# ISOELECTRIC FOCUSING OF ISOENZYMES OF HUMAN LIVER α-L-FUCOSIDASE

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## 1. Introduction

Normal mammalian tissues contain two forms of α-L-fucosidase, distinguishable by their gel-filtration behaviour as a macromolecular fucosidase I and a low mol. wt fucosidase II [1,2]. They can be also distinguished by their pH–activity profiles and by differing responses to heat denaturation. The recent introduction of the fluorigenic substrate 4-methylumbelliferyl α-L-fucoside allows a more detailed study by electrophoretic methods and it is clear that within these two main forms there is some further heterogeneity. Gel electrophoresis of α-fucosidases from serum and from cultured cells have revealed up to six electrophoretic forms [3], and isoelectric focusing of pig-kidney enzymes has shown that fucosidases I and II from this organ are both heterogeneous [4]. In this paper we report the multiple forms that can be demonstrated by isoelectric focusing of human liver enzymes in polyacrylamide gel and in thin layers of Sephadex.

## 2. Experimental

### 2.1. *Materials*

Ampholines (pH range 3.5–10) were purchased from LKB Produkter AB Bromma, Sweden, and Sephadex G-50 Superfine for thin layer use from Pharmacia (G.B.) Ltd., London, W.5. Acrylamide and methylene *N,N'*-bis-acrylamide was from B.D.H. Chemicals Ltd., Poole, Dorset, and 4-methylumbelliferyl α-L-fucoside from Koch-Light Laboratories, Colnbrook, Bucks.

### 2.2. *Enzyme preparations*

Homogenates of human liver (50% w/v) were prepared in distilled water, incorporating, when required, a final concentration of 0.1 mM phenylmethylsulphonyl fluoride as protease inhibitor. The supernatants after centrifuging for 15 min at 9000 rev/min in an MSE 18′ refrigerated centrifuge were used as an initial source of the enzyme. The enzyme was also further purified by the affinity chromatography procedure that has been reported previously [5], and fucosidases I and II were separated by gel filtration procedures as already published [1].

### 2.3. *Acrylamide gel focusing*

Slabs of 6% polyacrylamide gel incorporating either 1% or 2% ampholines and 0.3% methylene *N,N'*-bis-acrylamide were polymerised in plastic trays (12 cm × 15 cm × 0.5 cm) as described by Vesterburg [6]. The slabs were cooled at 4°C and samples applied either directly onto the gel surface or on strips of filter paper. Carbon electrodes soaked in 1 M NaOH (cathode) or 1 M $H_2SO_4$ (anode) were placed directly on the surface of the gel and focusing proceeded for 15–18 hr at 150 V. The location of focused isoenzyme was detected by applying to the surface a sheet of chromatography paper soaked in buffered 4-methylumbelliferyl α-L-fucoside (1 mM in 100 mM phospate-citrate buffer pH 5.5). Liberated 4-methylumbelliferone could be seen under u.v. illumination and the fluorescence was intensified if necessary by treating the gel surface with 0.5 M glycine-NaOH buffer pH 10.4. Proteins were stained with Coomassie brilliant blue R [6] and the pH gradient determined by cutting out samples of the gel and eluting in boiled

distilled water overnight before measuring the pH.

2.4. *Thin-layer focusing*

Thin layers of Sephadex G-50 superfine using 1% ampholine solutions were prepared as described by Radola [7] and allowed to dry partially to a matt surface before placing in a humidity chamber for isoelectric focusing. The sample was applied directly on the surface and electrical contact was made by cellulose acetate strips. The presence of focused α-L-fucosidase isoenzymes was detected by lightly spraying the surface with the buffered substrate mixture described above and viewing under u.v. light. The areas of activity could be scraped off, suspended in water and centrifuged free of Sephadex for further investigation.

2.5. *Column isoelectric focusing*

Human liver supernatant was focused using a sucrose gradient in an Ampholine type 8100 column (LKB Instruments Ltd., London, S.E. 20 UK) as previously described [8]. The subsequent fractions were assayed for α-L-fucosidase activity fluorimetrically [9].

2.6. *Neuraminidase treatment*

A 50% (w/v) homogenate of human liver was prepared in 5 mM phosphate-citrate buffer pH 4.0 and centrifuged as before. The supernatant (100 μl) was incubated with 25 units of *Vibrio cholerae* neuraminidase (Calbiochem B grade) for 5 hr at 37°C and the sample then focused without further treatment. Controls of untreated supernatant, and supernatant treated with boiled neuraminidase were run at the same time. An affinity purified sample of liver α-fucosidase was similarly examined.

## 3. Results and discussion

Isoelectric focusing in columns (fig.1) indicates that human liver α-L-fucosidase is heterogeneous to an extent not previously revealed by ion-exchange or gel-filtration data. Four main components, with pI values

