NEURAMINIDASE DEFICIENCY IN THE MOUSE

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Received 9 October 1979

1. Introduction

The liver of mouse strain SM/J expresses unique electrophoretic patterns of the lysosomal enzymes acid phosphatase (EC 3.1.3.2) [1], α-mannosidase (EC 3.2.1.24) [2] and arylsulfatase B (EC 3.1.6.1) [3]. For each of these enzymes, the more electronegative forms are enhanced whereas less negatively charged forms are depleted or absent. Genes involved in post-transcriptional processing of lysosomal enzymes have been implicated and each were mapped to approximately the same region of chromosome 17 [2–4]. The SM/J electrophoretic patterns can be converted to that of control mouse strains by pretreatment with bacterial neuraminidase (EC 3.2.1.18). These data have led us to consider the possibility that SM/J carries a unique allele for a single gene, perhaps involved in the production of neuraminidase, and that other lysosomal enzymes are processed differentially as dictated by alleles at this locus. Here we report profound neuraminidase deficiency in the liver of SM/J mice.

2. Materials and methods

2.1. *Materials*

N-Acetylneuraminic acid (type IV) and bovine serum albumin (Cohn fraction V) were purchased from Sigma Chemical, St Louis, MO. The sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) was synthesized in our laboratory [5]. Inbred C57BL/6J and SM/J mice were obtained from the Jackson Laboratory, Bar Harbor, ME.

2.2. *Animals and tissues*

Mice (5–10 weeks old) were killed by cervical dislocation. Liver, kidney, heart, lung and brain were dissected and immediately placed on ice. Homogenates (0.1 g tissue/ml water) were prepared at 0°C in a Potter-Elvejhem homogenizer (3 min treatment) fitted with a Teflon pestle. To prevent rapid inactivation of neuraminidase the homogenate was immediately made 12 mM in $CaCl_2$. With $CaCl_2$, the homogenate can be stored for months at −60°C without loss of neuraminidase activity. Freshly prepared or stored homogenates were used for neuraminidase assay.

2.3. *Neuraminidase assay*

Neuraminidase activity was assayed at 25°C according to [5] with 0.1 mM sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) as substrate, except that a 0.2 M acetate buffer (pH 4.4) was used in the incubation medium and that the reaction was stopped after 20 min by addition of 1 ml absolute ethanol. Protein was then centrifuged at 2000 × *g* for 10 min, the supernatant (1 ml) was collected and mixed with 0.1 ml 1 M NaOH just before reading fluorescence with an Aminco-Bowman model 768-H spectrofluorometer (excitation, 365 nm; emission, 450 nm).

Total sialic acid content of C57BL/6J and SM/J livers was determined after hydrolysis in 0.05 M H_2SO_4 at 80°C for 1 h. Free sialic acid was measured according to [6] after purification by passage through a Dowex 1 × 8 column (200–400 mesh, acetate form, 0.6 × 4 cm). The column was washed with distilled water and sialic acid was then eluted with 4 ml 0.84 M formic acid. The sialic acid containing fraction was

lyophilized and the residue was taken up in 0.2 ml water.

2.4. K_m app and K_i determinations

Apparent Michaelis-Menten constant, K_m app, was determined under standard assay conditions with sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) except that substrate concentration was varied between 0.08 and 0.4 mM. A Lineweaver-Burk plot was used for graphical determination of K_m app [7].

Apparent inhibition constant, K_i, for *N*-acetylneuraminic acid was determined using 2–10 mM inhibitor concentrations, and 0.05, 0.10 and 0.20 mM substrate concentrations, by the method in [8].

2.5. *Protein concentration*

This was determined by the method in [9] with bovine serum albumin as standard. Before assay, protein was dissolved in 0.1 M NaOH.

3. Results

Although neuraminidase is a relatively labile enzyme, minimal loss of activity occurred during homogeneization of mouse liver. Neuraminidase activity was assayed at 30 s intervals during the homogenization procedure and maximum activity was obtained after the first 30 s of treatment. However, a satisfactory homogenate was obtained only after a 3 min treatment. At least 80% of the initial enzyme activity was still present after 3 min. It is critical that the homogenates be made 12 mM in $CaCl_2$ immediately after homogenization because neuraminidase is lost rapidly (half-life 1.1–1.3 h) in absence of $CaCl_2$. The optimal concentration was found to be 12 mM $CaCl_2$ in which conditions half-life of neuraminidase was 14–15 h. The neuraminidase activity was stable during the assay since linearity with incubation time (≤ 30 min) and amount of homogenate protein (≤ 1.2 mg/ml) was obtained.

Neuraminidase activity was significantly lower than control (strain C57BL/6J) in liver of SM/J mice and (C57BL/6JxSM/J)F_1 hybrids, 17% and 48%, respectively ($P < 0.01$, table 1). Enzyme activity was also reduced to 35% of control in SM/J lung ($P < 0.05$) but for other organs, the lower activity found in SM/J mice was not statistically significant ($P > 0.05$). Mixtures of equal volumes of control and SM/J liver homogenates gave intermediate neuraminidase activity ruling out the presence of inhibitors or activators. Liver homogenates from C57BL/6J and SM/J did not differ significantly in their sialic acid content, 23.8 ± 4.9 μmol/g protein (mean ± SD) for control liver and 20.7 ± 5.1 for SM/J liver ($P > 0.05$, $n = 9$).

Two neuraminidase components, A and B, are present in control mouse liver which can be distinguished on the basis of their stability at 0°C in absence of $CaCl_2$ (fig.1). The more labile component (A) has a half-life of 1.1–1.3 h (calculated between 0 and 1 h incubation at 0°C) and represent 82–85% of total liver neuraminidase activity. For comparison purposes, a preparation of the B component was obtained essentially free of A activity by incubation of a fresh homogenate for 24 h at 0°C without $CaCl_2$.

