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Note

Rapid molecular weight determination for native glucocerebrosidase from human placenta using high-performance liquid chromatography

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Glucocerebrosidase (GCase) is a membrane-bound lysosomal hydrolase. Defects in the activity of this enzyme result in storage of glucocerebroside in reticuloendothelial tissues. The disorder is called Gaucher disease.

GCase is generally extracted from membranes with detergents. Estimations of "subunit" M_r on sodium dodecyl sulfate-polyacrylamide gel systems (SDS–PAGE)[1–3] yielded values ranging from 56,000 to 75,000. When whole tissue extracts were examined by gel exclusion chromatography (Sephadex G-200)[4] with a running buffer of Triton X-100 in 5 m*M* phosphate buffer, a M_r of 160,000–200,000 was observed for the fibroblast enzyme and 150,000 for the spleen in either 5 or 150 m*M* NaCl. Partially purified placental enzyme[5], examined on a similar column in 20 m*M* citrate–phosphate buffer containing cutscum and glycerol, was eluted at a volume corresponding to a M_r of 240,000, whereas a whole tissue placental extract revealed a 240,000 peak flanked by multiple activity peaks. GCase was thus thought to exist *in vivo* as a tetramer (in placenta) or perhaps a dimer (in spleen and fibroblasts). These studies suffered from a lack of sufficient and suitable protein standards, from working too close to the void volume of the Sephadex and from a failure to examine a completely purified protein, no longer in association with other proteins.

We have recently purified placental GCase to homogeneity by an affinity chromatography technique[1] and have examined the M_r of purified enzyme by the new and rapid procedure of high-performance liquid chromatography (HPLC). The technique is highly sensitive and obviates the necessity of making detectable derivatives, multiple activity assays and laborious elution volume measurements. Each run is complete in *ca.* 20 min. Only microgram quantities of protein are required[6,7].

EXPERIMENTAL

The HPLC system consisted of a Beckman 421 microprocessor, a single piston pump (Beckman Model 110A), variable wavelength detector (Beckman Model 155-40) set at 220 nm, a 250-μl sample injection loop and an Altex Model CRIA integrator-printer. The two columns used in series were Beckman size exclusion aqueous Spherogel, Micropak TSK Types SW 3000 and 4000, 7.5 mm × 30 cm, particle size and exclusion limits, M_r, being 10 μm and $3.0 \cdot 10^5$ and 13 μm and over $7.0 \cdot 10^5$ respectively.

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An in-line silica precolumn was used. Such columns consist of a hydrophilic phase bonded to microparticulate silica so that the nature of this surface keeps protein–silanol interactions to a minimum for neutral or acidic proteins[8]. GCase has an isoelectric point close to 5[1]. Organic modifiers can also be used to reduce hydrophobic interactions[8]. The flow-rate was maintained at 0.9 ml/min, and the separations were performed at room temperature.

When the six molecular weight standards (Pharmacia) were separated on this system (Fig. 1) using a running buffer of 100 m*M* sodium phosphate and 50 m*M* NaCl at pH 6, a linear relationship was maintained between retention time (directly related to elution volume) and log M_r between M_r 669,000 and 13,700.

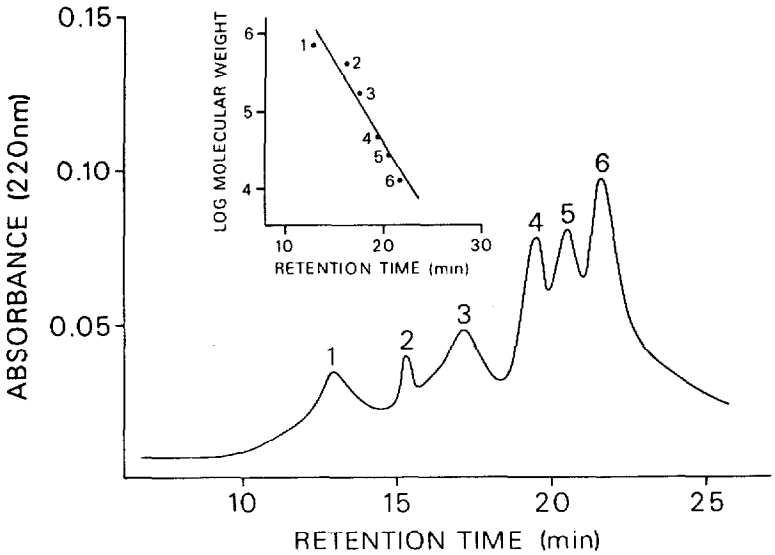

Fig. 1. HPLC separation of six molecular weight standards: 1 = thyroglobulin, M_r 669,000: 2 = ferritin, M_r 440,000; 3 = aldolase, M_r 158,000; 4 = ovalbumin, M_r 45,000; 5 = chymotrypsinogen A, M_r 25,000; 6 = ribonuclease, M_r 13,700. Conditions were as described in the text.

