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Properties of β-Glucosidase in Cultured Skin Fibroblasts from Controls and Patients with Gaucher Disease

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INTRODUCTION

The clinical symptoms of Gaucher disease, like those of several other inborn errors of metabolism, show considerable variation in severity from one patient to another. Three forms of the disease (infantile, juvenile, and adult or chronic) have been distinguished on the basis of clinical parameters and age of onset [1, 2]. The infantile form is characterized by hepatosplenomegaly and neurologic abnormalities during the first year of life [1]; among juvenile patients, neurologic signs appear later [2]. Patients with the chronic form suffer no neurologic symptoms, although hepatosplenomegaly, anemia, and bone abnormalities are usually observed. The severity of these symptoms and the consequent degree of physical impairment vary widely from one chronic patient to another [3]. Chronic Gaucher disease, like infantile Niemann-Pick disease and Tay-Sachs disease (two other sphingolipidoses), is more common among Ashkenazi Jews than in any other ethnic group [4].

Five patients have been described [5] in a group of interrelated black American families who, on the basis of clinical parameters, do not fall into any of the three groups described above. Three black patients, who appear to have a similar, rapidly progressing form of the disease, were studied in the course of the work described in this report. They have been designated 'juvenile variant' patients.

Despite this clinical heterogeneity, the basic metabolic defect appears to be the same in all Gaucher patients. Accumulation of glucocerebroside, primarily in cells of the reticuloendothelial system, occurs as a result of deficiency of the lysosomal hydrolase glucocerebrosidase [6, 7], an enzyme which catalyzes the hydrolytic cleavage of glucocerebroside into ceramide and glucose. This enzyme also has a general β-glucosidase activity and can cleave artificial substrates [8, 9]. Deficiency of β-glucosidase activity has been demonstrated in a variety of cells and tissues from Gaucher patients [7, 10–13], and subnormal activity has been found in leukocytes and cultured skin fibroblasts from obligate heterozygotes [10–12, 14].

Little is known about the possible biochemical or genetic basis for the clinical

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variation among Gaucher patients. It has been suggested that the neurologic involvement and rapid progression in infantile patients result from a more complete attenuation of glucocerebrosidase activity than that observed in chronic patients [15] or from the inability of infantile patients to catabolize the steroid glycoside deoxycorticosterone-β-glucoside [16].

To distinguish the different clinical forms of Gaucher disease at the enzyme level and to investigate possible causes of the observed clinical heterogeneity, we examined a number of properties of the residual β-glucosidase activity in patients with various forms of the disease. Because skin fibroblasts from patients and controls can be cultured under the same closely defined conditions, thereby minimizing the possibility of individual differences occurring as a secondary consequence of the disease process, they are a particularly useful source of the enzyme for comparative studies of the type described in this report.

METHODS

Patients and Fibroblast Cultures

Nine chronic Gaucher patients—M. M., M. K., J. C., M. F., E. F., J. P., L. J., L. Z., and P. N.—were seen at Mount Sinai Hospital. All patients were Ashkenazi Jews except J. C. who was of Italian descent, although pedigree analysis raised the possibility of Jewish ancestry. Diagnosis was made initially on the basis of clinical parameters and bone marrow biopsy. The severity of the clinical symptoms was generally related to the age of onset. Hepatosplenomegaly, anemia, and bone pain were most pronounced in those patients with the earliest age of onset. None of these patients showed any signs of neurological involvement. Skin biopsies were taken from the inner forearm, and fibroblast cultures were established (see below). Age at biopsy ranged from 9 years (J. C.) to approximately 50 years (P. N.).

Fibroblast cultures from patients A. T. and C. T. were obtained from Dr. Peter Mamunes of the Medical College of Virginia, Virginia Commonwealth University. The patients, brothers from an American black family, were found to have marked hepatosplenomegaly and Gaucher cells in the bone marrow at 1–2 years of age. Skin biopsies were taken at this time. The patients are now 3-(C. T.) and 2-years-old (A. T.) and show no neurological abnormalities. Fibroblasts from patient B. H. were obtained from Dr. William S. Sly, Washington University School of Medicine. This black patient had an accelerated course ending in liver disease and myelofibrosis with death in the sixth year of life. A skin biopsy was taken at age 5. Fibroblast cultures GM 852 (chronic Gaucher, Ashkenazi Jewish, biopsy at 20 years) and GM 877 (classical infantile Gaucher, white non-Jewish, biopsy at 1 year) were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey.