Table 1
Neuraminidase activity in mouse organs[a]

Organ	Mouse Strain		
	C57BL/6J	C57BL/6JXSM/J (F_1)	SM/J
Liver	3.37 ± 0.80 (10)[b]	1.62 ± 0.91 (4)[c]	0.56 ± 0.13 (5)[c]
Kidney	7.89 ± 1.93 (4)	7.45 ± 4.39 (2)	6.00 ± 1.44 (5)
Heart	0.37 ± 0.18 (5)	0.42 ± 0.17 (3)	0.35 ± 0.15 (4)
Lung	0.68 ± 0.27 (4)	0.56 ± 0.10 (3)	0.24 ± 0.11 (4)[d]
Brain	0.61 ± 0.06 (3)	–	0.53 ± 0.13 (3)

[a] Neuraminidase activity expressed in μmol 4-methylumbelliferone released. h^{-1} . g $protein^{-1}$

[b] Mean ± SD. Figures in parentheses refer to the number of animals used. Significantly different from the C57BL/6J strain at [c] $P < 0.01$ and [d] $P < 0.05$

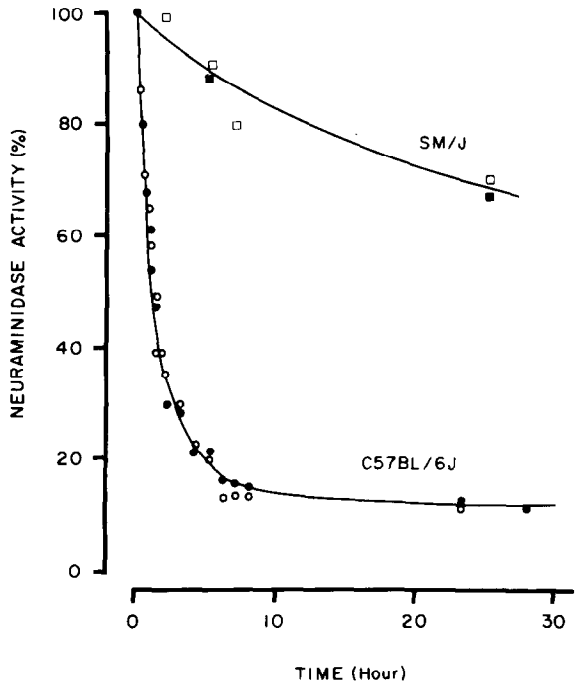

Fig.1. Effect of preincubation time at 0°C on neuraminidase activity in two control C57BL/6J (○,●) and two SM/J mice (□,■). The crude homogenate in water was preincubated for different intervals of time, without substrate, and then assayed at 25°C under standard assay conditions.

The half-life of the B component was similar to that of residual neuraminidase activity in SM/J liver (half-life, 41–44 h). Both B component and SM/J enzyme also showed identical pH optima (3.8), K_m app with sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) as substrate (55–63 μM), and K_i with the competitive inhibitor *N*-acetylneuraminic acid (1.6 mM). The pH optima for total neuraminidase of control liver was 4.4, the K_m app, 28–30 μM and K_i, 0.8 mM.

4. Discussion

We report neuraminidase deficiency in liver of mouse strain SM/J using sodium (4-methylumbelliferyl-α-D-*N*-acetylneuraminate) as substrate (table 1). The other SM/J organs are also affected but to a lesser extent than liver. The neuraminidase deficiency in SM/J strain is unique as indicated by a survey of 21 different mouse strains [10].

Two neuraminidase components (A,B) were identified in C57BL/6J liver on the basis of their different stabilities at 0°C. Available evidence suggests that SM/J liver is deficient in the more labile component A. Residual neuraminidase activity in SM/J liver (17%) approximately corresponded to the liver B content of strain C57BL/6J (15–18%), and both SM/J enzyme and B component showed similar half-life at 0°C (41–44 h) and kinetic properties. It is not known whether a structural gene of neuraminidase or a gene for neuraminidase activity or transformation of B into A form is involved in SM/J. The half-reduced neuraminidase activity in liver of F_1 hybrids (table 1) is consistent with a single structural gene hypothesis but does not eliminate other alternatives. A paper is in preparation where genetic aspects of neuraminidase deficiency will be fully discussed [10].

SM/J liver expresses unique electrophoretic patterns of acid phosphatase [1], α-mannosidase [2] and arylsulfatase B [3] where electronegative, sialylated forms of these enzymes are enhanced as compared to controls. However, there is apparently no gross alteration of sialic acid metabolism in SM/J liver since total sialic acid content was slightly, but not significantly, lower than controls. The observation that the normal electrophoretic patterns can be restored by in vitro treatment with bacterial neuraminidase suggests the possibility that the neuraminidase deficiency is responsible for abnormal electrophoretic patterns of lysosomal enzymes in SM/J liver. Needleman et al. [11] suggested that neuraminidase plays a role in the post-transcriptional modification of lysosomal enzymes by progressive cleavage of terminal sialic acid residues in the lysosomes, or during transfer of lysosomal enzymes to the lysosomes.

It is not clear at present how the biochemical abnormalities in SM/J mice are related to recently described neuraminidase deficiency syndromes in man: mucolipidosis type I [12–14], and the cherry-red spots myoclonus syndrome with [15] and without dementia [16,17]. However, the SM/J mouse can be useful as a model for enzyme therapy of these diseases, and for understanding the role of neuraminidase in the processing post-transcriptional modifications of lysosomal enzymes.

Acknowledgements

We thank Mrs Marie Debau for technical assistance. This work was supported by grants from the Medical Research Council of Canada and The National Foundation-March of Dimes.

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