Modification of subunit interaction in membrane-bound acid β-glucosidase from Gaucher disease

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The radiation inactivation method has been used to determine the molecular mass of membrane-bound acid β-glucosidase (EC 3.2.1.21) in situ, in normal human spleen and in that of two patients with type I Gaucher disease: the molecular mass in Gaucher spleen is about double (125 000 ± 8900) of that found in the normal spleen (67 000 ± 7700) which is compatible with the existence of subunit coupling in the muted acid β-glucosidase. From the results, we conclude that subunit interaction is altered in mutant acid β-glucosidase and that this may be due to a direct effect of the mutation.

Radiation inactivation method *Membrane-bound acid β-glucosidase* *Gaucher disease*

1. INTRODUCTION

Gaucher disease is the most common inherited disorder that primarily affects Ashkenazi Jewish people [1]. The genetic defect in this disease consists in a deficiency of a membrane-bound lysosomal acid β-glucosidase which catalyzes glucocerebroside hydrolysis. This enzyme has been purified and characterized from human placenta and spleen after solubilization by detergent [2–6]. It appears to be a tetramer composed of identical subunits of molecular mass between 57 000 and 67 000 by SDS–polyacrylamide gel electrophoresis [2,5–7]. Similar subunit M_r-values were obtained for purified acid β-glucosidase of normal (66 000) and Gaucher spleen (57 000) [5]. However, the physical state of the enzyme in the membrane, and the effect of the Gaucher mutation on subunit interaction are unknown.

The radiation inactivation method allows us to determine in situ molecular mass of membrane-bound enzymes without prior solubilization or purification [8,9]. We have used this method to determine the M_r of membrane-bound acid β-glucosidase, and to probe subunit interaction in normal human spleen and in that of patients affected with type I Gaucher disease.

Abbreviations: NBD, 12-[*N*-methyl-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)] aminododecanoic acid; MU, 4-methylumbelliferyl

2. MATERIALS AND METHODS

Homogenates from two normal spleens and two type I Gaucher patients (0.15 g tissue/ml) were prepared in deionized water using an Ultraturax homogenizer (at 4°C) and centrifuged at 220 000 × *g* for 1 h. The membrane fractions were resuspended in the initial volume of water and used for the irradiation experiments. In some experiments, 0.25% (v/v) Triton X 100 was added to the membrane fractions to solubilize the acid β-glucosidase activity [10].

Aliquots (1 ml) of the membrane preparations were lyophilized in 1.5 ml Eppendorf microfuge tubes. The tubes were flushed with nitrogen and irradiated at room temperature (26 ± 2°C) in a ^{60}Co irradiator (Gammacell model 220, Atomic Energy of Canada, Ottawa). The irradiator was

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calibrated with enzymes of known radiation sensitivities as in [11]. The tubes were placed in a special support designed to provide isodose exposure at about 0.1 Mrad/h [12]. Three tubes were irradiated for each dose and control non-irradiated tubes were run concurrently. The logarithm of remaining enzyme activity was plotted *vs* radiation dose and a straight line was fitted through the points using the least-squares method. The molecular mass was determined using a modification of the equation in [9]:

$$M_r = (1.48 \times 10^6)\, \mu_D,$$

where μ_D is the enzyme activity decay coefficient corresponding to the slope of the least-squares line.

Acid β-glucosidase activity was assayed with the MU-β-glucoside substrate in the presence of Triton X-100 and taurocholate [10,13,14] to inhibit non-specific β-glucosidase, with the NBD–glucosylceramide substrate as in [15] and with the [^{3}H]-glucosylceramide substrate as in [10]. *N*-Acetylhexosaminidase was assayed as in [16] and sphingomyelinase as in [17].