All fibroblast cultures were grown in RPMI 1640 medium supplemented with 15% fetal calf serum, glutamine, and antibiotics as previously described [17]. Cells were harvested by sequential exposure to trypsin and ethylenediaminetetraacetic acid at early confluence and approximately 24 hr after a change of medium [17]. Cultures were maintained by splitting 1 to 4 at intervals of about 1 week. All experiments were carried out with cells harvested between the 3d and 10th passage. Periodic checks for mycoplasma contamination of cell cultures maintained in this laboratory were negative.

Solubilization of Fibroblast β-Glucosidase

Fibroblast β-glucosidase is predominantly membrane bound and can be solubilized by various detergents. Good recovery of activity is obtained with a mixture of Triton X-100 (Sigma, St. Louis, Mo.) and crude sodium taurocholate (British Drug Houses, Poole, England). The following procedure was used for experiments described in this report. The cell pellet from one 75 cm^2 flask was suspended in 0.3 ml distilled water in a glass tube and frozen/thawed five times

in a dry ice/acetone bath and a 37°C water bath. The extract was adjusted to 1 ml with water, transferred to a 1 ml conical glass centrifuge tube, and spun for 15 min at 30,000 *g* and at 4°C. The supernatant was removed by aspiration and discarded. The pellet was resuspended in 1 ml 0.06% (v:v) Triton X-100, 0.2% (w:v) crude sodium taurocholate in distilled water and incubated at room temperature for 15 min. The sample was then centrifuged at 30,000 *g* at 4°C for 30 min. The supernatant, containing the solubilized β-glucosidase, was removed and stored on ice. All experiments were carried out on the day of preparation of the enzyme, although the sample could be stored overnight at 4°C with little loss of activity.

A slightly different preparation procedure was used for the estimation of specific enzyme activity and K_m values. The cells were lysed by freeze/thawing as described, and a 0.2 ml aliquot was removed and mixed with 0.7 ml distilled water and 0.1 ml 0.6% Triton X-100, 2% crude sodium taurocholate. The suspension was incubated for 15 min at room temperature, centrifuged for 30 min at 30,000 *g*, and the supernatant removed and assayed. The protein concentration of the frozen/thawed cell extract, prior to the addition of detergents, was determined by the method of Lowry et al. [18]. Specific activity was calculated as: (enzyme activity per ml solubilized preparation × 5)/mg protein per ml cell extract. The measurement of protein in the original cell extract, rather than in the detergent-containing cell supernatant, was found to give more reproducible specific activity values. This method for calculating specific activity allows a comparison to be made between the specific activity of the detergent-solubilized enzyme and that of the membrane-bound enzyme, assayed as described previously [19].

β-Glucosidase Assay

β-Glucosidase activity was assayed with 4-methylumbelliferyl-β-D-glucopyranoside (MUGlu, Koch-Light Labs, Colnbrook, England). The assay mixture was made up as follows: 5 mM MUGlu in water, 60 μl; 0.2 M sodium phosphate–citric acid buffer, pH 6.0, 30 μl; 0.12% Triton X-100, 2% crude sodium taurocholate, 10 μl; enzyme preparation, 20 μl. The assay mixture was incubated at 37°C for 30 min (controls) or 120 min (Gaucher cells). The reaction was terminated by addition of 4 ml 0.085 M glycine-carbonate buffer, pH 10.0. The fluorescent 4-methylumbelliferone released during the assay was quantitated in a Beckman model 772 ratio fluorometer against 4-methylumbelliferone standards. Crude sodium taurocholate caused a small increase in background fluorescence and thus all assay blanks contained this material in an identical concentration to that in the reaction tubes. No significant variation was observed among different batches of crude taurocholate (British Drug Houses), either in background fluorescence or in the degree of stimulation of enzyme activity.

The reaction progressed linearly for at least 120 min with enzyme from both control and Gaucher fibroblasts. For pH curve determinations, the pH of the phosphate-citrate buffer was varied from 4.5 to 7.0 by mixing 0.2 M sodium phosphate and 0.1 M citric acid to the required pH.

