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A NEW GROUP OF GLUCOCEREBROSIDASE ISOZYMES FOUND IN HUMAN WHITE BLOOD CELLS

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Summary

Multiple isozymes of glucocerebroside-β-glucosidase (EC 3.2.1.45) from normal white blood cell preparations have been demonstrated utilizing flat-bed isoelectric focusing on a granulated gel. Normal human leukocyte preparations contain significant glucocerebrosidase isozyme activities with pI's between 3.2 and 3.9. These novel, acidic pI species have an average D[1-^{14}C]glucocerebroside to 4-methylumbelliferyl-β-D-glucopyranoside substrate ratio greater than 40:1. β-D-Glucocerebrosidase activity of these low pI species is markedly reduced or absent in white blood cell preparations from patients with Type I Gaucher's disease.

Introduction

The importance of isoenzymes in the pathogenesis of metabolic diseases is well documented (1,2). In patients with Gaucher's disease, a deficiency of β-D-glucocerebrosidase results in the tissue accumulation of excessive amounts of glucocerebroside (3). Until recently, attempts to demonstrate the presence of isozymes of either membrane bound β-D-glucocerebrosidase or β-glucosidase by electrophoretic techniques have been hampered by poor penetration of the enzymes into the gel beds (4-8). Granulated gels provide a very useful isoelectric focusing medium since they permit essentially unrestricted migration of proteins in the liquid phase. Utilizing isoelectric focusing with Ultrodex^{R}, Maret et al (9) demonstrated the occurrence of several forms of β-D-glucocerebrosidase (pI 5.0 and pI 6.5). In this paper we document the presence of additional isozymes of

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β-D-glucocerebrosidase in the pI range of 3.2 to 3.9 in human white blood cell preparations.

Materials and Methods

Ultrodex^R and ampholines 2.5 - 4, 4 - 6, and 6 - 8 were obtained from LKB Instruments, Inc. (Rockville, MD). Pharmalyte 2.5 - 5 was obtained from Pharmacia (Piscataway, NJ). 4-Methylumbelliferyl-β-D-glucopyranoside (MUGp) was purchased from Koch-Light (Colnbrook, Bucks, England). Cutscum (iso-octylphenoxypolyoxyethanol) and Triton X-100 were obtained from Fisher Chemical Co. (Silver Spring, MD) and New England Nuclear (Boston, MA), respectively.

Venous blood samples were obtained from normal individuals and from patients with Type I Gaucher's disease. The blood sample was mixed with 0.2 volume 5% Dextran in normal saline and then the cells were allowed to settle for 1-2 h at room temperature. The supernatant was removed and the resulting suspended white cells were centrifuged for 15 min at 600 X g at 4°C. After lysing residual red cells with distilled water for 1.5 min, isotonicity was restored with 3.6% NaCl and the white cell pellet was obtained by centrifugation as above. The cell pellet was suspended in 2 ml of buffer (600 mM potassium phosphate buffer, pH 5.9 and 10 mg/ml Cutscum) and sonicated 4 times for 10 sec at 4°C. The sonicated suspension was centrifuged at 48,000 X g for 20 min and the supernatant was kept at 4°C until application of the sample to the gel bed.

Preparative flat-bed isoelectric focusing in Ultrodex^R was performed using a LKB 2117 Multiphor apparatus (at constant power of 8W for 16 h) as described by Radola (10) and Winter et al (11). A print of the focused gel was obtained by overlaying the Ultrodex^R with 5 mm X 25 mm strips of Whatman No. 1 filter paper for 10 min. The pH profile of the focused gel was measured with a #ES37615 Ingold Electrode, (Brinkmann Instrument, NY).

The Whatman print strips were transferred to tubes containing 25 μl potassium phosphate buffer (600 mM, pH 5.9, 1% Triton X-100), 150 μl distilled water, and 5 μl of D-[1-^{14}C]glucocerebroside (7.5 mg/ml in 50 mg/ml sodium taurocholate). Following incubation at 37°C the liberated D-1-^{14}C glucose was measured as previously described (12). Glucosidase activity was measured using 5 mM 4-MUGp in 0.05 M citrate-phosphate buffer, pH 4.5.

