COMPARISON OF THE ACIDIC LIPID REQUIREMENT OF CONTROL AND

TYPE 1 GAUCHER'S DISEASE LIVER AND BRAIN GLUCOCEREBROSIDASES

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INTRODUCTION

All patients with Gaucher's disease, regardless of whether they
have the non-neuronopathic form (type 1) or one of the neuronopathic
forms (types 2 and 3), are profoundly deficient in lysosomal
glucocerebrosidase activity. That the activity of glucocerebrosidase
from normal spleen, liver, and brain has a near absolute lipid
requirement is conveniently demonstrated by extracting the enzyme
preparation sequentially with a bile salt (e.g., sodium cholate) and
ice-cold n-butanol (1). This process, by delipidating the enzyme,
renders glucocerebrosidase inactive. The inactive enzyme from spleen
of controls and patients with type 1 Gaucher's disease can be
extensively reconstituted with exogenous acidic lipids (e.g.,
phosphatidylserine, galactocerebroside-3-sulfate, GM1) or the bile
salt sodium taurodeoxycholate (2,3). The mutant glucocerebrosidase of
the more severely affected type 2 patients, either before or after
sodium cholate extraction and n-butanol delipidation, cannot be
activated by the inclusion of bile salts or any of the above-mentioned
acidic natural membrane lipids in the assay medium.

Recently, several studies have indicated that the biosynthesis of
mature mammalian glucocerebrosidase is a multistep process which
involves limited proteolysis, glycosylation and deglycosylation and
possibly other reactions necessary for the targeting of the enzyme to
its proper subcellular location. This raises the possibility that
tissue-specific differences in the processing reactions within a given
species cause the resultant glucocerebrosidases present in various
organs to be structurally and kinetically distinct, thereby giving
rise to differences in the enzymes' affinity for substrate as well as
specificity with regard to the lipid requirement.

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The availability of autopsy tissues from a case (P.T.) of type 1 Gaucher's disease provided us with the opportunity to compare some of the properties of liver and brain glucocerebrosidases from control tissues and the corresponding tissues of a patient with non-neuronopathic Gaucher's disease. This report shows that while brain and liver glucocerebrosidases may be physically very similar, their ionic characteristics and lipid requirements can be quite different.

MATERIALS AND METHODS

Chemicals and reagents. 4-Methylumbelliferyl-β-D-glucopyranoside (MUGlc), bacterial alkaline phosphatase, and sodium taurodeoxycholate were obtained from Sigma Chemical Co. (St. Louis, MO, USA); phosphatidylserine and bovine sulfatide (galactocerebroside-3-sulfate, stearate-containing) were obtained from Supelco (Bellefonte, PA, USA). Ampholines (pH 3–10) were obtained from Bio-Rad (Richmond, CA, USA). All other reagents were of highest purity available.

Preparation of glucocerebrosidase. Liver and brain obtained at autopsy from individuals free of central nervous system disease served as the source of control glucocerebrosidase. Liver and brain obtained at autopsy from the case of type 1 Gaucher's disease described below served as the source of mutant glucocerebrosidase. Tissues were frozen (-43°C) for 1–12 mo until preparation of the enzyme by sequential sodium cholate and n-butanol extractions, as described elsewhere (4).

β-Glucosidase assay. The standard β-glucosidase assay contained 4 mM MUGlc, 0.1 M sodium acetate, pH 5.5 and various amounts of lipid activator, as specified in the text, in a final volume of 0.1 ml. Reactions were carried out at 37°C for 1 h and fluorescence was determined as described elsewhere (5).

Separation techniques. Sucrose density gradient ultracentrifugation (4), isoelectric focusing (6) and HPLC-gel filtration (7) of glucocerebrosidase preparations were performed as indicated.

Protein determination. Protein concentration was estimated according to the method of Bradford (8) using bovine serum albumin as standard.

Case history. P.T. was age four when excessive bleeding followed a tonsillectomy. Splenomegaly prompted a bone marrow exam three years later resulting in the diagnosis of Gaucher's disease. Splenectomy was carried out at age 28 because of thrombocytopenia and abdominal pain. Bone crises began in the left leg the following year. A traumatic fracture of the left femur occurred at age 36. Despite treatment with an intramedullary nail the fracture went onto nonunion. A femoral homograft was implanted one year later. The remainder of this patient's life was marked by a detached retina, progressive liver enlargement, cyanosis, and secondary erythrocytosis. Her final admission occurred at age 42 following a traumatic fracture of the left humerus. Her terminal course was marked by lethargy, cyanosis, and renal failure.

At age 30, her leukocyte β-glucosidase activity was determined using the pH 5.5-sodium taurocholate assay of Peters et al. (5); her β-glucosidase value was 5% of control (0.22 vs. 4.24 nm/mg/h). Her serum acid phosphatase activity (5150 nm/ml/h) was elevated to six times that of the control mean (847 nm/ml/h).