Reaction mixtures for inhibition experiments contained: 5 mM MUGlu in water containing 10 mg/ml crude sodium taurocholate, 30 μl; 0.2 M sodium phosphate–citric acid buffer, pH 6.0, 20 μl; inhibitor solution or buffer, 75 μl; enzyme preparation, 25 μl. The following inhibitor solutions were used: deoxycorticosterone-β-glucoside (Sigma), 1 mM in 0.05 M sodium phosphate–citric acid buffer, pH 6.0, containing 0.06% Triton X-100; and deoxycorticosterone (Sigma), 1 mM in buffer as above.

Heat Inactivation

The buffer solution for heating experiments was made up as follows: 0.2 M sodium phosphate–citric acid, pH 6.0, 5 vols; distilled water, 4 vols; 0.6% Triton X-100, and 2% crude sodium taurocholate, 1 vol. Crystalline bovine serum albumin (Armour Pharmaceutical, Kankakee, Ill.) was added to a final concentration of 2 mg/ml. Equal volumes of this buffer solution and solubilized fibroblast β-glucosidase were mixed and 110 μl aliquots dispensed into glass tubes. The tubes were sealed and heated in a 50°C water bath for up to 30 min. Heating was terminated by placing the tubes on ice. β-glucosidase activity was then assayed as described above with the exception that 50 μl aliquots of the enzyme were used.

Density Gradient Centrifugation

Four ml linear gradients were prepared by mixing 5% and 20% (w:w) sucrose solutions made up in 0.05 M sodium phosphate–citric acid buffer, pH 6.0 and containing 0.05% Triton X-100. Aliquots (0.1 ml) of solubilized fibroblast β-glucosidase were layered onto the gradients which were then centrifuged for 19 hr at 10°C and 40,000 rpm in a Beckman SW-56 swingout rotor (final $\omega^2 t = 1.15 \times 10^{12}$ rad^2 sec^{-1}). Fractions of 0.2 ml were taken and assayed for β-glucosidase activity.

RESULTS

Specific Activity

The specific activity of solubilized β-glucosidase in Gaucher fibroblasts ranged from 6.6% to 16.5% of control values in the chronic patients (group 1, table 1) and from 4.1% to 5.8% in the juvenile variant and infantile patients (groups 2 and 3, table 1). The difference in the specific activities of the enzyme in these two patient populations is significant ($P < .001$). Among patients in group 1 (chronic form), a weak correlation

TABLE 1

PROPERTIES OF DETERGENT SOLUBILIZED β-GLUCOSIDASE IN CULTURED SKIN FIBROBLASTS FROM GAUCHER PATIENTS AND CONTROLS

PATIENTS	SEX AND AGE OF ONSET (Yrs.)	SPECIFIC ACTIVITY (pmol/min/mg)	HALF-LIFE, 50°C (min)	pH RATIO	INHIBITION (%) DC*	INHIBITION (%) DC-β-glu†
Chronic Form (Group 1):						
M.K.	F, 4	113 (2)	7.4 (3)	1.6	n.t.‡	n.t.
M.M.	F, 5	172 (2)	7.0 (2)	1.5	n.t.	n.t.
J.C.	M, 5	161 (2)	5.6 (3)	1.7	21.2 (2)	26.2 (3)
M.F.	M, 16	187 (3)	8.8 (4)	1.8	10.3	13.8 (2)
E.F.	F, 17	129 (2)	9.3 (2)	2.4	19.9 (2)	31.7 (2)
GM 852	M, 20	148	6.6	n.t.	···	28.0
J.P.	F, 30	167 (5)	12.7 (3)	1.5	18.1	29.6
J.L.	M, 36	242 (2)	6.8 (2)	2.0	12.0	17.9
L.Z.	F, 36	284 (2)	8.1	n.t.	25.3	29.3 (2)
P.N.	M, 50	218 (4)	6.3 (2)	1.4	24.7	22.2 (2)
Average ± SD	···	182 ± 50	7.9 ± 1.9	1.7 ± 0.3	18.8 ± 5.4	24.8 ± 5.9
Juvenile Variant (Group 2):						
A.T.	M, 1	100 (2)	21.7	1.0	3.4	39.3 (3)
C.T.	M, 2	70 (2)	20.4 (3)	1.3	5.2	32.8 (2)
B.H.	M, 2	n.t.	18.1 (2)	n.t.	n.t.	n.t.
Infantile (Group 3):						
GM 877	M, 1/2	72 (2)	17.2 (2)	1.1	7.2	44.9 (2)
Controls:						
Average ± SD	···	1,724 ± 410 (No. = 10)	18.1 ± 2.7 (No. = 7)	1.15 ± 0.08 (No. = 7)	5.7 ± 2.3 (No. = 6)	40.3 ± 4.3 (No. = 11)

