Journal of Chromatography, 428 (1988) 203–205
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4163

Letter to the Editor

Human urinary β-mannosidase: behaviour towards two chromatographic supports

Sir,

In a previous study [1] we reported on the presence of only one form of β-D-mannosidase (EC 3.2.1.25) in human urine on the basis of anion-exchange chromatography on DEAE-Trisacryl. Its chromatographic behaviour was different from those observed for most other glycosidases usually fractionated in several forms on ion exchangers [2, 3]. The inability to separate different forms of β-D-mannosidase by DEAE-Trisacryl chromatography could be explained by an important loss of activity during an experiment such as that reported for human placental enzyme [4]. Thus we investigated the chromatographic behaviour of β-D-mannosidase during affinity chromatography on Concanavalin A-Sepharose.

EXPERIMENTAL

Urine was collected from healthy adults in the morning and kept freeze-dried until use.

β-D-Mannosidase activity

The enzymatic activity was determined with 4-methylumbelliferyl-β-D-mannopyranoside (Sigma, St. Louis, MO, U.S.A.). This substrate was used at a 0.0015 *M* concentration in 0.1 *M* citrate–phosphate buffer (pH 4.3). After incubation at 37°C, the liberated 4-methylumbelliferone was estimated fluorimetrically. Results are expressed as nmol of hydrolysed substrate per min and per ml of urine or enzymatic fraction.

Crude enzymatic extract preparation

Urine (2 l) was concentrated by ammonium sulphate precipitation (80% saturation), as previously described [1].

Affinity chromatography on Concanavalin A-Sepharose

The procedure was adapted from that described for urinary N-acetyl-β-D-glucosaminidase by Salvatore et al. [5].

0378-4347/88/$03.50 © 1988 Elsevier Science Publishers B.V.

A Concanavalin A-Sepharose (Pharmacia, Bois d'Arcy, France) column (6 cm×1.14 cm) was equilibrated at 4°C with a 0.025 *M* sodium phosphate buffer (pH 6.0) containing 0.15 *M* sodium chloride, at a flow-rate of 30 ml/h. After the crude extract was applied, the column was first washed with the equilibrating buffer (60 ml) and then with the same phosphate buffer containing 0.5 *M* sodium chloride until no absorbance at 280 nm was recorded. Chromatography was then performed at room temperature while the effluent was collected at 4°C. A 30-ml wash with phosphate buffer containing 1 *M* sodium chloride was applied, followed by elution with 0.5 *M* methyl-α-D-mannopyranoside in 0.025 *M* phosphate buffer containing 1 *M* sodium chloride at a flow-rate of 42 ml/h.

DEAE-Trisacryl chromatography

Chromatography was performed as previously described for serum and urine [1].

Chromatofocusing

Chromatofocusing was performed at room temperature on an anion exchanger PBE 94 (Pharmacia) equilibrated with a 0.025 *M* imidazole buffer (pH 7.4). The gradient (pH 7.4–4) was generated by a PB 74 buffer (pH 4.0) (Pharmacia) (1.5 times the column volume). After the sample had been adsorbed, elution was performed at room temperature with the same buffer at a flow-rate of 42 ml/h. Fractions were collected at 4°C.

RESULTS AND DISCUSSION

Concanavalin A-Sepharose chromatography of human urine revealed both bound and unbound β-mannosidase activities (Fig. 1). This result is opposite to that we obtained when urine was chromatographed on an anion exchanger [1]. To investigate this discrepancy, each fraction from Concanavalin A-Sepharose chromatography was chromatographed on DEAE-Trisacryl. The bound form was adsorbed, then eluted with 0.16 *M* sodium chloride. This chromatographic behaviour is the same as reported for the previously isolated urinary β-mannosidase [1]. Under the same experimental conditions, we were unable to detect any β-mannosidase activity after DEAE-Trisacryl chromatography of the unbound form. These results may be related to an instability of this enzyme during ion-exchange chromatography, as reported for human placental β-mannosidase [4].

The loss of activity of the unbound form during DEAE-Trisacryl chromatography explains why only one form is obtained when crude urine is chromatographed on this support [1].

