerular filtration barrier [6]. It can also interfere with the catabolism of low M_r plasma glycoproteins reabsorbed into the tubular cells [7]. Thus it was of interest to study the effect of rat kidney cortex sialidase on oligosaccharides whose sequences are found in the plasma and glomerular basement membrane glycoproteins or in the cell membrane glycolipids [8–10] and to look at the influence of the oligosaccharide structure on the enzyme's activity.

2. MATERIALS AND METHODS

2.1. *Substrates*

Sialyllactose from bovine colostrum (Sigma, St Louis, MO) was reduced by NaB^3H_4 (Amersham, England, 756 mCi/mmol), and then sialyl α2⟶3[^{3}H]lactitol and sialyl α2⟶6[^{3}H]lactitol were isolated by descending chromatography on Whatman no.3 paper for 5 days with ethyl acetate–pyridine–water (12:5:4, v/v) [11].

Disialyllactose, purified from bovine colostrum [12], and 6 sialyloligosaccharides, isolated from the urine of a patient with mucolipidosis I (S_I, S_{II}, S_{III}, S_{IV}, S_V and S_{IX}) [13] were gifts from J.C. Michalski and G. Strecker (table 1). Each of these compounds was reduced by NaB^3H_4 and isolated by descending chromatography on Whatman no.3 paper for 16 h with ethyl acetate–pyridine–acetic acid–water (5:5:1:3, v/v) [14]. Further purification was achieved by high-voltage electrophoresis on 57 cm long Whatman 3MM paper for 80 min at 4 kV with 95 mM pyridine acetate buffer (pH 5.4) in order to remove any desialylated contaminants.

The sialic acid content of each substrate was determined by the thiobarbituric acid method after

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

Table 1

Comparison of rat kidney sialidase activity on various radiolabeled sialyloligosaccharides, at identical total sialyl residue concentrations

Substrate			Enzyme activity[a]	
Origin and abreviation	Structures	Specific radioactivity ($Ci \cdot mol^{-1}$)	Specific (pmol NeuAc/min per mg protein)	Relative
Colostrum	αNeuAc2→3βGal1→4Glc-itol-1[^{3}H]	72.3	16	100
Colostrum	αNeuAc2→6βGal1→4Glc-itol-1[^{3}H]	72.3	8.2	51
Colustrum	αNeuAc2→8αNeuAc2→3βGal1→4Glc-itol-1[^{3}H]	51.4	19.8	124
Urine S_I	αNeuAc2→3βGal1→4βGlcNAc1→2αMan1→3βMan1→4GlcNAc-itol-1[^{3}H]	10.2	5.2	32
Urine S_{II}	αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3βMan1→4GlcNAc-itol-1[^{3}H]	8.7	1.0	6
Urine S_{III}	αNeuAc2→6βGal1→4GlcNAc-itol-1[^{3}H]	14.5	4.2	26
Urine S_{IV}	αNeuAc2→8αNeuAc2→6βGal1→4GlcNAc-itol-1[^{3}H]	30.9	6.7	42
Urine S_V	αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3 / βGal1→4βGlcNAc1→2αMan1→6 > βMan1→4GlcNAc-itol-1[^{3}H]	22.7	1.5	9
Urine S_{IX}	αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3 / αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→6 > βMan1→4GlcNAc-itol-1[^{3}H]	12.9	2.3	14

[a] Final concentration of bound sialic acid in each substrate was 6.7 μM. Incubation was carried out with 600 μg lysosomal enzyme protein

hydrolysis in 0.1 N sulfuric acid at 80°C for 1 h [15]. The specific radioactivities of all the substrates prepared and their sequences are given in table 1.

2.2. *Enzyme preparation*

Ten normal male Wistar rats of 350 ± 16 g (SE) were anesthetized with ether and bled from the abdominal aorta. The kidneys were removed and put on ice. The cortices were isolated and homogenized with 0.45 M sucrose (1:8, w/v). The homogenate was then fractionated by differential centrifugation [16]. Four fractions were obtained by 3 successive centrifugations: the nuclear pellet (5 min at 650 × *g*); a crude lysosomal pellet (20 min at 12000 × *g*), the microsomal pellet and the soluble fraction (60 min at 105000 × *g*). The lysosomal fraction, which contained most of the sialidase activity on sialyl α2⟶3[^{3}H]lactitol and sialyl α2⟶6[^{3}H]lactitol and also most of the acid phosphatase activity, was used for this study since solubilization of the enzyme by various treatments prior to further purification has been found to be relatively inefficient [4,5]. The lysosomal fraction was dialyzed against 75 mM sodium acetate buffer (pH 4.2). Its protein content was measured by the Lowry technique after dissolving in 0.1 N NaOH at 37°C for 2 h [15].

