Isoenzymes of Membrane-Bound β -Glucosidase of Human Spleen

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Normal human spleen contains two forms of membrane-bound β -glucosidase, distinguishable by their thermostability and kinetic properties. The spleen from a patient with adult Gaucher's disease was deficient in the major, thermolabile, form of the enzyme.

Glucocerebrosidase of human tissue is a membrane-bound enzyme that catalyses the hydrolysis of the artificial substrate, 4-methylumbelliferyl β -D-glucoside, and of the natural substrate, glucocerebroside (Ho, 1973). Its deficiency results in the lysosomal storage disorder Gaucher's disease (Brady *et al.*, 1965). Several variants of the disease are distinguished on the basis of clinical severity, age of onset and neuronal involvement: type 1 (adult form), type 2 (infantile form) and type 3 (juvenile form) (Frederickson & Sloan, 1972). In addition, enzyme assays (with the 4-methylumbelliferyl substrate) of β -glucosidase in Gaucher's-disease tissue do not always reveal the expected deficiency (see, e.g., Butterworth & Broadhead, 1978). The biochemical basis for this clinical, enzymic and genetic heterogeneity is still obscure. Although multiple forms of many human glycosidases have been documented (Robinson, 1978), such polymorphism has not been described for membrane-bound β -glucosidase, with the possible exception of the enzyme from cultured cells (Turner *et al.*, 1977). We now report that human spleen contains two clearly distinct forms of membrane-bound β -glucosidase, of which the major form is deficient in adult Gaucher's disease.

Materials and Methods

Post-mortem samples of human tissue were stored at -20°C . We are grateful to Dr. D. Patrick for a sample of spleen from a patient with adult Gaucher's disease. Tissue homogenates (20%, w/v) were prepared in water at 4°C with a Teflon/glass homogenizer and centrifuged (at $500g_{\text{av}}$ for 10 min at 4°C) to remove debris. The resulting supernatant was further centrifuged (at $25\,000g_{\text{av}}$ for 30 min at 4°C), and the pellet (referred to below as 'crude membrane fraction') was washed with water, recovered by re-centrifuging (at $25\,000g_{\text{av}}$ for 30 min

at 4°C), and suspended in water (5 ml/g of tissue). Membrane-bound β -glucosidase was assayed with 4-methylumbelliferyl β -D-glucoside (Sigma Chemical Co., Poole, Dorset, U.K.) by incubating substrate (1 mM in 0.1 M-sodium acetate buffer, pH 5.8) (0.4 ml) and enzyme (0.1 ml) for 30 min at 37°C . The reaction was stopped with 0.4 M-glycine/NaOH buffer, pH 10.4 (2.0 ml), and the fluorescence of the liberated aglycone was measured in an MSE Spectro-Plus instrument (MSE Scientific Instruments, Crawley, Sussex, U.K.) with an excitation wavelength of 365 nm and a cut-off filter of 440 nm. Glucocerebrosidase was assayed essentially as described by Dale *et al.* (1976), but the incubation mixture contained 0.3 mM-glucocerebroside in 0.2 M-sodium citrate buffer, pH 6.0, containing 0.9% sodium taurocholate (grade A; Calbiochem, Bishops Cleeve, Herts., U.K.) (0.3 ml) and enzyme (0.1 ml). For thermostability studies, the membrane fraction (protein concentration 2.7 mg/ml) suspended in water was heated at 52.5°C , and the residual activity was compared with that of a sample kept on ice.

Results and Discussion

The membrane-bound β -glucosidase activity of the Gaucher's-disease spleen, 4.2 nmol/h per mg of protein, was 18.4% that of the normal spleen (22.8 nmol/h per mg of protein), when assayed with the 4-methylumbelliferyl substrate. The pH optimum of the residual Gaucher's-disease enzyme was 5.1, whereas that of the normal enzyme was 5.8. The activity of the soluble β -glucosidase in the normal human spleen, 25% of the enzyme activity in the total homogenate, was identical with that in the Gaucher's-disease spleen (7.0 nmol/h per mg of protein). The results presented below relate only to membrane-bound enzyme.

