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MODIFICATIONS OF THE MOLECULAR WEIGHT OF MEMBRANE-BOUND NONSPECIFIC β-GLUCOSIDASE IN TYPE 1 GAUCHER DISEASE DETERMINED IN SITU BY THE RADIATION INACTIVATION METHOD

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The radiation inactivation method has been used to compare the molecular weight of the nonspecific membrane-bound β-glucosidase in situ in normal human spleen and in that of two patients with Gaucher disease type 1. We report, in type 1 Gaucher spleen, the presence of a high molecular weight component (557000) in addition to the normal low molecular weight component (97800). The various possible hypotheses explaining this high molecular weight component are discussed.

Multiple molecular forms of β-glucosidase have been described in human spleen [1–4] and we have recently reported distinctive properties of the three isoenzymatic groups [5]. Glucosylceramide-β-glucosidase (acid β-glucosidase, EC 3.2.1.45) hydrolyses the natural substrate (glucosylceramide) and synthetic substrates (*p*-nitrophenyl-β-D-glucoside, methylumbelliferyl-β-D-glucoside) in the presence of sodium taurocholate [1] and is deficient in Gaucher disease [1, 6–10]. In contrast, the two nonspecific β-glucosidases hydrolyse synthetic substrates but not glucosylceramide, are inhibited by taurocholate and are not deficient in Gaucher disease [1–4, 10–14]. Little is known on the structural modifications of β-glucosidases occurring in Gaucher disease. Recently, Ginns et al. [15] suggested a defect of glucosylceramide-β-glucosidase processing in Gaucher disease and we reported a modification of the apparent molecular weight of the membrane-bound glucosylceramide-β-glucosidase [16] using the radiation inactivation method. Until now, no data are available concerning the molecular structure of the membrane-bound nonspecific β-glucosidase neither in normal subjects nor in Gaucher patients.

The radiation inactivation method allows to determine the molecular weight of membrane-bound enzymes without prior solubilization or purification as previously demonstrated [17–19]. We used this method (which has stood the test of time), in order to compare the structure of the nonspecific membrane-bound β-glucosidase in normal and in Gaucher spleens.

Membrane preparations from two normal adult human spleens and from two patients with non-neuropathic type 1 adult Gaucher disease were prepared as previously reported [16]. Irradiation method was performed on lyophilized membrane preparations at room temperature (26 ± 2°C) in a ^{60}Co irradiator (Gammacell model 220, Atomic Energy of Canada, Ottawa) as described by Beauregard and Potier [19] and Beauregard et al. [20]. Membrane-bound nonspecific β-glucosidase assay using methylumbelliferyl-β-glucoside was

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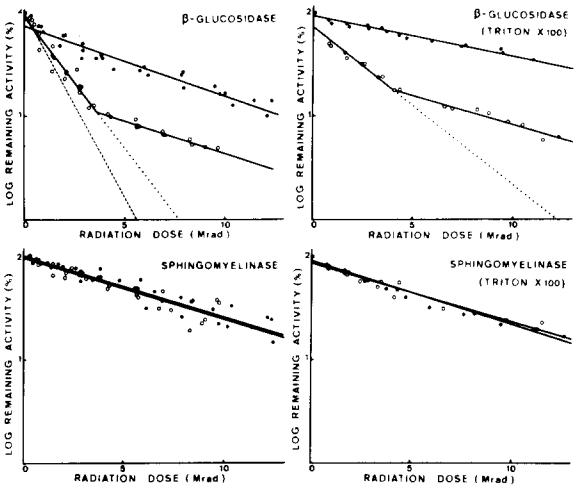

Fig. 1. Radiation inactivation of membrane-bound nonspecific β-glucosidase and acidic sphingomyelinase (control enzyme) in normal (●———●) and Gaucher (○———○) spleens. The fitted lines are derived from a constrained least-square analysis. Each value recorded is the mean of triplicate determination; all values reported in the figure represent the data from four separate experiments (experiments without Triton X-100) or from two experiments (experiments with Triton X-100). Dashed line (------) corresponds to the corrected line obtained by subtraction of interference due to the low component. In contrast, for the molecular weight determination in the presence of Triton X-100, the correction is hindered by a too large interference between the low and the high component as shown by the dotted line (·····). Initial activities of membrane-bound nonspecific β-glucosidase and sphingomyelinase were, respectively, 9.1 ± 1.7 and 8.3 ± 1.5 from normal spleen and 8.3 ± 1.4 and 9.4 ± 2.1 for Gaucher spleen (in nmol/ml per mg protein).

performed as previously described [4,5]. β-*N*-Acetylhexosaminidase was assayed by the method of Okada and O'Brien [21] and sphingomyelinase according to Wenger [22] with the previously reported modifications [23,24].