2.3. *Assay for sialidase activity*

Each of the substrates was incubated at 37°C with 600 μg lysosomal protein in 150 μl of 50 mM (final concentration) sodium acetate buffer (pH 4.2) containing 0.45 μmol sodium azide. The reaction time was 5–60 min according to the substrate in order to measure initial velocity. The reaction was stopped by heating at 100°C for 5 min. All the substrates were incubated in the same series. In

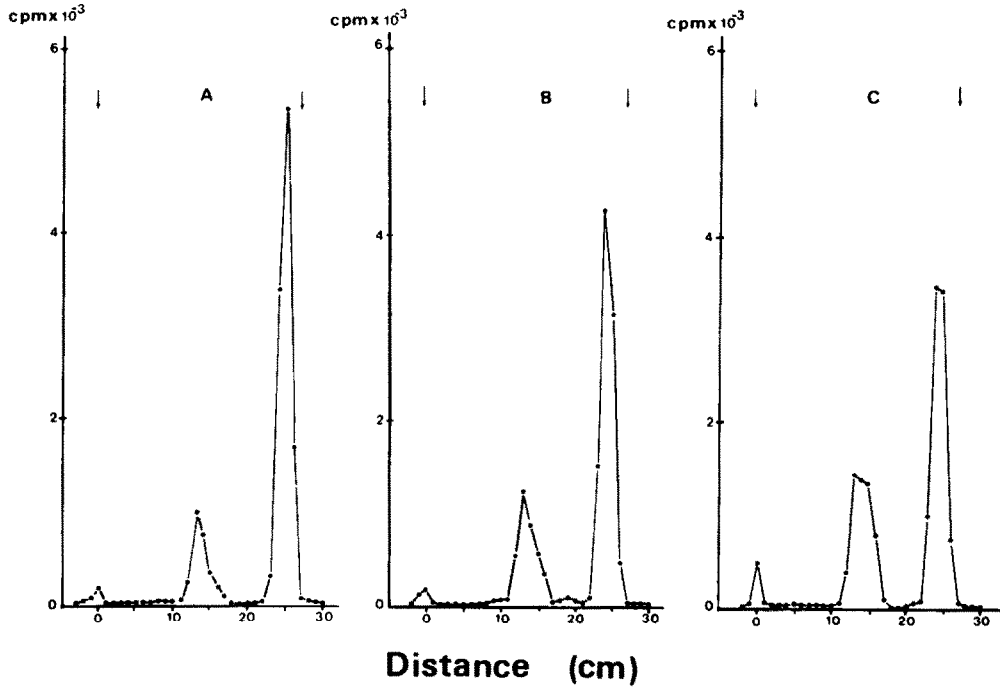

Fig.1. Cleavage of αNeuAc2⟶8αNeuAc2⟶3[^{3}H]lactitol (6.7 μM) by rat kidney lysosomal sialidase (600 μg protein) as revealed by high-voltage electrophoresis under conditions detailed in section 2. The arrows indicate the positions of lactose and NeuAc standards. (A) Enzymatic incubation for 5 min; specific releases of αNeuAc2⟶3[^{3}H]lactitol and [^{3}H]lactitol, calculated after control substraction, were 50 and 5 pmol, respectively. (B,C) Enzymatic incubations for 10 and 20 min, respectively; product-specific releases were 90 and 7 pmol after 10 min, 136 and 14 pmol after 20 min. The 5 min incubation was considered for the determination of initial velocity: 12 pmol·min^{-1} sialic acid released.

control tubes each substrate was also incubated with heat-denatured enzyme. The content of each tube was centrifuged at 9400 × g and the supernatant deposited on 57 cm Whatman 3MM paper for high-voltage electrophoresis. This was performed for 80 min at 4 kV with 95 mM pyridine acetate buffer (pH 5.4). The bands were cut into 0.5-cm pieces and each eluted with 1 ml water. The radioactivity was determined by liquid scintillation counting in a Packard Tricarb spectrometer using Picofluor (Packard, Downers Grove, IL) as scintillator. The peaks of radioactivity corresponding to the substrate – most anionic – and to the product(s) – neutral and eventually intermediary in case of disialylated substrate – were evaluated and their proportions calculated (fig.1). The coefficient of variation of triplicate determinations was 8%.