RESULTS AND DISCUSSION

GCase was injected immediately as it emerged from our affinity column. The enzyme was not concentrated or treated in any way before injection. In Fig. 2 the elution profile is compared to the separate elutions of lysosomal hexosaminidase (HEX) A and B from human placenta, purified in our laboratory[9]. The elution times of HEX A and B (17.74 and 18.45 min) correspond to $M\sigma$ of 120,000 and 87,000 respectively, in agreement with previously published values which ranged from 100,000 to 140,000; GCase, at 18.76 min, thus has a M_r of 74,000. These results did not change upon use of the following buffers: 1, 100 m*M* phosphate, 200 m*M* NaCl, pH 6; 2, 100 m*M* phosphate, 200 m*M* NaCl, 10% ethylene glycol, pH 6; 3, 100 m*M* phosphate, 50 m*M* NaCl, 10% ethylene glycol, pH 6; 4, 100 m*M* phosphate, 200 m*M* NaCl, 0.1% sodium taurocholate, pH 5 (230 nm). However, as the ionic strength decreased (50 m*M* phosphate, 50 m*M* NaCl) the apparent M_r of GCase increased. Finally, when salt was omitted from the buffer and detergents were present (100 m*M* phos-

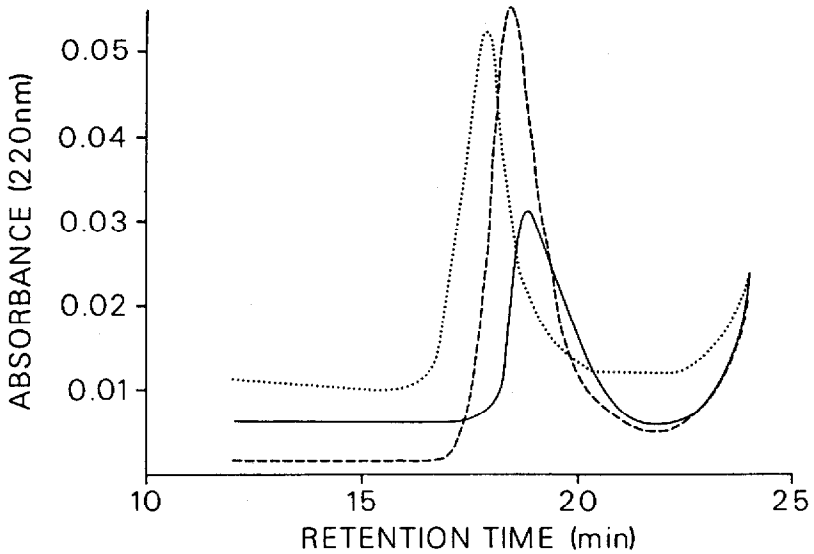

Fig. 2. HPLC analysis of GCase (——, 3 μg), HEX A (···, 4 μg) and HEX B (– – –, 4 μg). Conditions were as described in the text.

phate, 10% ethylene glycol; 100 m*M* phosphate, 0.1–0.5% sodium taurocholate; 50 m*M* phosphate, 0.1% sodium taurocholate), the apparent M_r of both GCase and HEX B increased to 174,000. We believe this is due to an artefactual aggregation phenomenon because the change is also seen with HEX B, an enzyme which has been characterized as to M_r in many laboratories[9]. It is interesting that HEX B behaves

