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Biochemistry

Human acid β-glucosidase: Isolation and amino acid sequence of a peptide containing the catalytic site

(Gaucher disease/glucocerebrosidase/conduritol B epoxide/enzyme inhibitors)

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ABSTRACT **Human acid β-glucosidase (D-glucosyl-*N*-acylsphingosine glucohydrolase, EC 3.2.1.45) cleaves the glucosidic bonds of glucosylceramide and synthetic β-glucosides. The deficient activity of this hydrolase is the enzymatic defect in the subtypes and variants of Gaucher disease, the most prevalent lysosomal storage disease. To isolate and characterize the catalytic site of the normal enzyme, brominated ^{3}H-labeled conduritol B epoxide (^{3}H-Br-CBE), which inhibits the enzyme by binding covalently to this site, was used as an affinity label. Under optimal conditions 1 mol of ^{3}H-Br-CBE bound to 1 mol of pure enzyme protein, indicating the presence of a single catalytic site per enzyme subunit. After V_8 protease digestion of the ^{3}H-Br-CBE-labeled homogeneous enzyme, three radiolabeled peptides, designated peptide A, B, or C, were resolved by reverse-phase HPLC. The partial amino acid sequence (37 residues) of peptide A (M_r, 5000) was determined. The sequence of this peptide, which contained the catalytic site, had exact homology to the sequence near the carboxyl terminus of the protein, as predicted from the nucleotide sequence of the full-length cDNA encoding acid β-glucosidase.**

Human acid β-glucosidase (D-glucosyl-*N*-acylsphingosine glucohydrolase, EC 3.2.1.45), a lysosomal enzyme, cleaves the β-glucosyl linkage in glucosylceramide (GC) as well as synthetic β-glucosides (1, 2). This membrane-associated glycoprotein is a homomer whose mature glycosylated subunit has a M_r of 67,000–73,000 (3–5). The enzyme is hydrophobic and requires detergents, negatively charged lipids, and/or a "co-glucosidase" for optimal hydrolysis of GC or synthetic substrates (6–9). Detailed studies of the effects and interactions of a variety of enzyme modifiers have indicated that the active site of the enzyme contains at least three domains with differing specificities: (*i*) the catalytic site, a hydrophilic pocket that recognizes β-glucosyl moieties and conduritol B epoxide (CBE); (*ii*) an aglycon binding site that is hydrophobic and has affinity for the alkyl chains of GC; and (*iii*) a hydrophobic third domain (9) or "allosteric" site (10) that interacts with negatively charged lipids to increase hydrolytic rates or cationic sphingosyl moieties of enzyme inhibitors (9). It has been proposed that these three domains function in the binding and orientation of substrates and release of products, respectively (8, 9). Defects of acid β-glucosidase function and/or processing and stability lead to the accumulation of GC and the resultant subtypes and variants of Gaucher disease, an inherited lysosomal storage disease (11–14). The most prevalent form of Gaucher disease, type 1, has been estimated to occur in about 1 in 2500 individuals of Ashkenazi Jewish descent (15).

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Recent kinetic and immunologic studies of the residual enzymatic activity from tissues of affected patients with various subtypes and variants of Gaucher disease have indicated several allelic mutations, which result in two general categories of abnormal acid β-glucosidases: those that have (*i*) a normal response to enzyme effectors but abnormal stability and/or processing and (*ii*) an abnormal response to a variety of active-site directed effectors (11, 12). The second category of mutant enzymes have been found exclusively in the non-neuronopathic (type 1) subtypes of Gaucher disease, primarily among affected Ashkenazi Jewish patients.

CBE and its brominated derivative (Br-CBE) are inhibitors that bind covalently to acidic amino acids in the catalytic sites of glucosidases from several different species (16–19). Compared to the normal human enzyme, increased concentrations of these suicide inhibitors were required to inhibit the residual enzyme from tissues of Ashkenazi Jewish Gaucher disease type 1 patients (11). These findings suggested that the mutation(s) in this subtype of Gaucher disease may have resulted from an amino acid substitution(s) in or near the active site of acid β-glucosidase.