3. RESULTS AND DISCUSSION

The activity of kidney cortex lysosomal sialidase on the various radiolabeled oligosaccharides was compared at the same concentration of total bound sialic acid (6.7 μM). The results are summarized in table 1.

Comparison of results for substrates which differ only by isomery at the α-sialyl linkage, i.e., the two sialyllactitols, αNeuAc2→3βGal1→4Glc-itol and αNeuAc2→6βGal1→4Glc-itol, and the hexasaccharides, αNeuAc2→3βGal1→4βGlcNAc1→2αMan1→3βMan1→4GlcNAc-itol (S_I) and αNeuAc2→6βGal1→4βGlcNAc1→2αMan1→3βMan1→4GlcNAc-itol (S_{II}), shows that the enzyme is about 2-times more active on the α2→3 than on the α2→6 isomer of sialyllactitol and 5-times more active on S_I than on S_{II}.

Hydrolysis velocity is higher with the trisaccharide, αNeuAc2⟶6βGal1⟶4GlcNAc-itol (S_{III}), than with the hexasaccharide S_{II} which differs only by the additional sequence αMan1⟶3βMan1⟶4GlcNAc-itol. Enzyme activity diminishes with increasing oligosaccharide chain length.

The sialidase is 3-times more active on sialyl-α2⟶6-lactitol than on sialyl α2⟶6-*N*-acetyllactosaminitol. Substitution of a glucitol residue by an *N*-acetyl-glucosaminitol lowers the enzyme activity.

There is little difference between the enzyme activities on the two biantennary substrates, S_V (monosialylated) and S_{IX} (disialylated), at the same concentration of bound sialic acid. The slightly higher activity on S_{IX} might be explained by the immediate availability of the second sialic acid for enzymatic catalysis.

With respect to the disialylated substrates possessing two adjacent sialyl residues, αNeuAc2⟶8αNeuAc2⟶3-lactitol and αNeuAc2→8αNeuAc2⟶6-*N*-acetyllactosaminitol, a short enzymatic incubation yielded mainly a monosialylated product (91 and 97%, respectively) (fig.1). Thus the rate of hydrolysis reflects the rate of cleavage of the αNeuAc2⟶8 linkage. The ac-

Table 2

Comparison of rat kidney sialidase activity on disialylated oligosaccharidic substrates possessing an αNeuAc2→8 linkage with that on their monosialylated homologues, at identical terminal sialyl residue concentration

Substrate	Relative activity[a]
αNeuAc2→3βGal1→4Glc-itol-1[^{3}H]	100
αNeuAc2→8αNeuAc2→3βGal1→4Glc-itol-1[^{3}H]	227
αNeuAc2→6βGal1→4GlcNAc-itol-1[^{3}H]	26
αNeuAc2→8αNeuAc2→6βGal1→4GlcNAc-itol-1[^{3}H]	81

[a] Final concentration of each substrate was 6.7 μM. Incubation was carried out with 600 μg lysosomal enzyme protein. Enzyme activity was determined as pmol·min^{-1} terminal NeuAc released and expressed relatively to that on sialyl α2→3-lactitol (9.6 pmol·min^{-1})

tion of the enzyme on these disialylated substrates may be compared with that on the monosialylated homologues with the former at the same sialyl residue concentration (table 1) or at the same total substrate concentration, equivalent to an identical terminal sialyl residue concentration (table 2). αNeuAc2⟶8αNeuAc2⟶3-lactitol is cleaved at a faster rate than αNeuAc2⟶3-lactitol: the increase in velocity is 25% at the same sialyl residue concentration and 100% at the same total substrate concentration. Similarly, for αNeuAc2⟶8αNeuAc2⟶6-*N*-acetyllactosaminitol, the increases are 60 and 300%, respectively. Thus the enzyme was more active in hydrolysing the αNeuAc2⟶8 linkage than the αNeuAc2⟶3 and αNeuAc2⟶6 linkages.