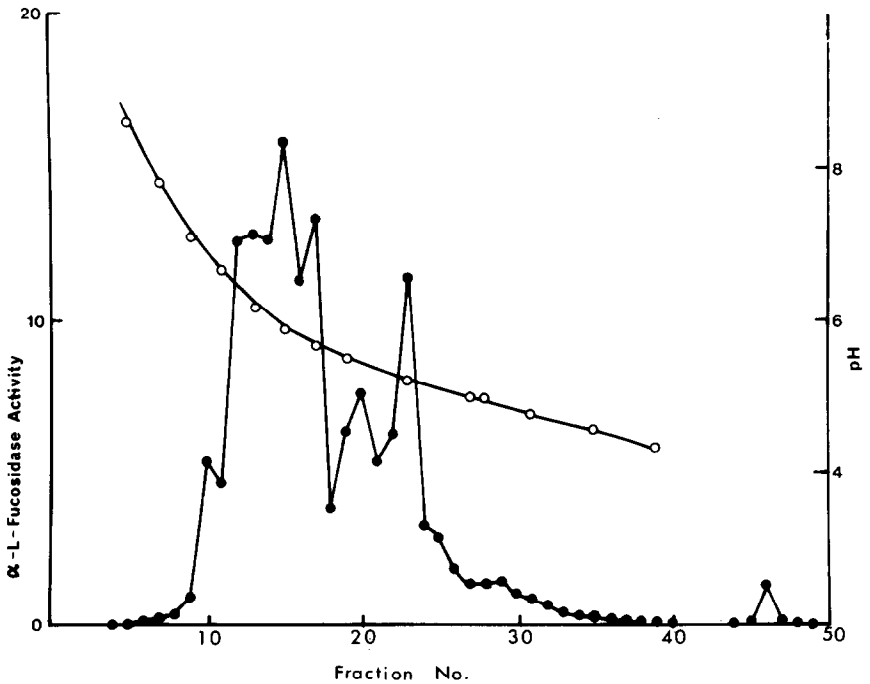


Fig.1. Column isoelectric focusing of human-liver α-L-fucosidases assayed with 4-methylumbelliferyl α-L-fucoside, (•—•) enzyme profile; (o—o) pH gradient.

between 5.0 and 6.5, can be clearly distinguished from the profile. A similar heterogeneity was shown by Wiederschain [4] and Alhadeff et al. [10]. The presence of multiple forms is particularly evident after polyacrylamide gel focusing. The number of bands seen depended on the individual liver sample and on the length of time it was allowed to react with the 4-methylumbelliferyl substrate. All samples of liver had three strong bands of activity with p*I* values 6.3, 5.9 and 5.6, and a fourth band p*I* 6.4 was frequently seen (fig.2a). Prolonged contact with the substrate revealed further bands, and up to nine components have been found in this way between the p*I* range 4.9 to 6.4 (fig.2b). The results were unaffected by the ampholine concentration or by the initial positioning of the sample. A comparison of two samples from the same liver, one of which had no added protease inhibitor, showed no visible differences. Samples of human placenta, kidney, serum and urine also show multiple forms similar to those in liver, but the relative proportions of each may vary from tissue to tissue.

An attempt was made to estimate the quantitative contribution of each component to the overall activity of a liver sample that showed four major bands. These bands, with p*I* 6.4, 6.2, 5.9, and 5.6, had 18%, 25%, 24% and 15% of the total activity, the remaining activity being largely accounted for by two other minor components. The results must be only regarded as semiquantitative, being obtained by removal of individual bands from a polyacrylamide gel and assay by the standard method [9]. No allowance was made for any differences in instability during the separation technique, but all forms appear to be of similar stability in view of the way they survive through various separation processes.

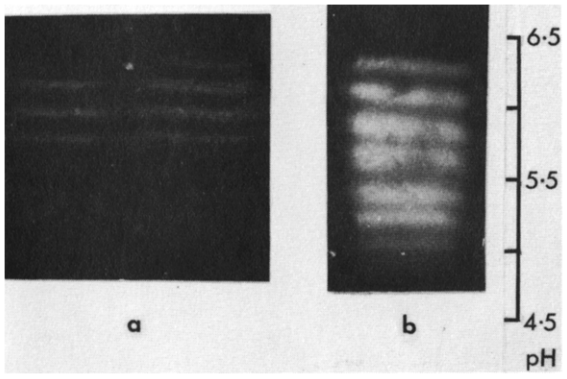


Fig.2. Isoelectric focusing of human liver α-L-fucosidases in acrylamide gel. (a) Individual livers, showing three and four major bands; (b) whole homogenate, developed to reveal weaker bands of activity, as described in the text.

Three major bands with p*I* 6.2, 5.9, and 5.6, which were obtained by electrofocusing of a liver sample in thin layer Sephadex, were scraped off, resuspended in water and the Sephadex centrifuged down. The resulting solutions were refocused in polyacrylamide and now each migrated as a single band that had retained its characteristic p*I*. Each of the forms thus appears to have a separate and stable identity, does not give rise to other forms by conformational changes, and their origin does not appear to be due to partial proteolysis during the extraction procedure.

In agreement with earlier reports [3,10], neuraminidase treatment made no difference to the electrophoretic mobility of the major forms of the enzyme, but some of the minor bands of lower p*I* value appeared to be transformed to new components when liver homogenate or purified enzyme was treated in this way. Samples of fucosidase I and II, separated by gel filtration, each gave a pattern identical to that obtained with the homogenate from which it was made. The individual components do not therefore segregate on a size basis. Our most highly purified sample of α-L-fucosidase, prepared by affinity chromatography and chromatographing as fucosidase I, was similarly heterogeneous.

The nature of the multiple forms of α-L-fucosidase remains unexplained. If it is due simply to variations in sialic acid content then a good deal of the substituent must be resistant to neuraminidase attack under the conditions used here. Some further structural features may be important, and Turner et al. [3] have discussed the possibility of genetic polymorphism. Our observation that the number of major bands varies from one individual liver to another may support this. The evidence of isoelectric focusing of fucosidase I and II leads us to ascribe these forms to different states of aggregation of the same group of isoenzymes.

**Acknowledgement**

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