The properties of the two urinary forms were characterized. Both forms have the same optimum pH (5) but different pH_i values: 5.60 for the unbound form and 4.30 for the bound one. We found no evidence for a form with neutral optimum pH as described for goat liver [6].

The chromatographic behaviour towards Concanavalin A-Sepharose suggests the presence in urine of two forms with different carbohydrate compositions. In his work Dawson [6] suggests that goat liver contains two structurally different

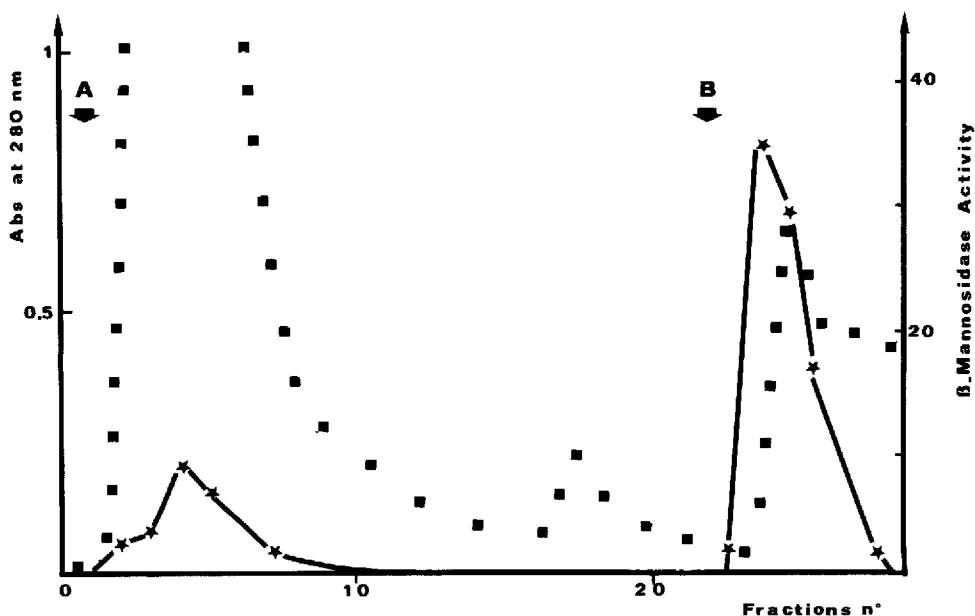

Fig. 1. Concanavalin A-Sepharose chromatography of urinary β-mannosidase. (A) Equilibrating buffer; (B) A+0.5 M α-D-methylmannoside. (■) Absorbance at 280 nm; (★) β-mannosidase activity.

forms of β-mannosidase: a lysosomal one (bound on Concanavalin A-Sepharose) with optimum pH 5–5.5 and a cytosolic one, unbound, with an optimum pH in neutral range. As the optimum pH values of the two urinary forms are identical, and in the acidic range, it seems difficult to conclude that the unbound form is not of lysosomal origin.

According to Paigen and Peterson [7], several lysosomal enzymes are actively secreted in urine. Further studies on the renal β-mannosidase are in progress in order to elucidate the origin of urinary enzymes.

Laboratoire de Chimie Biologique, U.E.R. de Biologie Humaine et Expérimentale, Université René Descartes, 4 Avenue de l'Observatoire, 75006 Paris (France)

B. RICARD
M. BERNARD
M.J. FOGLIETTI*
F PERCHERON

1 M. Bernard, M. Sioud, F. Percheron and M.J. Foglietti, Int. J. Biochem., 18 (1986) 1065.
2 M. Bernard, M.J. Foglietti and F. Percheron, Clin. Chim. Acta, 116 (1981) 91.
3 M. Paraire, R. Bourbouze, F.Ch. Baumann and F. Percheron, Clin. Chim. Acta, 129 (1983) 233.
4 Ch. Noeske and G. Mersmann, Hoppe-Seyler's Z. Physiol. Chem., 364 (1983) 1645.
5 D. Salvatore, S. Bonatti and P. Dinatale, Bull. Biol. Med., 9 (1984) 111.
6 G. Dawson, J. Biol. Chem., 257 (1982) 3369.
7 K. Paigen and J. Peterson, J. Clin. Invest., 61 (1978) 751.

(Received December 18th 1987)