To gain further insight into the structural basis of the kinetic properties of the normal and mutant acid β-glucosidases, ^{3}H-labeled Br-CBE (^{3}H-Br-CBE) was used to affinity-label the catalytic site of the normal enzyme. After proteolysis of the labeled homogeneous enzyme, a peptide containing the catalytic site of human acid β-glucosidase was purified and its amino acid sequence was determined. The amino acid sequence of this peptide was colinear with the predicted amino acid sequence near the carboxyl terminus of a recently isolated human cDNA that encodes this enzyme (20).

MATERIALS AND METHODS

Materials. The following were obtained commercially: human and bovine serum albumin (Sigma); 4-methylumbelliferyl β-D-glucopyranoside (Research Products International, Mount Prospect, IL); NBD-dodecanoic acid (Molecular Probes, Junction City, OR); *Staphylococcus aureus* V_8 protease (V_8 protease, Miles); Vydac protein C-4 column (4.6 × 250 mm; The Nest Group, Southboro, MA); Protosol (New England Nuclear). Acetonitrile and trifluoroacetic acid were HPLC grade. All other chemicals were reagent grade or better.

GC was purified from spleens of Gaucher disease patients (21) and NBD-dodecanoyl-GC was prepared from glucosyl-

Abbreviations: CBE, conduritol B epoxide; ^{3}H-Br-CBE, brominated ^{3}H-labeled CBE; GC, glucosylceramide.

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sphingosine (22). Br-CBE, ^{3}H-Br-CBE (8000 cpm/pmol), and *N*-alkyl-deoxynojirimycin-Sepharose were synthesized as described (23, 24).

Enzyme Purification, ^{3}H-Br-CBE Labeling, and Proteolytic Cleavage. Human placental acid β-glucosidase was purified to homogeneity by affinity chromatography using *N*-alkyl-deoxynojirimycin-Sepharose (24). Aliquots (1–2 nmol) of the enzyme were labeled with ^{3}H-Br-CBE as follows: the enzyme, in 0.5 ml of buffer A (0.04 M citrate/0.05 M phosphate, pH 5.5, containing 4 mM 2-mercaptoethanol and 5 mM EDTA) and 60–80% ethylene glycol, was incubated at 22°C for 96 hr with a 10-fold molar excess (0.15 ml) of ^{3}H-Br-CBE (800 cpm/pmol). Control experiments indicated that the enzyme was stable under these conditions. To this solution, sufficient 1.25 M Tris·HCl, pH 6.8, 5% $NaDodSO_4$, and concentrated ethylene glycol were added to achieve the following final concentrations: 0.125 M Tris·HCl, pH 6.8/0.5% $NaDodSO_4$/50% ethylene glycol (buffer B). This solution then was heated to 100°C for 2 min. After cooling to 22°C, freshly prepared V_8 protease in buffer B (2–8 μl) was added to a 17-fold excess of enzyme protein (wt/wt) and incubated at 37°C for 96 hr. Control experiments demonstrated that ^{3}H-Br-CBE did not bind to V_8 protease. V_8 protease in the digests was inactivated by heating at 100°C (2 min) in the presence of 2% 2-mercaptoethanol for $NaDodSO_4$/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE) or by freezing at −20°C prior to reverse-phase HPLC.

Isolation of Radiolabeled Peptides. The V_8 protease digests of the radiolabeled-acid β-glucosidase were defrosted and subjected to reverse-phase HPLC (Waters Associates) by using a protein C-4 column in 0.05% trifluoroacetic acid. The peptides were eluted with a programmed nonlinear 0–80% acetonitrile gradient (see *Results*) at a flow rate of 0.7 ml/min. In pilot runs, the gradient was optimized to provide maximal resolution of the peptides. The eluted absorbance peaks (A_{280} or A_{214}) were collected in Teflon tubes and the radioactivity was determined in aliquots from each tube. Analytical $NaDodSO_4$/PAGE (12.5%) (25) and the silver-staining technique (26) were used to monitor the purity of the peptides. The amino acid sequence of one of the pure radiolabeled peptides was determined by using gas-phase sequencing techniques (27). Hydropathy profiles and probable secondary structural assignments were calculated by using the data of Kyte and Doolittle (28) and the program of Corrigan and Huang (29), respectively.