3. RESULTS

The radiation inactivation of membrane-bound acid β-glucosidase, assayed with both MU-β-glucoside and NBD–glucosylceramide as substrates is shown in fig.1 for normal and Gaucher spleens. Corresponding M_r values for acid β-glucosidase and control enzymes, *N*-acetylhexosaminidase and sphingomyelinase, are given in table 1. The majority of residual acid β-glucosidase activity in the Gaucher spleen (about 17% of normal activity) corresponds to a molecule about 2-times larger in size than that in the normal spleen. It is also possible that the Gaucher spleen contains one or more higher M_r aggregates of the enzyme since, as shown in fig.1, a rapid loss of acid β-glucosidase activity was observed at low radiation doses. However, the molecular mass of these acid β-glucosidase components cannot be determined with precision under our experimental conditions since only a relatively small amount is present in the Gaucher spleen. Because of these difficulties, we used the slope of the least-squares lines (μ_D) depicted in fig.1 (the enzyme activity decay as a function of radiation

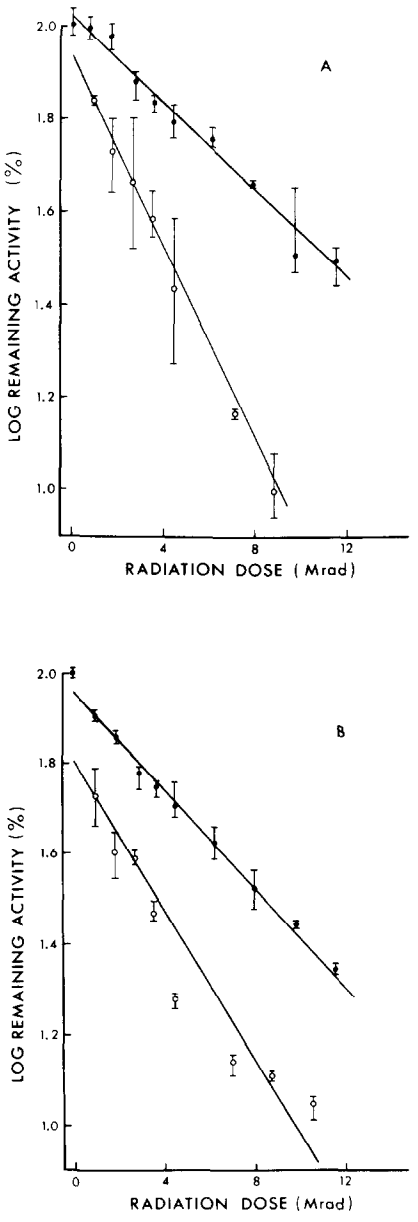


Fig.1. Radiation inactivation of acid β-glucosidase in normal (●) and Gaucher (○) spleens as determined with NBD–glucosylceramide (A) and 4-MU-β-glucoside (B) as substrates. Initial activities of acid β-glucosidase were as follows: with the NBD–glucosylceramide substrate, 420 nmol.h^{-1}.g tissue^{-1} for normal spleen, and 59 nmol^{-1}.h^{-1}.g tissue^{-1} $^{-1}$ for the Gaucher spleen; with the 4-MU-β-glucoside substrate, 1300 nmol.h^{-1}.g tissue^{-1} for normal spleen and 250 nmol^{-1}.h^{-1}.g tissue^{-1} for the Gaucher spleen. Typical experiments are shown.

Table 1

M_r of membrane-bound acid β-glucosidase and control enzymes by radiation inactivation

Enzyme		Normal spleen		Gaucher spleen	
		μ_D (Mrad^{-1})	M_r	μ_D (Mrad^{-1})	M_r
Acid β-glucosidase					
MU-β-glucoside	MB[b]	0.052 ± 0.005[a]	78 000 ± 6800	0.106 ± 0.015	160 000 ± 23 000
	S[b]	0.048, 0.052	72 000 ± 77 000	0.078, 0.081	115 000, 120 000
NBD-glucosylceramide	MB	0.045 ± 0.005	67 000 ± 7700	0.084 ± 0.006	125 000 ± 8900
	S	0.037, 0.043	64 000, 55 000	0.078, 0.104	116 000, 154 000
N-Acetylhexosaminidase		0.068 ± 0.002	101 000 ± 3600	0.067 ± 0.007	100 000 ± 9900
Sphingomyelinase		0.057 ± 0.008	86 000 ± 12 000	0.059 ± 0.009	90 000 ± 14 000

[a] Mean ± SD of 4 different experiments from 2 normal and 2 type I Gaucher spleens
[b] MB, membrane-bound; S, solubilized enzyme in presence of 0.25% (v/v) Triton X-100

dose) to calculate molecular mass according to a modified equation in [9] (see section 2).

