Discussion Letter

BIOCHEMICAL BASIS OF SIX DIFFERENT TYPES OF SIALIDOSIS

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1. Sialidosis

Presently, six different inborn diseases can be defined as sialidosis. The semiology of mucolipidosis II (I-Cell disease) is well known: the occurrence of numerous cytoplasmic inclusions in cultured fibroblasts, the increase of the activity of several lysosomal hydrolases (except β-glucosidase and acid phosphatase) in extracellular fluids contrasting with a decrease of the same activities in cultured fibroblasts are the most significant elements of diagnosis [1–3]. An elevated excretion of urinary sialyl-oligosaccharides has been observed [4,5], with a total lack of α-neuraminidase activity in leukocytes, while the other hydrolase activities are normal [6]. This defect of α-neuraminidase activity has been also found in cultured fibroblasts [7,8], but was not characteristic, in this case, of a primary defect, since the other hydrolases were also defective. This primary defect in α-neuraminidase is in good agreement with many other observations which have been made on mucolipidosis II such as an increase of electronegativity of secreted hydrolases compared to intracellular or normal plasma hydrolases [9], and failure of their uptake by different types of cells [10]. Recognition markers of secreted glycoproteins, including acidic hydrolases, have been identified for different types of tissues as galactose, *N*-acetylglucosamine or mannose [11–13], and an α-neuraminidase deficiency might explain the abnormal exocytosis of hydrolases in ICD fibroblasts by masking the recognition marker.

Mucolipidosis III can be considered as an attenuated form of mucolipidosis II. A partial defect of leukocyte α-neuraminidase activity (15% of the normal value) and a moderate excretion of urinary sialyl-oligosaccharides (10–20-fold the normal level) were also characterized [14,15].

Other known mucolipidosis present quite different pathogenesis. Mucolipidosis I is characterized by a profoundly diminished activity of α-neuraminidase and abnormal accumulation of sialic acid-containing compounds in cultured fibroblasts [8]. Nevertheless, the hydrolase activity abnormalities found in mucolipidosis II and III are here not present. Structures of sialyl-oligosaccharides accumulating in urine have been described [16] and are identical to those of mucolipidosis II.

Mucolipidosis W. (provisionally named by the initial of the patient) is very similar to mucolipidosis I, with additional renal insufficiency [17]. An accumulation of urinary sialyl-oligosaccharides and a total lack of leukocyte α-neuraminidase activity have been observed [4,5].

Patients De P. [18] and N. (Guazzi, Federico, Carlemagno, Michalski and Strecker) are adolescent and adults, without significant symptoms, except cherry-red macular spot and moderate neurological troubles. In these two 'new' types of mucolipidosis (which are probably attenuated forms of mucolipidosis I), a massive excretion of urinary sialyl-oligosaccharides has been observed and an α-neuraminidase deficiency was confirmed for the two patients De P. [5,15].

Abbreviations: Na, *N*-acetylneuraminic acid; Gal, galactose; Man, mannose; GNAc, *N*-acetylglucosamine; β-endo-GlcNase, β-endo-*N*-acetylglucosaminidase

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Table 1
α-Neuraminidase activity[a] in leukocytes (nmol/h/mg protein)

		Substrate	
		α-AcNeu-(2→6)–R	α-AcNeu-(2→3)–R
1. Concentration of substrate		20 nmol/ 200 μl	
Normal subjects		0.128–0.488 (0.330; n = 30)	n.d.[b]
Sialidosis A			
MLP II	case no. 1	0.00	n.d.
	case no. 2	0.020	n.d.
MLP III		0.045	n.d.
Sialidosis B			
MLP W.		0.006	n.d.
MLP De P.	brother	0.036	n.d.
	sister	0.045	n.d.
2. Concentration of substrate		400 nmol/200 μl	320 nmol/200 μl
Normal subjects		13.77–29.60 (19.60; n = 12)	8.00–12.90 (12.40; n = 12)
Sialidosis A			
MLP II	case no. 3	2.00	1.60
	case no. 4	0.02	2.45
Sialidosis B			
MLP W.		0.00	9.92
MLP I	case no. 1	0.00	10.90