Quantitation of Acid β-Glucosidase Active Sites. The relationship between the amount of enzymatic protein (pmol) and ^{3}H-Br-CBE bound to the enzyme was determined by incubating various amounts of active homogeneous acid β-glucosidase with a large excess of ^{3}H-Br-CBE (10 μM final concentration) in buffer A containing 0.6% human serum albumin. For these studies, the enzyme was diluted with buffer A to an ethylene glycol concentration of <1%. Control studies demonstrated that under these conditions human serum albumin was required to maintain enzyme stability at 22°C for up to 24 hr. Complete inactivation of the enzyme by ^{3}H-Br-CBE was achieved by 2 hr at 22°C. To ensure that all sites that could bind ^{3}H-Br-CBE were saturated, the reaction mixture was allowed to stand at 22°C for 24 hr. The enzyme–^{3}H-Br-CBE complexes were immunoprecipitated quantitatively with monospecific rabbit anti-human acid β-glucosidase IgG and *Staphylococcus aureus* protein A (12). The resultant supernatants were reprecipitated with additional IgG and protein A until no additional increase in precipitated radioactivity was observed; immunoprecipitation usually was quantitative after a single cycle. The resultant pellets were washed by resuspension and centrifugation (10,000 × *g*; 40 min) twice in phosphate-buffered saline containing 1% bovine serum albumin, 0.5 M NaCl, and 0.05% Tween 20 and then twice in phosphate-buffered saline containing 0.05% Tween 20. The washed pellets were dissolved in 100 μl of Protosol in 900 μl of water (24 hr at 22°C) and the radioactivity was determined. The data are presented as the pmol of homogeneous enzymatic protein based on the protein concentration (30) and the estimated molecular weight (55,000) for the unglycosylated enzyme subunit as calculated from the amino acid composition (20, 24). These results were in close (±10%) agreement with calculations of the enzymatic protein concentration (pmol) based on the turnover number (140 nmol of substrate hydrolyzed per hr per pmol of enzyme) of the homogeneous enzyme using 4-methylumbelliferyl β-D-glucopyranoside as substrate (unpublished results).

Determination of the IC_{50} Values for Br-CBE. The IC_{50} values for Br-CBE (i.e., the concentration of Br-CBE required to achieve 50% inhibition of the original enzymatic activity) were determined as follows: the final incubation mixture (0.2 ml) contained 0.04 M citrate, 0.05 M phosphate (pH 5.5), 4 mM 2-mercaptoethanol, 5 mM EDTA, 4 mM Triton X-100, 4.65 mM sodium taurocholate, and the required amounts of Br-CBE (11) and 4-methylumbelliferyl β-D-glucopyranoside or GC [NBD-dodecanoyl-GC/splenic GC, 1:19, mol/mol (22)]. Reactions were initiated by the addition of homogeneous enzyme in amounts determined to hydrolyze <2% of the substrate. After 1 hr, the reactions were terminated and the fluorescence intensity of the products was determined (22).

RESULTS

Interaction of Br-CBE and Acid β-Glucosidase. Fig. 1 shows the direct dependence of the IC_{50} values for Br-CBE on the concentration of the substrates, 4-methylumbelliferyl β-D-glucopyranoside or GC, in incubation mixtures containing homogeneous acid β-glucosidase. These results and similar data (9) obtained with CBE and δ-gluconolactone, a competitive inhibitor, provide evidence for the specific binding of Br-CBE to a catalytic site of the enzyme. To establish the number of ^{3}H-Br-CBE binding sites on acid β-glucosidase, known amounts (pmol) of enzyme were inactivated completely by a 1500- to 2500-fold excess of ^{3}H-Br-CBE (10 μM) and then separated from the unreacted inhibitor by quantitative immunoprecipitation. The mole quantity of ^{3}H in the washed immunoprecipitates was correlated with that of pure enzymatic protein. Fig. 2 demonstrates the 1:1 (mol/mol) stoichiometry of homogeneous enzymatic protein and bound ^{3}H-Br-CBE, indicating the presence of a single catalytic site per enzyme subunit. Covalent binding of ^{3}H-Br-CBE to acid β-glucosidase was suggested by the coelution of the enzymatic protein and radioactivity on reverse-phase HPLC (Fig. 3). For these experiments, the enzyme and ^{3}H-Br-CBE (1:10, mol/mol) were incubated for 96 hr at 22°C. Under these conditions, ≈13% of the enzymatic protein was labeled by