The radioactive [^{3}H]glucosylceramide substrate was also used to assay the acid β-glucosidase activity in irradiated normal spleen preparations. We report a M_r similar to that found with the NBD-glucosylceramide (~60 000). However, this substrate was not sensitive enough to detect the low acid β-glucosidase activities obtained after radiation inactivation of the Gaucher spleen enzyme (not shown).

Consistently higher M_r-values were found for acid β-glucosidase assayed with the MU-β-glucosidase than with the NBD-glucosylceramide substrate (table 1); although the difference was not statistically significant (Student's t-test, $p > 0.05$, $N = 4$). It must be pointed out that the latter substrate is more specific to acid β-glucosidase whereas the former is also hydrolyzed by a non-specific membrane-bound spleen β-glucosidase which has a M_r of ~100 000 by radiation inactivation (unpublished data). Although this non-specific β-glucosidase is largely inhibited by the addition of taurocholate and Triton X-100 to the assay medium it could still interfere with the assay of acid β-glucosidase using the MU-β-glucoside substrate [10,13,14].

brane by 0.25% (v/v) Triton X-100, prior to irradiation, did not alter the sizes of the molecules in both normal and Gaucher spleens, respectively (table 1).

The control enzymes *N*-acetylhexosaminidase (soluble) and sphingomyelinase (membrane-bound) had about the same M_r in the normal and Gaucher spleens (table 1). The M_r of spleen *N*-acetylhexosaminidase corresponded to that of the oligometric form of the enzyme as reported by other authors in human tissues (100 000, [18]). With the spleen sphingomyelinase, we report a M_r-value slightly higher than that of the subunit of the purified human brain (70 000, [19]) and placentral (70 500, [20]) enzymes.

4. DISCUSSION

The subunit M_r of purified acid β-glucosidase, as determined by SDS–polyacrylamide gel electrophoresis, was reported to be around 60 000 in the human placenta [2], 66 000 in the normal human spleen and 57 000 in the Gaucher spleen [5]. In normal cultured skin fibroblasts 3 different polypeptides with M_r-values 63 000, 61 000 and 56 000 reacting with an antibody prepared against purified placental acid β-glucosidase were reported [7]. They reported that in type I Gaucher fibroblasts, only the 56 000 M_r form was present. With the radiation inactivation method, we report for the normal spleen acid β-glucosidase a M_r corresponding about, considering an experimental error of 10–15% [8], to that of the enzyme subunit as determined by other authors using SDS–polyacrylamide gel electrophoresis [2,5–7]. Since the size of

the subunit of residual acid β-glucosidase in spleen [5] and cultured skin fibroblasts [7] of type I Gaucher patients is only slightly different from that of the normal enzyme, the results of the radiation inactivation experiment suggest that two subunits are coupled in the mutant enzyme. However, the nature of the subunits involved, with respect to the different types identified in cultured skin fibroblasts in [7], cannot be specified due to the experimental error in M_r determination.

Coupled subunits have also been found for spleen *N*-acetylhexosaminidase (the M_r corresponds to that of the oligomeric form of the enzyme [18]) but not for sphingomyelinase (table 1), has been suggested [8] that the radiation inactivation method is sensitive to the size of the functional unit of a molecule (the minimal structural assembly necessary for the expression of a given biological function) or reflects the degree of coupling between subunits which would be responsible for energy transfer after a direct hit. A distinct possibility is that subunit interaction may be required for the expression of biological activity [8]. The destruction of one subunit in an oligomer may be responsible for the loss of activity in all associated subunits. This interaction may be necessary to maintain the conformation of the active site or of another enzyme binding site implicated in the activation or stabilization of the enzyme. Although we cannot choose between these possibilities at the present time, the results of the radiation inactivation of normal and Gaucher spleen acid β-glucosidase clearly involve subunit interaction in the mutant enzyme.