NOTE.—Patients are listed individually and divided into three groups on the basis of clinical symptoms. The age of onset was taken to be the age at which splenomegaly or anemia were first noted or at which severe bone pain was first experienced. Diagnosis of the disease was usually made shortly after the onset of symptoms. Figures in parentheses next to some of the experimental results indicate the number of separate experiments in which that parameter was measured. Where no parentheses are present, only a single determination was made. For all properties shown in the table, the difference in means between patients in group 1 and controls is statistically significant ($P < .001$; $t \geq 4.90$).

* DC = deoxycorticosterone (0.5 mM).

† DC-β-glu = deoxycorticosterone-β-D-glucopyranoside (0.5 mM).

‡ n.t. = not tested.

was noted between specific enzyme activity and age of onset of the disease ($r = 0.71$; $.05 > P > .02$). In fibroblast cultures from six obligate heterozygotes for Gaucher disease (five chronic and one infantile), the specific β-glucosidase activity was intermediate between that of patients and controls. Average specific activity in this heterozygote sample was 1,091 ± 143 (± SD; range 842–1,280). Thus, detergent-solubilized enzyme preparations are suitable for both diagnosis of the disease and detection of heterozygotes.

It should be noted that the specific activity of the enzyme in chronic Gaucher patients was, on average, 10.6% of control activity. This is similar to the activity obtained previously by assaying the enzyme in the membrane-bound form, in the absence of detergents and at pH 4.0 [19]. The solubilization procedure clearly does not alter the relative activity of β-glucosidase in control and Gaucher fibroblasts. Comparison of the activities in table 1 with our previous data on the membrane-bound enzyme [19] shows that solubilization results in a slight increase in activity per mg cell protein in both control and Gaucher fibroblasts.

Thermostability

Fibroblast β-glucosidase was progressively inactivated by heating at 50°C (fig. 1). The enzyme from controls underwent an exponential loss of activity with an average half-life of 18.1 min (range: 14.5–22.2 min). As shown in figure 1, the enzyme from the chronic Gaucher patients was inactivated at a consistently faster rate. For nine of these patients, the average half-life of the enzyme at 50°C fell in the range 5.6–9.3 min (table 1). The enzyme from the remaining chronic patient was inactivated more slowly, with a half-life of 12.7 min (table 1). In most enzyme preparations from these patients, the rate of inactivation was more rapid during the first 5 min of heating than thereafter. By extrapolation (fig. 1), this initial, rapid inactivation accounts for about 15% of the original β-glucosidase activity in chronic patients. Although the proportion of enzyme inactivated during the first 5 min of heating varied from one preparation to another, up to a maximum of 39%, no consistent individual variation was noted. The origin of this rapidly inactivated enzyme is unclear. It was found, however, that when enzyme preparations were frozen at −20°C overnight, the proportion of rapidly inactivated enzyme increased, although the initial enzyme activity and the rate of inactivation from 5 min to 30 min were unaltered. To eliminate variation due to this rapid, initial inactivation, half-lives were calculated from the linear exponential part of the decay curve, from 5 min to 30 min.

In contrast to the results above, enzyme preparations from the three juvenile variant patients and the infantile patient were inactivated at a consistent rate over the entire heating period with half-lives within the control range (fig. 1 and table 1). Similarly, the half-life of the enzyme from an obligate heterozygote for chronic Gaucher disease was within the control range.

pH Optimum

The pH optimum of detergent-solubilized β-glucosidase from normal fibroblasts was pH 6.0. An identical optimum was obtained for the detergent-solubilized enzyme from peripheral leukocytes and long-term lymphoid lines [19] and placenta [8]. The pH