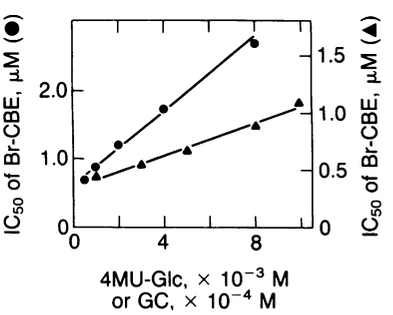

FIG. 1. The dependence of the IC_{50} values for Br-CBE on the concentrations of the substrates 4-methylumbelliferyl β-D-glucopyranoside (4MU-Glc) (●) or GC (▲) in the incubation mixtures. Homogeneous acid β-glucosidase from placentae was the enzyme source and incubations were terminated after 1 hr.

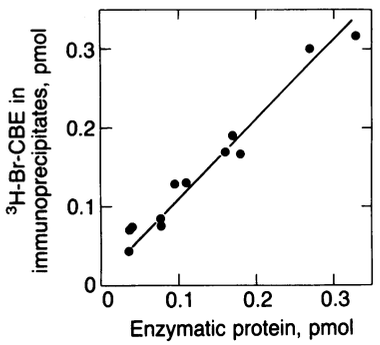

FIG. 2. The relationship of homogeneous acid β-glucosidase protein (pmol) to ^{3}H-Br-CBE (pmol) after complete enzyme inactivation by the inhibitor. Radioactivity bound specifically to the enzyme was determined after quantitative immunoprecipitation using rabbit anti-acid β-glucosidase IgG. Unweighted linear regression analysis by the least-squares method gave a slope of 0.984 with a correlation coefficient of 0.988.

^{3}H-Br-CBE. Rechromatography of the labeled enzyme on reverse-phase HPLC resulted in ≈25% loss of radioactivity associated with the protein. Autoradiographs of the NaDodSO$_4$/polyacrylamide gel of the pure enzyme after labeling with ^{3}H-Br-CBE demonstrated a single labeled protein species at $M_r \cong 67{,}000$ (Fig. 3 *Inset*). The finding of a single NH_2-terminal amino acid sequence (24) and a single protein species on reverse-phase HPLC (Fig. 3) or NaDodSO$_4$/PAGE (data not shown) provided evidence for the homogeneity of the acid β-glucosidase preparations.

Isolation and Amino Acid Sequence of a Peptide Containing the Catalytic Site. After labeling the homogeneous acid β-glucosidase with ^{3}H-Br-CBE and then digestion with V_8 protease, the radiolabeled peptides were resolved by reverse-phase HPLC using programmed nonlinear acetonitrile gradients. A typical HPLC profile of the digests is shown in Fig. 4. Three peptides, designated peptide A, B, or C, which eluted at ≈32%, ≈40%, or ≈42% acetonitrile, respectively, contained the ^{3}H label. To obtain optimal resolution of the labeled and unlabeled peptides, only 1- to 2-nmol aliquots of the digested labeled enzyme were subjected to HPLC. This approach was necessary because chromatography of the isolated peptides on HPLC resulted in large losses of the ^{3}H label from these peptides. In addition, short exposures (1–2 min) of the purified labeled peptides to neutral or basic buffers containing amines or urea also resulted in loss of the radiolabel. Furthermore, the radioactive label was released during cleavage of the enzyme by cyanogen bromide using several different procedures (17–19, 31). Incubation of peptide A with trypsin in a variety of buffers did not result in cleavage.

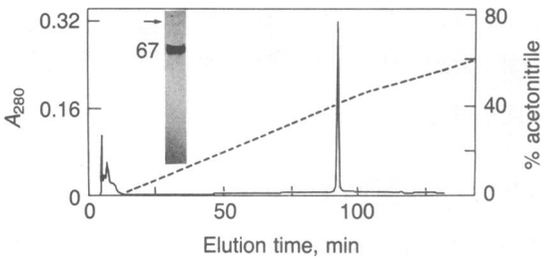

FIG. 3. Reverse-phase (protein C-4 column, 4.6 × 250 mm) HPLC elution profile of 5.4 nmol of purified acid β-glucosidase. The pure enzyme eluted at 41.0% acetonitrile. The peaks at 6–10 min were absorbing compounds in the application buffer. (*Inset*) Autoradiograph of the ^{3}H-Br-CBE labeled enzyme (M_r, ≅67,000) after NaDodSO$_4$/PAGE (12.5%). Arrow indicates origin.