Results and Discussion

Isoelectric focusing on flat beds of granulated gels permits the separation of large, high molecular weight species that do not enter low porosity media. Representative preparative mode isoelectric focusing patterns of glucocerebrosidase from one normal and two Type I Gaucher's white blood cell preparations are shown in Figure 1. In addition to the previously described molecular forms at pI 5.0 and 6.5 (9), white cell preparations from normal individuals contained isozymes of glucocerebrosidase in the pI interval of 3.2-3.9. Previous workers were not able to resolve the more acidic species using a pH 4-8 ampholine gel system. White blood cell

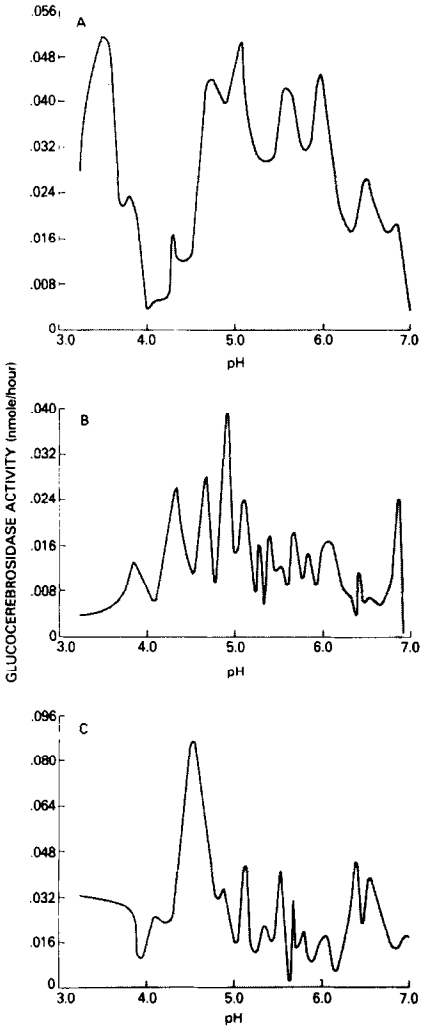

Figure 1 - Isoelectric focusing of glucocerebrosidase from normal (A) and Type I Gaucher (B,C) white blood cells. Enzyme activities were obtained as described in the text. Approximately 1.5 X 10^9 white blood cells were applied to each gel.

isoelectric focusing patterns from patients with Type I Gaucher's disease showed marked deficiency or absence of the low pI isozymes in the range of 3.2-3.9. Figures 1b and 1c illustrate white blood cell glucocerebrosidase isozyme patterns from two different patients. Although total glucocerebrosidase activity is decreased in white cell preparations from Type I Gaucher's disease, our isoelectric focusing patterns suggest that the activity of glucocerebrosidase isoenzymes in the 3.2-3.9 pI interval

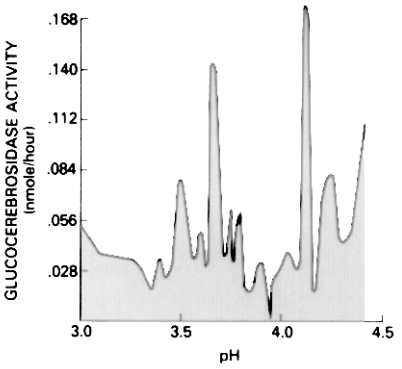

Figure 2 - Isoelectric focusing with a narrow pH gradient of glucocerebrosidase from normal white blood cells. Enzyme activities were obtained as described in the text.

is more selectively depressed. Figure 1c also demonstrates that the non-specific glucosidase (pI 4.2-4.6) found in Type I Gaucher's white cells may have significant activity for glucocerebroside.

Higher resolution of isozyme patterns can be obtained by isoelectric focusing within a narrower pH range. Figure 2 illustrates the multiplicity of species in the glucocerebrosidase isoelectric focusing pattern that can be seen in the pI interval of 3.2-3.9.

Highly purified placental glucocerebrosidase (species that have isoelectric points in the pH range of 4.8-7.3) has a natural to artificial substrate ratio of approximately 20 to 1 (12). The low pI (3.2-3.9) glucocerebrosidase isozymes reported in this paper have an average substrate ratio of greater than 40 to 1. We can conclude that the placental glucocerebrosidase preparation contains additional general β-glucosidase activity or has forms having broader substrate specificity. Work done in this laboratory indicates that the latter conclusion is more likely (F. S. Furbish, unpublished observations). Preliminary investigations also indicate that these acidic glucocerebrosidase isozymes are concentrated in mononuclear white cell fractions and crude spleen extract. The greater specificity for glucocerebroside and the marked difference between Type I

Gaucher's and normal white blood cell isozyme activity in the low pI range suggests that these acidic pI isozymes may reflect the primary genetic defect in Type I Gaucher's disease. The consistency of these initial observations will be examined in a larger group of patients.

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