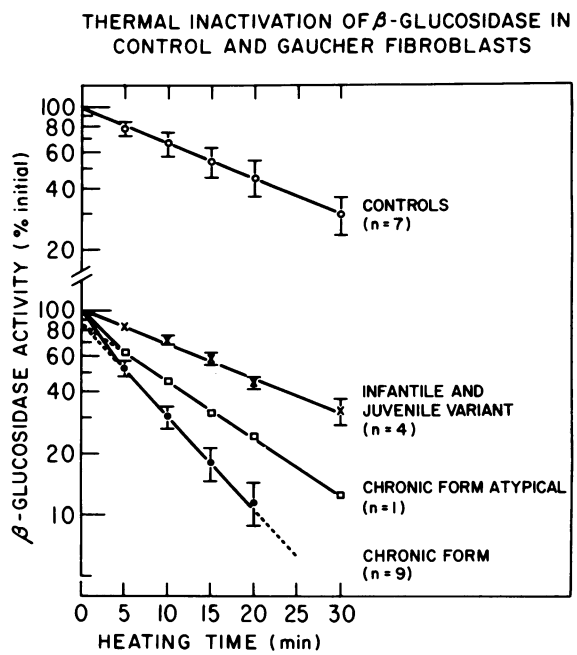

FIG. 1.—Inactivation of detergent-solubilized fibroblast β-glucosidase by heating at 50°C. Activity is expressed as a percentage of the activity of the unheated sample. Each curve is an average obtained from several different experiments as indicated. *Vertical bars* represent ± 1 standard deviation.

curve of the enzyme from the eight chronic Gaucher patients tested showed, in each case, a decrease in relative activity at pH values below the optimum (fig. 2). This change in the shape of the pH curve can be conveniently quantitated by the ratio of activity at pH 6.25 to activity at pH 5.5. As shown in table 1, the average control value for this ratio was 1.15 with a range of 0.95–1.21. The enzyme from a single obligate heterozygote for chronic Gaucher disease gave a value of 1.07. The eight chronic Gaucher patients tested had an average value of 1.7 with a range of 1.4–2.4, which is significantly different from that of the control population ($P > .001$). The pH curves of the enzyme from two of the juvenile variant patients and the infantile patient, were indistinguishable from that of the control enzyme. In these patients, the values for the pH 6.25:pH 5.5 activity ratio fell within the normal range (table 1).

We have previously reported [19] that solubilization of fibroblast β-glucosidase with Triton X-100 and sodium taurocholate causes a shift in the pH optimum from pH 4.5 (membrane-bound enzyme) to pH 6.0 (solubilized enzyme). In view of this, we determined whether the altered pH curve obtained with the solubilized enzyme from Gaucher fibroblasts was also apparent when the enzyme was assayed in the membrane-bound form (i.e., in the absence of detergents). In three chronic patients tested, the pH curve of the membrane-bound enzyme showed a slight but consistent shift towards more alkaline values (fig. 3). This change can be quantitated, as above, by comparing the ratio of activities at pH values on either side of the optimum, in this case pH 5.1 and pH 4.0. Using our published results [19] for activity at these two pH

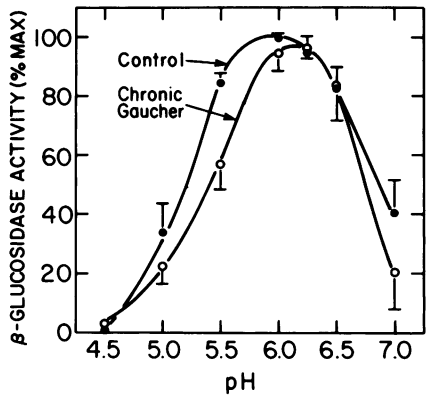

FIG. 2.—Activity at different pH values of detergent-solubilized β-glucosidase from control and Gaucher fibroblasts. The curves shown represent, respectively, the mean values obtained from seven controls and from eight patients with the chronic form of Gaucher disease. For each sample, the β-glucosidase activity at each pH was calculated as a percentage of the activity at the pH optimum. These values were then averaged to give the curves shown. *Vertical bars* above or below each point represent 1 standard deviation.

values, the ratio pH 5.1:pH 4.0 is 0.72 in controls (no. = 20), 0.70 in obligate heterozygotes (no. = 5), and 1.3 in patients with the chronic form of Gaucher disease (no. = 7).