[a] All the other hydrolase activities are normal or slowly increased, except β-galactosidase (15% of the normal value for MLP case no. 1)
[b] n.d. not determined

Table 2
α-Neuraminidase activity in cultured fibroblasts (nmol/h/mg protein)

	Substrate	
	α-AcNeu-(2→6)–R	α-AcNeu-(2→3)–R
Concentration of substrate	20 nmol/200 μl	40 nmol/200 μl
Control 1	0.436	0.339
Control 2	0.835	0.668
Sialidosis B[a]		
MLP De P. (brother)	0.020	0.267
MLP I (case no. 2)	0.00	0.507

[a] All the other hydrolase activities are normal or slowly increased

From a biochemical point of view, these six types of sialidosis can be divided into two classes: sialidosis type A, (mucolipidosis II and III) with an α-neuraminidase defect, a moderate excretion of urinary sialyl-oligosaccharides (10–20-fold the normal value for mucolipidosis III and 40–120-fold for mucolipidosis II) and a leakage of lysosomal hydrolases into extracellular fluids; sialidosis type B, (mucolipidosis I, W. De P. and N.) with an α-neuraminidase defect and massive excretion of urinary sialyl-oligosaccharides (500–1000-fold the normal value), but without lysosomal hydrolase exocytosis. On the other hand, it is interesting to note [5] that the accumulated compounds show a ratio of α-(2→6) to α-(2→3)-sialyl linkages of 1 for sialidosis A and of 10–30 for sialidosis B.

2. Enzymatic studies

All the above facts can be explained by the hypothesis that sialidosis A is due to a defect of both α-(2→3) and α-(2→6) neuraminidase activities, while sialidosis B is only characterized by the single defect of α-(2→6) neuraminidase activity.

In order to verify this hypothesis, we determined the α-neuraminidase activities using as natural substrates two sialyl-oligosaccharides isolated from the urine of the patients. After reduction with tritiated potassium borohydride, their formulae were as follows:

α-AcNeu-(2→6)–β-Gal-(1→4)–β-GlcNAc-(1→2)–α-Man-(1→3)–β-Man-(1→4)–GlcNAc-itol-1-^{3}H and

α-AcNeu-(2→3)–β-Gal-(1→4)–β-GlcNAc-(1→2)–α-Man-(1→3)–β-Man-(1→4)–GlcNAc-itol-1-^{3}H.

The neuraminidase activity was determined according to the procedure described [6]. The results we obtained (tables 1 and 2) confirm the hypothesis of a specific defect of α-(2→6) neuraminidase in the case of sialidosis B.