Fig. 5 shows that a single protein species was present in each of the isolated labeled peptides when subjected to 12.5% NaDodSO$_4$/PAGE. Based on the estimated labeling (8–15%) of the purified enzyme by ^{3}H-Br-CBE (Fig. 3), 60–75% recovery of ^{3}H was obtained in peptides A, B, and C. Although the HPLC profiles of the peptides in the enzyme digests were somewhat variable, the principal changes were in the relative amounts of each labeled peptide; peptide A always was present in the greatest amount. Since a 1:1 stoichiometry of enzymatic protein and ^{3}H-Br-CBE was obtained under full labeling conditions (Fig. 2), peptides B and C probably represent overlapping sequences with peptide A. However, the amino acid sequences of peptides B and C could not be determined because the former was NH_2-terminally blocked and the latter was obtained only in small amounts. Since one preparation of peptide A also was NH_2-terminally blocked, it was likely that the NH_2-terminal blocking of peptide B occurred during the cleavage and isolation procedure.

The amino acid sequence (37 residues) obtained from two different preparations of ^{3}H-Br-CBE-labeled peptide A (M_r, ≅5000) is shown in Fig. 6. Tentative assignments are shown in parentheses and X denotes an unidentifiable amino acid residue. Peptide A contained one methionine residue and ≈50% of the amino acids were hydrophobic. This sequence had exact homology to amino acid residues 429–465 predicted from a cDNA for human acid β-glucosidase (20), except for the second residue, which was predicted to be a serine (Fig. 6).

The precise amino acid to which ^{3}H-Br-CBE was bound (i.e., the catalytic site) was not identified with certainty, since the ester linkage to the peptide was labile in the presence of dimethylamine gas used during microsequencing. In separate experiments, we showed that trimethylamine liberates all ^{3}H from peptide A. Since it is likely that ^{3}H-Br-CBE binds to an

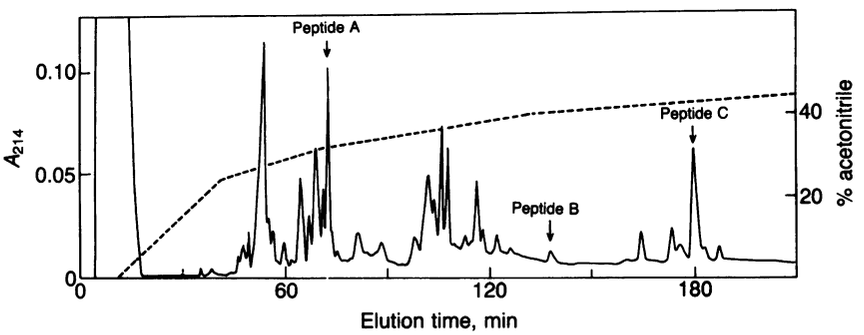

FIG. 4. Typical reverse-phase (protein C-4 column, 4.6 × 250 mm) HPLC elution profile of 1.5 nmol of a V_8 protease digest of ^{3}H-Br-CBE-labeled acid β-glucosidase. ^{3}H-labeled peptides, designated peptide A, B, or C, eluted at 32%, 40%, and 42% acetonitrile, respectively. The acetonitrile gradient in 0.05% trifluoroacetic acid was 0–24% from 12 to 42 min, 24–32% from 42 to 72 min, 32–40% from 72 to 132 min, and 40–45% from 132 to 222 min.

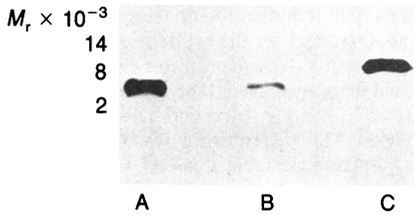

FIG. 5. NaDodSO$_4$/PAGE of radiolabeled peptides A (M_r, ≅5000), B (M_r, ≅4500), and C (M_r, ≅9500) obtained from reverse-phase HPLC.

acidic amino acid (16–18), three possible sites were indicated in this portion of peptide A: Asp^{14}, Asp^{16}, and Asp^{24}. Preliminary studies using solid-phase sequencing of peptide A indicated the presence of radioactivity only at Asp^{14}. However, only 0.5% of the total radioactivity bound to the polystyrene beads was recovered after the sequencing procedure. Thus, confirmation of this finding will be required to assign the catalytic site to Asp^{14} with certainty.

