Purification of an Acid α -Glucosidase by Dextran-Gel Filtration

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1. The behaviour of rat liver α -glucosidases on dextran gel (Sephadex G-100) columns was studied. A 'retardation' of an acid α -glucosidase activity was observed. This activity was identified as lysosome α -(1 $\rightarrow$ 4)-glucosidase. A single gel-filtration step resulted in a 700-fold purification of the enzyme. The same technique was also used to purify the acid α -glucosidase of human kidney. 2. The acid α -glucosidases of both tissues show very similar pH optima when tested with maltose or glycogen as substrate.

The α -glucosidases catalyse the hydrolysis of glycogen and oligosaccharides in mammalian tissues (Lejeune, Thinès-Sempoux & Hers, 1963; Torres & Olavarria, 1964; Brown & Brown, 1965). Two α -(1 $\rightarrow$ 4)-glucosidases, usually distinguished by their pH optima, have been found in the liver: the acid and the neutral α -glucosidases. The former enzyme is localized in the lysosomes and probably degrades glycogen from its outer chain ends to free glucose (Hers, 1963).

Hers (1964) has found that the lysosomes from rat livers contain an enzyme that splits α -(1 $\rightarrow$ 6)-glucosidic bonds and this fact supports the hypothesis that glycogen can be completely hydrolysed to glucose by the lysosomes. The neutral α -glucosidases of some mammals hydrolyse both glycogen and oligosaccharides (Lejeune *et al.* 1963; Torres & Olavarria, 1964); however, the human liver enzyme appears to be an oligo- α -(1 $\rightarrow$ 4)-glucan glucosidase having no capacity to hydrolyse glycogen (Brown & Brown, 1965). Both types of glucosidase exhibit a maltase activity (Lejeune *et al.* 1963; Torres & Olavarria, 1964; Brown & Brown, 1965).

Torres & Olavarria (1964) used the same purification procedure for both types of α -glucosidase from dog liver. The purification of the acid enzyme, however, required several steps and only very low recoveries of activity were achieved. The purpose of the present investigation was to develop a simpler method for the purification of acid α -glucosidase that would give good yields of a purer enzyme and could be applied without modification to extracts of many different tissues.

The elution volumes of proteins filtered through dextran-gel columns are generally functions of their molecular weights (Wieland, Duesberg & Determann, 1963; Whitaker, 1963; Andrews, 1964; Auricchio & Bruni, 1964). However, it is known

that some molecules are 'retarded' by Sephadex columns and at least two mechanisms are thought to be responsible for this: (a) adsorption and (b) charge effects (Flodin, 1962, p. 72). It has been proposed that the 'retardation' of α -amylase (EC 3.2.1.1) on dextran gel is due to enzyme-substrate-type interactions. This explanation is supported by the similarities in the structures of starch and dextran (Flodin, 1962, p. 63). Acid α -glucosidase has a weak dextranase activity (Rosenfeld, 1964) so that interactions between the protein and dextran gel would be expected to 'retard' the elution of this enzyme also from gel columns. It should thus be possible to separate the acid α -glucosidase from other proteins of similar molecular weight that are not 'retarded'. Our results show that the acid α -glucosidase is indeed 'retarded' and that by means of this property it is possible to purify it extensively from different tissues with good recovery of initial activity.

MATERIALS AND METHODS

Sephadex G-100 (lot no. To 1358; particle size 40–120 μ) was obtained from Pharmacia, Uppsala, Sweden. All other reagents were of analytical grade.

The α -glucosidase activity was determined by measuring the rate of formation of glucose from maltose (maltase activity) or of glucose from glycogen (in this paper indicated as glucoamylase activity) by modification of the method of Hers (1963): 1 mg. of the incubation mixture contained 4 mg. of maltose or 8 mg. of glycogen together with 60 μ -moles of buffer. The buffers used were acetic acid-sodium acetate, pH 3.5–5.5; citric acid-NaOH, pH 6.5; imidazole-HCl, pH 7.0 and 7.5. The reactions were allowed to proceed at 37° for 0.5–2 hr. and were then stopped by the addition of NaOH and ZnSO₄ (final concentrations 0.06 N and 0.03 M respectively). The glucose in the protein-free solution was determined by the method of Huggett & Nixon (1957) with the exception that the phosphate buffer was replaced by 0.5 M-tris-HCl buffer (Dahlqvist, 1961). The amounts of

glucose liberated were proportional both to protein concentration and to the incubation time. Protein concentration was measured by the method of Lowry, Rosebrough, Farr & Randall (1951), the modified reagent B introduced by Eggstein & Kreutz (1955) being used. Crystalline bovine plasma albumin was used as standard.