The larger M_r obtained for the Gaucher acid β-glucosidase does not seem to be due to a generalized membrane defect or the accumulation of lipids in the Gaucher spleen. The control enzymes, *N*-acetylhexosaminidase and sphingomyelinase, are not altered in the Gaucher spleen (table 1). In addition, Triton X 100-solubilized acid β-glucosidase had a similar M_r-value to the found in the intact membranes from normal and Gaucher spleens, respectively (table 1).

In conclusion, we report that the acid β-glucosidase in Gaucher spleen has a different structure as determined by the radiation inactivation method. It appears that subunit interaction (or coupling) is present in the Gaucher acid β-glucosidase so that energy transfer occurs when one of the subunits is hit by an ionizing radiation or that subunit interaction is necessary for the expression of residual enzyme activity. The radiation inactivation reveals a detail of the mutant enzyme structure, not detected by other methods, which could represent a direct effect of the type I Gaucher mutation on the enzyme.

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REFERENCES

[1] Desnick, R.J. (1982) Prog. Clin. Biol. Res. 95, 1–30.
[2] Pentchev, P.G., Brady, R.O., Hibbert, S.R., Gal, A.E. and Shapiro, D. (1973) J. Biol. Chem. 248, 5256–5261.
[3] Braidman, I.P. and Gregoriadis, G. (1977) Biochem. J. 164, 439–445.
[4] Dale, G.L. and Beutler, E. (1976) Proc. Natl. Acad. Sci. USA 73, 4672–4674.
[5] Pentchev, P.G., Brady, R.O., Blair, H.E., Britton, D.E. and Sorrell, S.H. (1978) Proc. Natl. Acad. Sci. USA 75, 3970–3973.
[6] Furbish, F.S., Blair, H.E., Shiloach, J., Pentchev, P.G. and Brady, R.O. (1977) Proc. Natl. Acad. Sci. USA 74, 3560–3563.
[7] Ginns, E.I., Brady, R.O., Pirrucello, S., Moore, C., Sorrell, S., Furbish, F.S., Murray, G.J., Tager, J. and Barranger, J.A. (1982) Proc. Natl. Acad. Sci. USA 79, 5607–5610.
[8] Kempner, E.S. and Schlegel, W. (1979) Anal. Biochem. 92, 2–10.
[9] Kepner, G.R. and Macey, R.I. (1968) Biochim. Biophys. Acta 163, 188–203.
[10] Maret, A., Salvayre, R., Negre, A. and Douste-Blazy, L. (1981) Eur. J. Biochem. 115, 455–461.
[11] Beauregard, G. and Potier, M. (1982) Anal. Biochem. 122, 379–384.
[12] Beauregard, G., Giroux, S. and Potier, M. (1983) Anal. Biochem. in press.
[13] Wenger, D.A. (1978) Adv. Exp. Med. Biol. 101, 707–717.

[14] Peters, S.P., Coyle, P. and Glew, R.H. (1976) Arch. Biochem. Biophys. 175, 569–582.
[15] Gatt, S., Dinur, T., Barenholz, Y., Leibovitz-Ben Gershon, Z., Rosenthal, J. Devine, E.A., Shafit-Zagardo, B. and Desnick, R.J. (1981) Methods in Enzymol. 72, 367–372.
[16] Okada, S. and O'Brien, J.S. (1969) Science 165, 698–700.
[17] Wenger, D.A. (1977) in: Practical Enzymology of the Sphingolipidoses (Glew, R.H. and Peters, S.P. eds) pp. 39–70, Alan R. Liss, New York.
[18] Geiger, B. and Arnon, R. (1976) Biochemistry 15, 3484–3493.
[19] Yamanaka, T. and Suzuki, K. (1982) J. Neurochem. 38, 1753–1764.
[20] Sakuragawa, N. (1982) J. Biochem. (Tokyo) 92, 637–646.