The calculated hydropathy indices and predicted secondary structure for the sequenced portion of peptide A are shown in Fig. 7. Interestingly, Asp^{14} and Asp^{24} are in relatively hydrophilic areas surrounded by two hydrophobic areas. Furthermore, Asp^{14} as well as Asp^{16} appear to be in an α-helical domain, while the residues surrounding Asp^{24} may be β-pleated sheets. The tentative assignments of Asn-Arg-(Ser) (residues 33–35) at the carboxyl terminus of peptide A indicated the presence of a site for *N*-glycosylation. This sequence was shown to be consistent with the predicted amino acid residues encoded by the nucleotide sequence of the acid β-glucosidase cDNA (Fig. 6). Compared to peptide A, which contained 50% hydrophobic amino acids, the amino acid composition of peptide B (M_r, ≅4500; data not shown), indicated only 31% hydrophobic amino acid residues. However, peptide B required an 8% higher acetonitrile concentration for elution from the reverse-phase HPLC than the more hydrophobic peptide A. This finding suggested that peptide A may be glycosylated and, therefore, eluted anomalously.

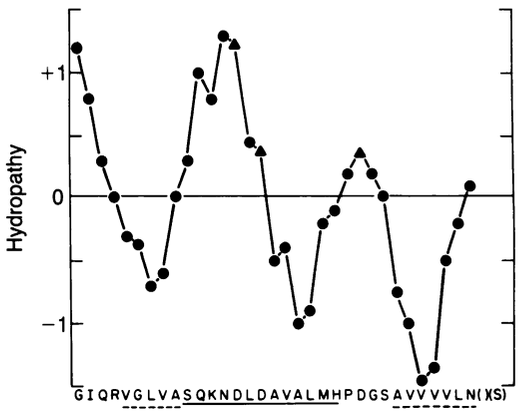

FIG. 7. Hydropathy profile (●) and the predicted secondary structure (---, β-pleated sheats; —, α-helixes shown below) of peptide A that contained the catalytic site of acid β-glucosidase. Asp^{14}, Asp^{16}, and Asp^{24} (▲) are possible ^{3}H-Br-CBE binding sites with Asp^{14} as the most likely candidate for the catalytic site.

DISCUSSION

Previous studies of human acid β-glucosidase have provided information concerning physical and kinetic properties (6, 8–13) and processing (14) of this lysosomal hydrolase in normal and Gaucher disease tissues. However, until the recent report of a complete cDNA sequence (20), little structural information had been available for the normal acid β-glucosidase. Therefore, the studies reported here were designed to characterize the primary structure of the human acid β-glucosidase catalytic site. These investigations were undertaken as a basis for subsequent structural analyses to correlate the kinetic abnormalities previously identified in the defective acid β-glucosidases in unrelated patients with the subtypes and variants of Gaucher disease (8, 11, 12). Toward this goal, homogeneous acid β-glucosidase was reacted with ^{3}H-Br-CBE to form an ester linkage at the catalytic site (23). The labeling was specific (Fig. 1) and the interaction was solely at the catalytic site in a 1:1 ratio (mol/mol) with enzymatic protein and ^{3}H-Br-CBE (Fig. 2). Amino acid sequencing of peptide A provided the primary structure of this peptide that contained the catalytic site, but the lability of the ester bond between the inhibitor and the peptide did not permit the certain identification of the amino acid to which ^{3}H-Br-CBE was bound. The amino acid sequence data were corroborated by the colinearity with the predicted sequence for residues 429–465 from the acid β-glucosidase cDNA (20). Indeed, independent confirmation of the authenticity of the cDNA clone encoding acid β-glucosidase was based, in part, on the homology of the predicted amino acid sequence and that of peptide A.