K_m Values

Solubilized β-glucosidase from both normal and Gaucher fibroblasts gave linear double-reciprocal plots over a range of substrate concentrations from 2.5 mM to 0.1 mM. K_m values were calculated from these plots using best-fit lines calculated by the least squares method. No significant differences were found in the K_m values of the

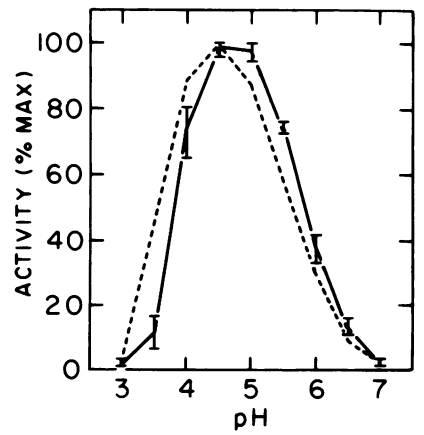

FIG. 3.—The pH curves of membrane-bound β-glucosidase from cultured skin fibroblasts. The membrane-bound enzyme was assayed in the absence of detergents as described previously [19]. The *solid line* connects the average values obtained with preparations from three patients. *Vertical bars* show the range of activities at each pH value. *Broken line* shows the pH curve of β-glucosidase from control fibroblasts assayed under identical conditions.

enzyme from normal and Gaucher cells. Values were as follows (mM ± standard deviation): controls, 2.7 ±'0.2 (no. = 4; range: 2.6–3.1); chronic patients, 3.5 ± 1.1 (no. = 8; range: 2.2–5.5); and juvenile variant and infantile patients, 2.8 ±'0.6 (no. = 4; range: 1.7–3.4).

Inhibition Studies

The steroid glycoside deoxycorticosterone-β-glucoside is a substrate for particulate and soluble β-glucosidases in human tissues [16] and is an effective inhibitor of placental glucocerebrosidase [20]. As shown in figure 4, deoxycorticosterone-β-glucoside also inhibits solubilized membrane β-glucosidase from normal fibroblasts. This inhibition is concentration dependent, and the results suggest that the mechanism is primarily competitive with an apparent K_i of approximately 0.4 mM.

At an inhibitor concentration of 0.5 mM and a substrate concentration of 1 mM, the reduction in activity of the enzyme from a series of control fibroblasts ranged from 34%–45%. Deoxycorticosterone itself was ineffective as an inhibitor under the same conditions. As shown in table 1, very similar results were obtained with the enzyme from the juvenile variant and infantile patients. In the eight chronic Gaucher patients who were tested, deoxycorticosterone-β-glucoside caused a smaller reduction in activity (14%–32%), while deoxycorticosterone was a considerably more effective inhibitor than against the control enzyme (table 1).

Density Gradient Sedimentation

Purified membrane β-glucosidase, prepared in solubilized form from placenta, has been reported to have a molecular weight, by gel-filtration, of approximately 240,000 [8]. We obtained a similar value for the purified placental enzyme. However, sedimentation studies on this enzyme preparation using sucrose density gradients, in the presence of the detergent Triton X-100, showed that the enzyme has an unusually low sedimentation coefficient of 4.1 *S* (B. M. Turner, unpublished results). As the

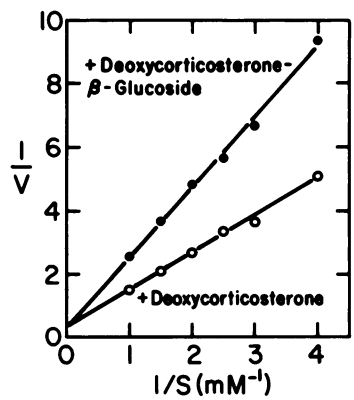

FIG. 4.—Double-reciprocal plots showing inhibition of normal fibroblast β-glucosidase by deoxycorticosterone-β-glucoside. Reaction mixtures contained the substrate 4-methylumbelliferyl-β-D-glucoside at concentrations from 1.0 to 0.25 mM together with 0.5 mM deoxycorticosterone or deoxycorticosterone-β-glucoside. Apparent K_m values are 3.6 mM in the presence of deoxycorticosterone and 7.5 mM in the presence of deoxycorticosterone-β-glucoside.

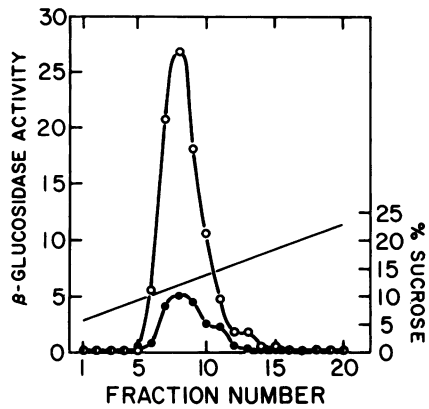

FIG. 5.—Sedimentation of detergent-solubilized β-glucosidase from control (○) or chronic Gaucher (●) fibroblasts through sucrose density gradients. Gradients were run in parallel under identical conditions as described in the text. Enzyme activity is expressed in fluorometer units per hr/20 μl.

sedimentation coefficient is a function of both molecular size and density, this low value is probably due to the low density of the enzyme.

