Subunit Composition of Human Liver α -L-Fucosidase

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The subunit composition of human liver α -L-fucosidase was studied by using three different procedures for treating and electrophoresing the purified enzyme. Although the presence of only one subunit was found by conventional procedures using sodium dodecyl sulphate and urea, two non-identical subunits with mol.wts. of 59000 and 54000 were found when the α -L-fucosidase was reduced and *S*-carboxymethylated with iodoacetate followed by electrophoresis on polyacrylamide gels containing 0.1% sodium dodecyl sulphate and 8.0M-urea.

The lysosomal hydrolase α -L-fucosidase (fucoside fucosidase, EC 3.2.1.51) is widely distributed in nature and is found in bacteria, protozoa, marine organisms and mammals [see Alhadeff & O'Brien (1977) for a review]. The enzyme is important in mammalian metabolism because it is involved in the degradation of many biologically active molecules containing L-fucose (Spiro, 1970; Kabat, 1956; Aminoff & Furukawa, 1970; Kuhn *et al.*, 1958; Watkins, 1966). Its importance in human biochemistry is evidenced by deficiency of its activity leading to the neurovisceral storage-disease fucosidosis (Van Hoof & Hers, 1968; Durand *et al.*, 1969). The existence of two major types of fucosidosis (Hirschhorn *et al.*, 1967) and at least one atypical case (Schafer *et al.*, 1971) suggests that genetic heterogeneity exists for this fatal disease. The similarity among affected sibs also suggests that the clinical differences are genetically determined (Gatti *et al.*, 1973; Kousseff *et al.*, 1973).

Many studies on human α -L-fucosidase have been prompted by its deficiency in fucosidosis and by the availability of a simple affinity-chromatographic procedure for its purification (Alhadeff *et al.*, 1974, 1975). The enzyme has been purified from several human tissues, including placenta (Alhadeff *et al.*, 1974; Turner, 1978), liver (Alhadeff *et al.*, 1975; Thorpe & Robinson, 1978), brain (Alhadeff & Janowsky, 1977) and serum (Alhadeff & Janowsky, 1978). Many investigators have studied the subunit composition of mammalian α -L-fucosidases with conflicting results (see the Discussion section). It is important that the confusion about the subunit composition of human α -L-fucosidase be clarified, not only for characterizing the normal enzyme, but also for elucidating the molecular basis for the

previously mentioned genetic heterogeneity displayed by fucosidosis patients.

In the present investigation we have used various conditions for separating and electrophoresing the subunits of human liver α -L-fucosidase and we have found two non-identical subunits with very similar molecular weights.

Experimental

General

All procedures were carried out at 0–4°C unless otherwise stated. Protein was determined by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as standard. α -L-Fucosidase activity was assayed by using either *p*-nitrophenyl α -L-fucopyranoside (Sigma, St. Louis, MO, U.S.A.) or 4-methylumbelliferyl α -L-fucopyranoside (Koch-Light, Colnbrook, Bucks., U.K.) as previously described (Alhadeff & O'Brien, 1977). A unit of α -L-fucosidase is the amount of enzyme that hydrolyses 1 nmol of substrate/min at 37°C. The agarose- ϵ -aminohexanoylfucosamine used in the affinity-chromatographic purification of α -L-fucosidase was purchased from Miles-Yeda (Elkhart, IN, U.S.A.).

Purification of human liver α -L-fucosidase

α -L-Fucosidase was purified to apparent homogeneity by a previously described affinity-chromatographic procedure (Alhadeff *et al.*, 1975) by using agarose- ϵ -aminohexanoylfucosamine from 504 g of human liver which appeared normal on gross pathological examination.

Isoelectric focusing

Isoelectric focusing was performed as previously described (Alhadeff *et al.*, 1974, 1975) on crude liver supernatant α -L-fucosidase (210 units) and on the

Abbreviation used: SDS, sodium dodecyl sulphate.

purified α -L-fucosidase (157 units). Ampholytes were purchased from LKB Produkter (Stockholm, Sweden).

Polyacrylamide-gel electrophoresis

The presence, number and molecular weights of subunits in the purified human liver α -L-fucosidase was studied by three methods.

(a) By the general method of Laemmli (1970) in 10% polyacrylamide disc gels (0.6 cm $\times$ 10.0 cm) containing 0.1% SDS. The protein was treated for 6 min at 80°C in 0.125 M-Tris/HCl, pH 6.8, containing 5% (v/v) β -mercaptoethanol and 1% (w/v) SDS.

(b) By the method (a) above except that 8.0 M-urea was included in the running gel and 5 mM-dithiothreitol was included in the buffer for treating the protein.

(c) By the method described in (b) above except that a 3% stacking gel (0.6 cm $\times$ 0.5 cm) was used and the protein was reduced and S-carboxymethylated with iodoacetate using the procedure of Crestfield *et al.* (1963) modified as follows: (1) the reaction volumes were reduced from 12.0 ml to 0.200–0.400 ml; (2) the reaction was not run under N₂ nor in the dark; (3) the reaction was terminated by dialysis at room temperature for 16 h against three changes of a total

Fig. 1. Sodium dodecylsulphate/polyacrylamide-gel electrophoresis of human liver α -L-fucosidase

See the Experimental section under 'Polyacrylamide-gel electrophoresis' (method a) for details. (a) 3.0 μ g of α -L-fucosidase; (b) 7.5 μ g of α -L-fucosidase; (c) 10.0 μ g of α -L-fucosidase.

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of human liver α -L-fucosidase (13.0 μ g) after reduction and S-carboxymethylation with iodoacetate

See the Experimental section under 'Polyacrylamide-gel electrophoresis' (method c) for details. Arrows indicate positions of two protein bands.

of 100 vol. of 0.01 M-Tris/HCl, pH 6.8, containing 1% (w/v) SDS, 5% (v/v) β -mercaptoethanol and 8 M-urea.