As shown in Fig. 1, the decay curves for normal and Gaucher membrane-bound nonspecific β-glucosidase were quite distinct. For the normal enzyme one straight line could be plotted and the molecular weight calculated from D_{37} was 97 800 ± 5800 (Table I). For the Gaucher enzyme, the decay curve was composed of two individual exponential decay lines (Fig. 1) suggesting a complex system with two components of different size: a high molecular weight component (557 000 ± 28 000) which was inactivated by exposure to low doses of radiation and a smaller component (101 000 ± 6400) the inactivation of which was apparent only after exposure to the higher radiation doses. This low molecular weight component corresponded to 10–15% of initial activity of the membrane-bound nonspecific β-glucosidase. Similar values were obtained for two different Gaucher spleens (Table I).

Irradiation of samples prepared in Triton X-100 showed a different molecular weight of the membrane-bound nonspecific β-glucosidase from normal and Gaucher spleens (Table I). Triton X-100 is a strong inhibitor of this enzyme [4] but the low concentrations used in these experiments (0.25% in the enzyme preparation and 0.12% in the assay mixture) results in 50% inhibition and thus the residual activity is sufficient for the enzyme activity determinations in the irradiation experiments.

β-*N*-Acetylhexosaminidase (soluble) and acidic sphingomyelinase (membrane-bound) control enzymes had about the same molecular weight in normal and Gaucher spleens (Table I). The decay curves for the membrane-bound control enzyme (Fig. 1) presented only one straight line in either normal and Gaucher spleen. Moreover, as indicated in Table I, Triton X-100 did not disturb the molecular weight of the two control enzymes.

The results, above quoted, showed, in the Gaucher spleen, the presence of a characteristic high molecular weight component of the nonspecific membrane-bound β-glucosidase whereas the low molecular weight component was present in the normal and Gaucher spleens.

The molecular weights of the low molecular weight component (97 800 and 101 000 respectively in normal and in Gaucher spleens) were significantly different from those of glucosylceramide-β-glucosidase (78 000 and 160 000, respectively [16]). The difference cannot be attributed to large interferences of glucosylceramide-β-glucosidase activity in the nonspecific β-glucosidase assay for two reasons: without detergent, the optimum pH of methylumbelliferyl β-glucoside hydrolysis is about 4.0 for glucosylceramide-β-glucosidase [9] but about 5.5–6.0 for the membrane-bound nonspecific β-glucosidase [4] and without activator (taurocholate or Triton X-100) glucosylceramide-β-glucosidase activity on methylumbelliferyl β-glucoside is very low [1,4]. Thus, these enzymes low molecular weight component of membrane-bound

TABLE I

MOLECULAR WEIGHT OF MEMBRANE-BOUND NONSPECIFIC β-GLUCOSIDASE AND CONTROL ENZYMES BY RADIATION INACTIVATION

Triton X-100: no addition (−) or addition (+) (0.25%) of Triton X-100 in the enzymatic preparation. The molecular weight (M_r) was determined using the equation of Kepner and Macey [17] $M_r = (6.4 \cdot 10^5) D_{37}$.

Enzyme	Triton X-100	Normal spleen			Gaucher spleen	
		D_{37} (Mrad)	M_r		D_{37} (Mrad)	M_r
Membrane-bound non-specific β-glucosidase	−	6.55 ± 0.37	97800 ± 5800 [a]	H [b]	1.15 ± 0.06 [a,c]	557000 ± 28000 [a,c]
				L [b]	6.35 ± 0.38 [a]	101000 ± 6400 [a]
	+	10.74	53300	H [b]	2.83, 2.55	226000, 251000
				L [b]	8.3, 6.5	77000 [d], 98000 [d]
N-Acetylhexosaminidase	−	6.44 ± 0.12 [a]	101000 ± 3600 [a]		6.46 ± 0.77 [a]	100000 ± 9900 [a]
	+	6.49	98600		6.9, 6.4	92900, 99900
Sphingomyelinase	−	7.57 ± 1.26 [a]	86000 ± 12000 [a]		7.76 ± 1.06 [a]	90000 ± 14000 [a]
	+	6.75, 7.52	94800, 85000		8.1	78600

[a] Mean ± S.D. of four separate experiments from two different spleens (two from normal subjects and two from patients with type 1 Gaucher disease).