When the crude membrane fraction from normal

spleen was heated at 52.5°C, β -glucosidase activity was rapidly lost (Fig. 1). After 3 h the remaining thermostable enzyme represented 20–25% of the initial activity; the exact proportion is difficult to measure, as the pH optimum of the thermostable enzyme was 5.0, compared with 5.8 for the total normal activity. The membrane-bound β -glucosidase of Gaucher's-disease spleen was stable at 52.5°C (Fig. 1). Similar curves to that for normal spleen enzyme were obtained with crude membrane fractions from other normal human tissues; the proportion of thermostable activity in each was: brain, 10%; kidney, 5%; liver, 5%.

The heat-stable β -glucosidase of normal spleen had a K_m value of 2.35 mM for the 4-methylumbelliferyl substrate, similar to that of the residual enzyme of Gaucher's-disease spleen (2.56 mM); the corresponding K_m value for the total

activity of normal spleen enzyme was 0.86 mM. The thermostable enzyme and the residual activity of Gaucher's-disease spleen enzyme were both inhibited by 1 mM-glucose (by 10–20%) and by 0.15 M-NaCl (by 20%), whereas the total normal enzyme was activated by each of these (by 30 and 10% respectively); 1 mM-mercaptoethanol inhibited the thermostable enzyme and the residual activity of Gaucher's-disease spleen enzyme (by 20%), but had no effect on the total normal enzyme. The glucocerebrosidase activity of the crude membrane fraction of normal spleen was 19 nmol/h per mg of protein. There was no detectable activity (<1.0 nmol/h per mg of protein) in the heat-stable membrane preparation from normal spleen, nor in the crude membrane fraction of Gaucher's-disease spleen.

Our results indicate that there are two forms of membrane-bound β -glucosidase in normal human tissues. They can be distinguished on the basis of several enzymic and physicochemical properties, which are summarized in Table 1. This is the first well-documented report of isoenzymes of membrane-bound human β -glucosidase; forms distinguishable by the effect of NaCl (Broadhead & Butterworth, 1977) or of treatment at pH 4.0 (Turner *et al.*, 1977) have been described, but they were not characterized with respect to substrate specificity.

The residual membrane-bound β -glucosidase activity of the Gaucher's-disease spleen was 18% (assayed with 4-methylumbelliferyl β -D-glucoside) or <5% (assayed with glucocerebroside) compared with that of the normal spleen. On the basis of physicochemical and kinetic studies of solubilized β -glucosidase from normal and Gaucher's-disease spleen, several groups have proposed that the genetic lesion in adult Gaucher's disease is a structural mutation (Pentchev *et al.*, 1978; Mueller & Rosenberg, 1979). The reason for the observed differences in the properties of the normal and the Gaucher's-disease enzyme may now be clear. The residual

Fig. 1. Heat-inactivation of membrane-bound β -glucosidase

The crude membrane fraction from normal human spleen (●) or Gaucher's-disease spleen (○) was heated at 52.5°C, and the remaining activity was assayed with 4-methylumbelliferyl β -D-glucoside at pH 5.8. Further details are given in the Materials and Methods section.

Table 1. Comparison of the two forms of membrane-bound β -glucosidase in normal human spleen
The substrate was 4-methylumbelliferyl β -D-glucoside, unless otherwise stated. For experimental details see the text.

Property	Major form	Minor form
Proportion of total activity assayed at pH 5.8 (%)	80	20
Stability at 52.5°C	Labile	Stable
pH optimum	5.8*	5.0
K_m (mM)	0.86*	2.35
Effect of glucose (1 mM)	Activation*	Inhibition
Effect of NaCl (0.15 M)	Activation*	Inhibition
Effect of mercaptoethanol (1 mM)	None*	Inhibition
Glucocerebrosidase activity (nmol/h per mg of protein)	19	<1.0

* These values were obtained with a crude membrane fraction comprising approximately 80% thermolabile and 20% thermostable form.

activity in the disease state (in the one case that we have studied) is the minor, thermostable, form that has no glucocerebrosidase activity, and that has structural and enzymic properties distinct from those of the major, thermolabile, enzyme. Thus the precise nature of the genetic lesion in adult Gaucher's disease cannot be stated with certainty.

The biological function of the minor membrane-bound β -glucosidase remains obscure. It might be identical with the microsomal β -glucosidase involved in glycoprotein biosynthesis (Hubbard & Robbins, 1979), or with a steroid β -glucosidase (Williamson *et al.*, 1977). Further work is required to establish whether there is any structural or genetic relationship between the two forms of membrane-bound β -glucosidase, and between these forms and the soluble β -glucosidase.

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