The higher activity observed here with the rat kidney sialidase on the α2⟶3 linkage than on the α2⟶6 linkage has also been found for other sialidases [11,18]. In contrast, the α2⟶8 linkage has been reported to be cleaved at a much slower rate than the α2⟶3 linkage by human liver lysosomal sialidase or by fowl plague virus and influenza A_2 virus sialidases using disialyllactose and αNeuAc2⟶3-lactose as substrates. However, Newcastle disease virus neuraminidase is almost as active on disialyllactose as on αNeuAc2⟶3-lactose [18]. The high rate of cleavage of the α2⟶8 linkage by rat kidney sialidase has been found here with two different disialylated substrates. Substitution of a glucose residue by an *N*-acetylglucosamine has been reported to increase the enzyme activity of viral neuraminidases [18], but here it has been found to lower the rat kidney enzyme activity. Thus it appears that substrate specificity varies according to the source of the sialidase. For the rat kidney enzyme a broad specificity has been found with respect to the type of αNeuAc linkage and to the type of oligosaccharide chain structure. This is in agreement with the reported action of rat kidney sialidase on brain ganglioside mixtures (containing sialyl α2⟶3 and sialyl α2⟶8 linkages and lactose residues) and on various sialylglycoproteins (containing sialyl α2⟶6 and sialyl α2⟶3 linkages and *N*-acetyllactosamine residues) [1,2]. Thus the enzyme could act on the gangliosides of kidney cell membranes and on the glycoproteins reabsorbed into the tubular cells or found in the kidney basement membranes.

ACKNOWLEDGEMENTS

We thank G. Strecker and J.C. Michalski for providing us with disialyllactose and the oligosaccharides from the urine of a patient with mucolipidosis I. This work was supported by the following institutions: Délégation Générale à la Recherche Scientifique et Technique, Université René Descartes, Faculté de Médecine Broussais-Hôtel-Dieu and Association pour le Développement de la Recherche en Biochimie-Paris.

REFERENCES

[1] Mahadevan, S., Nduaguba, J.C. and Tappel, A.L. (1967) J. Biol. Chem. 242, 1103–1113.
[2] Kirschbaum, B.B. and Bosmann, H.B. (1973) Nephron 11, 26–39.
[3] Typpy, J.V. and Palese, P. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1169–1178.
[4] Cohen-Forterre, L., Mozère, G., André, J. and Sternberg, M. (1983) in: Glycoconjugates (Chester, M.A., Heinegard, D., Lundblad, A. and Svensonn, S. eds) p.442, Rahms Press, Lund.
[5] Cohen-Forterre, L., Mozère, G., André, J. and Sternberg, M. (1984) Biochim. Biophys. Acta, in press.
[6] Brenner, B.M., Bohrer, M.P., Baylis, C. and Deen, W.M. (1977) Kidney Int. 12, 229–237.
[7] Maack, T., Johnson, V., Kan, S.T., Figueirido, J. and Sigulem, D. (1979) Kidney Int. 16, 251–270.
[8] Strecker, G. and Montreuil, J. (1979) Biochimie 61, 1199–1246.
[9] Spiro, R.G. (1967) J. Biol. Chem. 242, 1923–1932.
[10] Ng, S.S. and Dain, J.A. (1976) in: Biological Roles of Sialic Acid (Rosenberg, A. and Schengrund, C.L. eds) pp.59–102, Plenum, New York.
[11] Frish, A. and Neufeld, E.F. (1979) Anal. Biochem. 95, 222–227.
[12] Veh, R.W., Michalski, J.C., Corfield, A.P., Sander-Wewer, M., Gres, D. and Schauer, R. (1981) J. Chromatogr. 212, 313–322.
[13] Michalski, J.C., Strecker, G., Fournet, B., Cantz, M. and Spranger, J. (1977) FEBS Lett. 79, 101–104.
[14] Takasaki, S., Mizuochi, T. and Kobata, A. (1982) Methods Enzymol. 83, 263–268.
[15] Aminoff, D. (1961) Biochem. J. 81, 384–392.
[16] Shibko, S. and Tappel, A.L. (1965) Biochem. J. 95, 731–741.
[17] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–276.
[18] Corfield, A.P., Michalski, J.C. and Schauer, R. (1981) Perspectives in Inherited Metabolic Diseases 4, 3–70.