All electrophoretic runs were done in 0.05 M-Tris/0.38 M-glycine, pH 8.3, containing 0.1% SDS as the running buffer. Electrophoresis was at 1 mA/gel through the stacking gel (when present) and 1.5–2.0 mA/gel through the running gel. α -L-Fucosidase (3.0–13.0 μ g) and 5–20 μ g of standard proteins (ovalbumin, Sigma, 45000 mol.wt; aldolase, Sigma, 39500 mol.wt.; bovine serum albumin, Schwarz-Mann, 67000 mol.wt.; bovine liver catalase, Sigma, 57500 mol.wt.) were treated identically as described above and were electrophoresed for approx. 4 h at 4°C. The anionic marker dye Bromophenol Blue was run in each gel. The gels were stained for protein in 0.25% Coomassie Blue in water containing 9.2% (v/v) acetic acid and 45.5% (v/v) methanol. The gels were destained in water containing 7.5% (v/v) acetic acid and 25% (v/v) methanol.

Results

The affinity-chromatographic purification of α -L-fucosidase resulted in a 4969-fold purification with 73% yield. The final specific activity was 19876 nmol/min per mg of protein. Isoelectric focusing of the crude liver supernatant and the purified enzyme indicated that all isoelectric forms of the enzymes were purified. The purified α -L-fucosidase, which appeared homogeneous by polyacrylamide-gel disc electrophoresis (Alhadeff *et al.*, 1975), was not separated by gel filtration into high- and low-molecular-weight active forms (see the Discussion section) before the subunit studies.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis using method (a) (see the Experimental section) resulted in only one protein band for various amounts of α -L-fucosidase (3.0, 7.5 and 10.0 μ g) (Fig. 1), suggesting the presence of a single subunit of approx. 53000 mol.wt. Method (b) gave results similar to method (a), also suggesting the presence of a single subunit. However, when the α -L-fucosidase was reduced and *S*-carboxymethylated with iodoacetate (method c), the presence of two protein bands was detected after electrophoresis on gels containing 8.0M-urea and 0.1% SDS (see arrows, Fig. 2). When standard proteins were treated and electrophoresed in a manner identical with that for α -L-fucosidase, the plot of log molecular weight versus protein-migration distance in the gel yielded a straight line. The molecular weights of the two α -L-fucosidase subunits were determined from this plot to be 59000 $\pm$ 2000 and 54000 $\pm$ 2000.

Discussion

The subunit composition of human liver and brain α -L-fucosidase initially was determined to be a tetramer of four identical subunits of approx. 50000 mol.wt. (Alhadeff *et al.*, 1975; Alhadeff & Janowsky, 1977). However, a later preliminary study using thin SDS/polyacrylamide slab gels (0.1 cm) suggested the presence of two non-identical subunits with very similar molecular weights for both liver (53000 and 49000 mol.wts.) and serum (56400 and 54500 mol.wts.) α -L-fucosidases (Alhadeff & Janowsky, 1978). The only other published studies on human α -L-fucosidase have found one major subunit (54000 mol.wt.) and the possibility of a minor one (135000 mol.wt.) for the liver enzyme (Thorpe & Robinson, 1978) and one major subunit (53000 mol.wt.) and a minor one (25000 mol.wt.) for the placental enzyme (Turner, 1978). Studies on rat liver (Opheim & Touster, 1977), rat epididymal (Wright *et al.*, 1976) and monkey brain (Alam & Balasubramian, 1978) α -L-fucosidases all indicate the presence of a single subunit (of 55000, 54000 and 73500 mol.wts. respectively).

The majority of previous studies have found one

subunit and have suggested that α -L-fucosidase is a tetramer of four identical subunits. However, most of these studies were done using methodology comparable with that of our method (a), in which we were also only able to demonstrate the presence of one subunit even when small amounts (3.0 μ g) of α -L-fucosidase were used (Fig. 1a). When more drastic conditions are used for treating the α -L-fucosidase (method c), the presence of two non-identical subunits in approximately equal amounts are found. Our studies indicate that the methods used for determining the presence and number of α -L-fucosidase subunits critically affect the results and that other investigators should use several different methods in their studies on the subunit composition of mammalian α -L-fucosidases. Our present results are similar to those of Carlsen & Pierce (1972), who previously found two non-identical subunits (53700 and 49700 mol.wts.) for rat epididymal α -L-fucosidase. The presence of two different α -L-fucosidase subunits could provide the molecular basis for the genetic heterogeneity displayed by fucosidosis patients.

The presence of two different α -L-fucosidase subunits also raises the interesting question of the relationship of these subunits to the high- and low-molecular-weight active forms (200000 and 50000 respectively) of the enzyme, which are separable by gel filtration on Sephadex G-200 (Robinson & Thorpe, 1973; Wiederschain *et al.*, 1973). It is possible that only one of the subunits is enzymically active and corresponds to the low-molecular-weight active form of α -L-fucosidase. At present it is not known if the subunits are enzymically active due to the denaturing conditions necessary for effecting their separation. Since the high-molecular-weight form of α -L-fucosidase has an estimated molecular weight of 200000 and since we have found approximately equal amounts of two different subunits, our studies suggest that the enzyme is a tetramer composed of two pairs of non-identical subunits. Further studies are needed (1) to find non-denaturing conditions for separating the subunits, (2) to determine if the subunits are enzymically active and (3) to determine the exact relationship of these subunits to the high- and low-molecular-weight active forms as well as to the various isoenzymes of these forms (Thorpe & Robinson, 1975; Alhadeff & O'Brien, 1977).

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