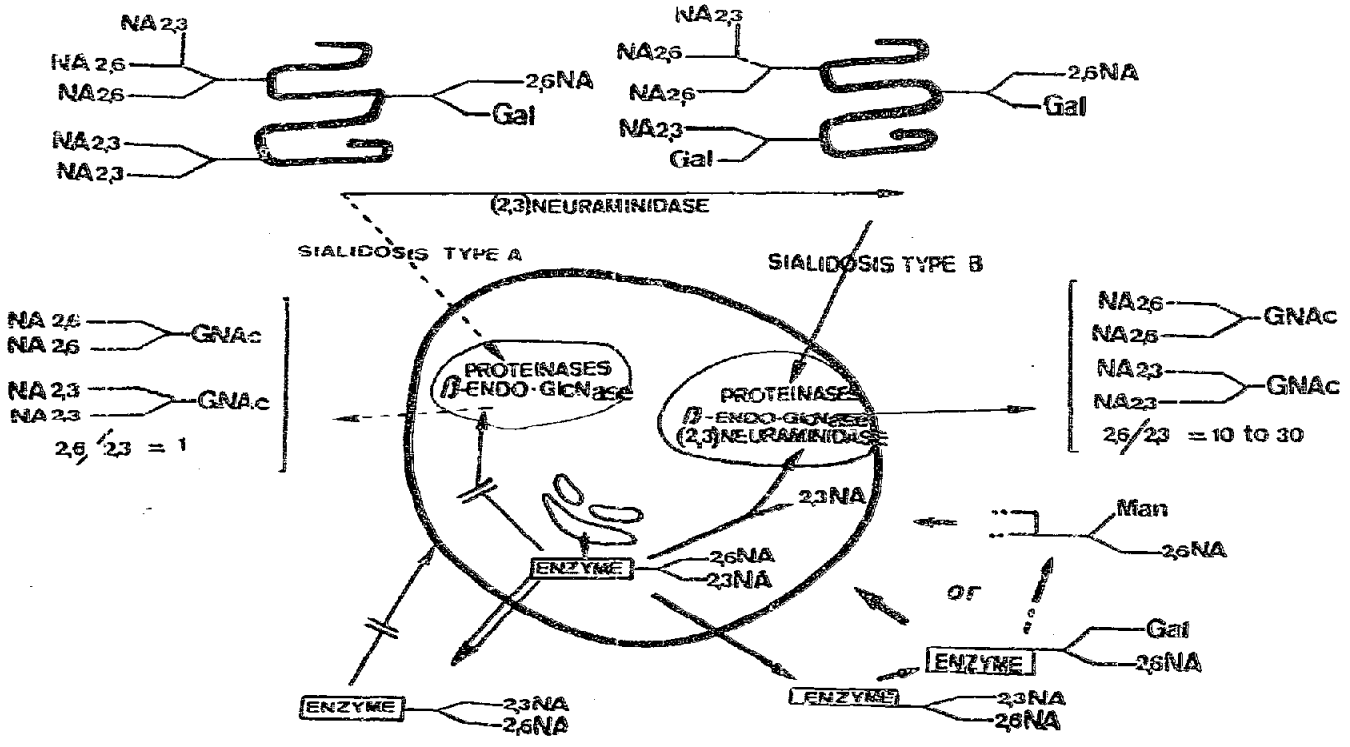

Fig.1. Catabolism of glycoproteins and packaging of lysosomal hydrolases in sialidosis A and B.

3. Discussion

It has been recently shown that plasma hydrolases are normally sialylated (except for β-glucosidase, β-xylosidase and acid phosphatase) in contrast to liver hydrolases [19]. It has been also shown that the release of only two sialyl residues on the twelve present in ceruleoplasmin was sufficient to result in endocytosis of the glycoprotein [11]. In view of these facts, we propose a scheme (fig.1) which might explain the differential etiology of the two types of sialidosis. The broken line (—‖→) indicates the failure of hydrolase packaging which characterizes sialidosis A. In the case of sialidosis B, a single defect of α-(2→6) neuraminidase activity allows a normal uptake of lysosomal hydrolases. The moderate excretion of sialyl-oligosaccharides in the case of sialidosis A is probably due to the slackening of glycoprotein endocytosis (dotted line, fig.1).

It has been also found that desialylation of ICD-excreted β-hexosaminidase with *Clostridium perfringens* neuraminidase did not enhance enzyme uptake by β-hexosaminidase-deficient, non-ICD cells [20]. However, Ullrich and von Figura [21] found that endocytosis of α- and β-*N*-acetylglucosaminidase by human skin fibroblasts was inhibited by D-mannose and L-fucose and that of β-glucuronidase by mannose. These facts do not confirm the hypothesis of a masking of recognition markers due to a primary defect in α-neuraminidase, since glycoproteins, and probably hydrolases, possess this common terminal structure:

α-AcNeu-(2→3/6)—β-Gal-(1→4)—β-GlcNAc-(1→2/4/6)—α-Man→R.

The desialylation of excreted hydrolases could not restore their endocytosis, in the case of cultured fibroblasts.

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