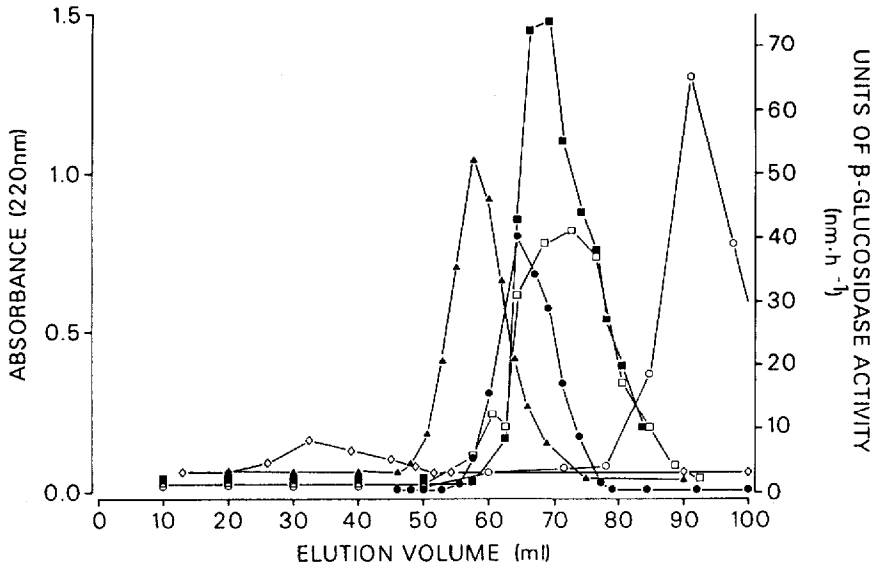

Fig. 3. Sepharose CL-6B analysis of GCase (□ or ■, two different preparations), HEX A (●), catalase (▲, 232,000), blue dextran (◇) and vitamin B_{12} (○). GCase was detected by activity[11], the others by absorbance at 220 nm.

in this fashion since this form of HEX is the more hydrophobic and tends to aggregate[10]. GCase, being a membrane protein, is also hydrophobic by nature.

GCase, HEX A, catalase, blue dextran and vitamin B_{12} were eluted separately from a 1.5 × 50 cm Sepharose CL-6B column, equilibrated with 100 m*M* phosphate, 50 m*M* NaCl, at pH 6 (Fig. 3). GCase emerges from the column later than, and therefore is smaller than, HEX A. This corroborates the HPLC results on the size of the enzyme.

After HPLC analysis of GCase, HEX A and HEX B, we analyzed the catalytic activity of the enzymes using 4-methylumbelliferyl substrates. The recovery of activity was highest for the least hydrophobic enzymes HEX A (90%) and lower for the more hydrophobic HEX B (67%) and GCase (40–50%). The retention times for HEX A and B give apparent M_r values for the native enzymes which are compatible with those in the literature and thus we believe that for GCase in its native state M_r is 74,000. When GCase is reduced and alkylated[1] it migrates on HPLC in 6 *M* guanidine hydrochloride (our data, not presented) and on SDS-PAGE[1] as a single peak with apparent M_r of 70,000 and 75,000 respectively. Our results indicate GCase is a monomer in its native active state.

This HPLC technique will prove useful in analyzing GCase from Gaucher tissue.

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REFERENCES

1 P. M. Strasberg, J. A. Lowden and D. Mahuran, *Can. J. Biochem.*, 60 (1982) 1025.
2 F. S. Furbish, H. E. Blair, J. Shiloach, P. G. Pentchev and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.*, 74 (1977) 3560.
3 E. I. Ginns, R. P. Brady, S. Pirruccello, C. Moore, S. Sorrell, F. S. Furbish, G. J. Murray, J. Jager and J. A. Barranger, *Proc. Nat. Acad. Sci. U.S.*, 79 (1982) 5607.
4 O. T. Mueller and A. Rosenberg, *J. Biol. Chem.*, 252 (1977) 825.
5 P. G. Pentchev, R. O. Brady, S. R. Hibbert, A. E. Gal and D. Shapiro, *J. Biol. Chem.*, 248 (1973) 5256.
6 T. Hashimoto, H. Sasaki, M. Auira and Y. Kato, *J. Chromatogr.*, 160 (1978) 301.
7 S. Rokushika, T. Ohkawa and H. Hatano, *J. Chromatogr.*, 176 (1979) 456.
8 *Guide to Protein and Peptide Chromatography*, Pamphlet 03-913886-00, Varian Associates, Palo Alto, CA, 1981.
9 D. Mahuran and J. A. Lowden, *Can. J. Biochem.*, 58 (1980) 287.
10 J. F. Tallman, R. O. Brady, J. M. Quirk, M. Villaba and A. E. Gal, *J. Biol. Chem.*, 249 (1974) 249.
11 P. M. Strasberg and J. A. Lowden, *Clin. Chim. Acta*, 118 (1982) 9.