[b] Components of high molecular weight (H) and low molecular weight (L) determined from decay curves.

[c] Corrected D_{37} and molecular weight (non-corrected values: $D_{37} = 1.68 \pm 0.11$ and $M_r = 381000 \pm 25300$; see Fig. 1).

[d] These values are overestimated because no correction can be calculated by the method of correction used in (c).

nonspecific β-glucosidase and glucosylceramide-β-glucosidase) seems to be structurally different. Triton X-100 effect on the normal enzyme suggests a dissociation in a functional target subunit, the molecular weight of which is about half of that without Triton X-100. Such an effect of Triton X-100 on the size of an enzyme has been previously reported by Rosenberry et al. [25]. The experimental values (slightly higher than the expected theoretical values) are in good agreement with recent results of Beauregard and Potier (unpublished data) showing that the apparent target size of membrane-bound enzymes increases by a factor of 1.27 in the presence of Triton X-100. The low molecular weight of the Triton-treated membrane-bound nonspecific β-glucosidase from normal spleen is not an artefact due to the interference of glucosylceramide-β-glucosidase in the membrane-bound nonspecific β-glucosidase assay since it was lower (53300) than that of glucosylceramide-β-glucosidase (74500) [16]. Thus a homodimeric structure for the normal membrane-bound nonspecific β-glucosidase seems to be quite possible and cannot be confused with the structure of glucosylceramide-β-glucosidase, first because the latter is not dissociated by Triton X-100 and second, because the molecular weight of these enzymes are different [16].

The high molecular weight component present in type 1 Gaucher spleen does not seem to be an artefact due to a general modification of the Gaucher membranes (i.e. accumulation of glucosylceramide) inducing an apparent increase of the molecular weight determined by radiation inactivation method, since sphingomyelinase is not altered in Gaucher spleens. On the other hand, a specific association between the enzyme and the substrate molecules (in this case, the difference of molecular weight corresponds to about 500 substrate molecules) seems to be improbable, because, generally, such interactions lipids-proteins exist for the membrane-bound enzymes without disturbance of molecular weight determined by the radiation inactivation method [26]. The existence of this high molecular weight component could be explained by several other hypotheses: polymerisation of the normal low molecular weight component enzyme, or binding of the normal enzyme with one (or more) high molecular weight proteins, or defect in its post-translational processing, or increase (perhaps by induction) of another β-glucosidase molecular form which is minor in normal

spleen. These hypotheses imply that the observed modification of the membrane-bound non-specific β-glucosidase is a secondary effect of the Gaucher mutation. In contrast, another hypothesis (involving a more direct consequence of the Gaucher mutation) could be the polymerisation of the muted glucosylceramide-β-glucosidase. However, this hypothesis assumes, at least, two aggregation states of the muted glucosylceramide-β-glucosidase, this first one corresponding to the dimer (125 000) previously reported [16], keeping a residual activity on the natural substrate (glucosylceramide) and on the synthetic one (methylumbelliferyl-β-glucoside in assay conditions of glucosylceramide-β-glucosidase), the second one corresponding to the polymer (high molecular weight component) reported here, completely inactive in assay conditions of glucosylceramide-β-glucosidase but still active on the synthetic substrate in the assay for nonspecific β-glucosidase.

We do not know if the molecular weight modification reported for spleens from two patients with type 1 non-neuropathic Gaucher disease is a general feature of this disease, including infantile and juvenile types, or is a general characteristic of the adult form or constitutes a new subtype group. The right hypothesis could be determined by molecular weight studies of more numerous cases in laboratories having organs from Gaucher patients with different types. At last the physiological function of this membrane-bound nonspecific β-glucosidase is still unknown as well as the pathophysiological significance of its molecular weight modification coinciding with that of glucosylceramide-β-glucosidase in Gaucher disease.

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