Fibroblast β-glucosidase solubilized from the particulate fraction of a cell extract as described above, showed the same characteristic sedimentation properties as the placental enzyme. As shown in figure 5, the normal enzyme sedimented as a single major peak which was slightly skewed towards the bottom of the gradient. This asymmetry was a consistent finding in a number of different enzyme preparations. The fibroblast enzyme from two chronic Gaucher patients (J. L. and P. N., table 1) was found to sediment as a single major peak at the same position as the normal fibroblast enzyme together, in both cases, with a minor overlapping peak, or shoulder, closer to the bottom of the gradient. The tendency of human β-glucosidase to form high molecular weight aggregates has been noted previously [8], and the asymmetric sedimentation profile of the fibroblast enzyme may be due to the presence of such aggregates. They clearly account for only a small proportion of the β-glucosidase activity from both normal and Gaucher cells.

DISCUSSION

The 14 patients studied in this investigation fall into the following three groups in terms of clinical parameters and ethnic origin: (1) 10 chronic patients, all (with one possible exception) of Ashkenazi Jewish origin; (2) three black patients with severe visceral symptoms, manifest from early childhood, but without neurological involvement; and (3) an infantile patient of white non-Jewish parentage. All skin fibroblast cultures from these patients had reduced activity of β-glucosidase, ranging from 4.1% to 16.5% of the mean control value. By solubilizing the enzyme from the membrane fraction of cell extracts with a mixture of Triton X-100 and sodium taurocholate and by using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside, sufficient β-glucosidase activity was obtained from the patients' cells for the accurate and reproducible characterization of the enzyme.

The β-glucosidase activity in cultured skin fibroblasts from patients with chronic Gaucher disease (group 1) was found to differ qualitatively from the control cells. The enzyme from the chronic patients was more thermolabile, had an altered pH curve, was less effectively inhibited by deoxycorticosterone-β-glucoside, and was more effectively inhibited by deoxycorticosterone. The K_m of the patients' enzyme for 4-methylumbelliferyl-β-glucoside and, in the two patients tested, the sedimentation coefficient, were unaltered. In fibroblasts from patients in groups 2 and 3, with clinically more severe forms of Gaucher disease, the enzyme was qualitatively indistinguishable from that in controls.

The residual β-glucosidase in fibroblasts from chronic Gaucher patients appears to be a structurally altered form of the enzyme present in normal cells. The results do not, however, define the nature of this alteration. Three different mechanisms by which a structurally altered enzyme may be generated are as follows: (1) an alteration in primary structure due to mutation of a gene coding for an enzyme subunit; (2) a defect in post-translational processing of the nascent peptide chain by, for example, glycosyl transferases responsible for attachment of carbohydrate residues; and (3) binding of a defective activator macromolecule to the enzyme.

Mechanism 2 seems unlikely in that enzymes involved in post-translational processing of β-glucosidase would not be expected to be specific for this enzyme, but rather would have a more general role in processing a number of different proteins. A defect at this stage would therefore be expected to have more far-reaching effects on protein metabolism.

Mechanism 3 must be considered in view of reports describing the isolation from spleen of glycoprotein activators of β-glucosidase [21, 22]. However, it has been reported that these activators can be isolated from both normal and Gaucher spleen and can be replaced, at least in part, by components present in crude sodium taurocholate [22]. In view of these findings and the fact that our assays were carried out in the presence of optimum concentrations of sodium taurocholate, it seems unlikely that an abnormal activator protein can account for our results. Thus, while these considerations are not conclusive, an alteration in the primary structure of the enzyme due to mutation of a structural gene is the most probable explanation for our results.

The altered enzyme in chronic Gaucher fibroblasts clearly differs from that present in fibroblasts from the infantile patient we studied. This difference can distinguish these two forms of Gaucher disease in early infancy and possibly prenatally using amniotic fluid cells. It should be noted, however, that in a recent report, Mueller and Rosenberg [23] describe a thermolabile form of β-glucosidase in two infantile patients. In view of the rarity of infantile Gaucher disease, considerable genetic heterogeneity is expected. Studies on larger numbers of infantile patients will be required to define the extent of this heterogeneity.