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                  1                   5                  10                  15
Peptide A         Gly Ile Gln Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala

Predicted         Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala

cDNA              GGC TCC CAG AGA GTG GGG CTG GTT GCC AGT CAG AAG AAC GAC CTG GAC GCA GTG GCA


                  20                  25                  30                  35
Peptide A         Leu Met His Pro Asp Gly  X  (Ala) Val Val Val Val Leu Asn Arg (Ser) X (Lys)

Predicted         Leu Met His Pro Asp Gly Ser  Ala  Val Val Val Val Leu Asn Arg  Ser Ser Lys

cDNA              CTG ATG CAT CCC GAT GGC TCT  GCT  GTT GTG GTC GTG CTA AAC CGC  TCC TCT AAG


                        40                  45                  50
Predicted         Asp Val Pro Pro Thr Ile Lys Asp Pro Ala Val Gly Phe Leu Glu

cDNA              GAT GTG CCT CCT ACC ATC AAG GAT CCT GCT GTG GGC TTC CTG GAG
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FIG. 6. Colinearity of the peptide A amino acid sequence with that predicted from the nucleotide sequence of a cDNA encoding human acid β-glucosidase. The nucleotide sequence was from ref. 20.

Peptide A was located near the carboxyl terminus of the amino acid sequence encoded by a full-length (496 amino acids) cDNA (20). The amino acid sequence of peptide A had no significant homology with any other protein, including V_8 protease (32), in the current SEARCH program data base (>2900 entries). Also, no obvious amino acid sequence homology exists between the catalytic site sequences of fungal or almond β-glucosidases (18, 19, 33) and that for the human enzyme. This lack of homology was consistent with the functional properties of the plant β-glucosidases, since the almond enzyme did not cleave GC (unpublished observation).

The primary structure of the peptide containing the catalytic site has several interesting features (Fig. 7) that may relate to the kinetic properties of the enzyme. The sequenced portion of the peptide A contained three acidic amino acids, all of which were aspartates. By composition analysis and comparison to the predicted amino acid sequence (Fig. 6), two additional acidic amino acid residues (aspartates) were in the unsequenced portion of peptide A. Since CBE has been shown to bind aspartate residues in the catalytic site of glucosidases from all species studied (16–19, 33), one of the aspartates most likely binds Br-CBE. Although the lability of the ester bond during gas-phase sequencing prevented the identification of the precise binding site, preliminary studies suggest that Asp^{14} is the catalytic site. It is interesting to speculate that the Br-CBE binding site and its surrounding residues may be the structural equivalents of the third domain or "allosteric" site, which have been defined by kinetic studies (9, 10). Since the properties of the third domain (9) suggest the presence of an anionic residue and a surrounding hydrophobic region, each of the aspartates at residues 14, 16, or 24 of peptide A had the necessary structure. In comparison, Asp^{38} and Asp^{45} were surrounded by more hydrophilic regions. In addition, a distinct hydrophobic domain, an aglycon binding site, for interaction with acyl chains of GC (9) has been proposed. The calculated hydropathy profile from the partial sequence of peptide A (Fig. 7) suggests that each of the three aspartates at residues 14, 16, or 24 had the required surrounding hydrophobic structure. In particular, Asp^{14} was predicted to be in a hydrophilic pocket flanked by two hydrophobic areas, conforming closely to the kinetic model that predicted the catalytic site would be in proximity to the hydrophobic aglycon binding site and the third domain (8, 9). Additional modeling studies will be required, once the Br-CBE binding site has been confirmed as Asp^{14}, for accurate predictions of the catalytic site's three-dimensional conformation. However, initial calculations suggest a high probability of α-helix formation (Fig. 7) in the region spanning amino acids 10–22 of peptide A. The presence of a *N*-glycosylation site, Asn-Arg-Ser, assigned to residues 33–35 of peptide A and the anomalous elution of this peptide suggested that glycosylation also may be important for proper active site conformation and, possibly, enzymatic activity (17).

In summary, these studies represent the isolation and characterization of a catalytic site from a human lysosomal glycosidase. These results also provide a baseline for similar approaches to define the molecular basis of the abnormal acid β-glucosidase function in human Gaucher disease (11, 12). Extension of these studies to elucidate the structure of the active sites of other lysosomal hydrolases may provide insight into the evolutionary relationships of these enzymes.

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