In nine of the 10 chronic Gaucher patients presented here, the properties of the residual β-glucosidase were found to be essentially the same, irrespective of the clinical severity of the disease. If it is assumed that different gene mutations are unlikely to cause the same alterations in several properties of the enzyme, then it is probable that these nine patients have the same mutant enzyme, coded for by the same mutant allele.

The only example of biochemical heterogeneity among the chronic Gaucher patients was found in patient J. P. The enzyme from this patient was heat inactivated at a rate which was consistently intermediate between that of the normal enzyme and the enzyme from other chronic Gaucher patients. The pH curve of this enzyme and its inhibition by deoxycorticosterone-β-glucoside also fell between the normal and patient means. A possible explanation for these intermediate values is that this patient is a double heterozygote who carries one mutant allele identical to that found in other chronic patients and a second allele which codes for an enzyme with reduced activity but otherwise normal properties. The clinical manifestations of the disease in this patient were mild, but not unusually so.

The degree of heterogeneity in the properties of the residual β-glucosidase in the chronic Gaucher patients we studied is less than that reported by Klibansky et al. [24]. Using peripheral leukocytes as the enzyme source and the natural substrate glucocerebroside, these workers found that glucocerebrosidase activity in cells from some patients had a reduced thermostability, thus providing evidence for a qualitative enzyme alteration in this form of the disease. However, this alteration in thermostability was observed in patients with an early age of onset and not in less severely affected individuals. Our own results provide no evidence for such a distinction. The reasons for this difference may lie in the use of different substrates for assay or in the use of different cell types. However, using the artificial substrate and a heat-inactivation procedure similar to that outlined above, we found that the residual β-glucosidase activity in peripheral leukocytes from chronic Gaucher patients is consistently more thermolabile than the control enzyme (B. M. Turner, in preparation). It may be significant that Klibansky et al. [24] heated their enzyme preparations for a fixed time period (20 min) and calculated the thermostability as the percentage activity loss over this period. This method may give more variable results than the calculation of half-life values, particularly in view of the rapid and variable loss of activity which occurs during the first 5 min of heating. This rapid, initial inactivation is characteristic of the enzyme from chronic Gaucher cells, and its significance remains unclear. We found that the proportion of rapidly inactivated enzyme is increased by freezing the enzyme preparation, although total enzyme activity is unaffected, which raises the possibility that a structural or conformational change in the native enzyme is involved.

SUMMARY

Membrane-bound β-glucosidase from cultured skin fibroblasts can be solubilized in an active form by treatment of membrane preparations with a mixture of Triton X-100 and sodium taurocholate. Several properties of the solubilized enzyme have been studied in fibroblasts from normal, healthy individuals and from 14 patients with different clinical forms of Gaucher disease. The patients studied were classified as follows: group 1 consisted of 10 chronic patients, all (with one exception) of Ashkenazi Jewish origin; group 2 consisted of three black American patients with severe visceral symptoms, manifest from early childhood, but with no apparent neurological involvement; and group 3 consisted of a single white patient with the classical infantile form of the disease.

Specific β-glucosidase activity ranged from 6.6% to 16.5% mean control value in group 1 patients and from 4.1% to 5.8% in groups 2 and 3. When compared with the enzyme from control fibroblasts, the enzyme from chronic Gaucher patients (group 1) was more rapidly inactivated at 50°C, had an altered pH curve, was less effectively inhibited by deoxycorticosterone-β-glucoside, and was more effectively inhibited by deoxycorticosterone. The enzyme from patients in groups 2 and 3 was qualitatively indistinguishable from the control enzyme in terms of these parameters. No differences in K_m (4-methylumbelliferyl-β-glucoside) or sedimentation coefficient were found between the β-glucosidases from control and Gaucher cells. The results demonstrate that cells from Ashkenazi Jewish patients with the chronic form of Gaucher disease contain a structurally altered form of β-glucosidase. This enzyme differs both from normal β-glucosidase and from the residual enzyme in patients of different ethnic origin and with clinically more severe forms of the disease.

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Erratum

In the paper "Compound Lateral Asymmetry in Human Chromosome 6: BrdU-Dye Studies of 6q12→6q14" by B. S. Emanuel (*Am J Hum Genet* 30:153–159, 1978), figures 3 and 4 have been transposed. The figure that appears over figure legend 3 is actually figure 4, and the figure over figure legend 4 is actually figure 3.