Sephadex G-100 columns (1.5 cm. diam. $\times$ 67 cm.; void volume 40 ml.; internal volume 74 ml.) were prepared as previously described (Auricchio & Bruni, 1966): the flow rates were approx. 10 ml./hr. A solution of 1 mM-EDTA, containing 25 mM-NaCl, adjusted with 2N-NaOH to pH 6.7, was used for chromatographic development of the Sephadex columns. All chromatographic experiments were done at 3°.

Rat liver. Wistar male rats were starved for 30 hr. and then killed by a blow on the head. The same solution used for chromatographic development of the gel columns was also used for perfusion and homogenization of rat livers. The livers were perfused and were then homogenized in an MSE homogenizer with 1 vol. of solution for 3 min. The homogenate was frozen and thawed and was then centrifuged at 105 000g for 60 min. at 4°. The supernatant was concentrated by dialysis under reduced pressure. Generally two ranges of protein concentration were used for the gel-filtration experiments: 25 mg./ml. or 280 mg./ml., or both. Samples (5–7 ml.) of the concentrate were pipetted on to the top of gel columns and subjected to gel filtration. In most cases samples of the eluted fractions were examined for their E_{280} values, for maltase activity at pH 3.6 and 7.5 (indicated as acid- and neutral-maltase activity respectively) and for glucoamylase activity at pH 3.6.

Human kidney. A sample of human kidney, which was found to be normal by both histological and electron-microscopic criteria, was removed and immediately frozen. After thawing the tissue was homogenized and filtered through a single layer of cheesecloth. The filtrate was centrifuged, concentrated and filtered through dextran gel in the same way as for rat liver extract. The eluted fractions were examined for their E_{280} values and for acid-maltase activity.

RESULTS

Rat liver preparations. The elution diagram of samples containing 25 mg. of protein/ml. showed coincident peaks of neutral- and acid-maltase activity and were eluted with most of the 280 m μ -adsorbing material (Fig. 1a, between 27 and 65 ml.). In the following fractions a low acid-maltase activity was detectable. This activity could be more clearly demonstrated when samples containing 280 mg. of protein/ml. were filtered. In these cases, besides the first peak of acid-maltase activity, a second peak of the same type of activity appeared (Fig. 1b, between 100 and 230 ml.). This peak, which was eluted after the total volume of the column and therefore evidently 'retarded', had the same elution volume as a peak of glucoamylase activity at the same pH (Fig. 1b). The material eluted between 170 and 230 ml. of the chromatogram described by Fig. 1(b) contained 20% of the applied acid-maltase activity and was 700-fold purified compared with the sample used for the gel filtration. The latter had about the same specific activity as the homogenate:

Fig. 1. Elution diagram of rat liver α -glucosidase activities on a Sephadex G-100 column (1.5 cm. $\times$ 67 cm.) equilibrated with a solution of 1 mM-EDTA containing 25 mM-NaCl, pH 6.7. Samples applied to the column were (a) 7 ml. containing 25 mg. of protein/ml. and (b) 5 ml. containing 280 mg. of protein/ml. The eluate was examined for E_{280} (O), for maltase activity at pH 3.6 ($\blacktriangle$) and at pH 7.5 ($\triangle$), and for enzymic activity forming glucose from glycogen at pH 3.6 ($\square$).

i.e. 1.5 m μ moles of maltose hydrolysed/min./mg. of protein.

In a single experiment, 9 ml. of a sample containing 290 mg. of protein/ml. was filtered through dextran-gel columns and the eluted fractions were tested for maltase activity at pH 7.5 and pH 4.5 (Fig. 2). The activity at pH 4.5 was also measured in the presence of 30 mM-turanose, a specific inhibitor of lysosomal α -glucosidase (Hers, 1963). In the presence of turanose the maltase activity was diminished, the second peak being almost completely inhibited.

Both the maltase and the glucoamylase activities of the purified enzyme were 90% inhibited by 30 mM-turanose at pH 4.5.

Fig. 4(a) shows the pH-dependence of glucose production from maltose and from glycogen by the purified enzyme. The pH optima were 4.5 and 5.0 respectively.

The enzyme was stable after freeze-drying and could be stored at -20° without loss of activity for at least 2 weeks.

Fig. 2. Elution diagram of rat liver α -glucosidase activities on a Sephadex G-100 column (1.5 cm. $\times$ 67 cm.) equilibrated with a solution of 1 mM-EDTA containing 25 mM-NaCl, pH 6.7. The sample applied to the column was 9 ml. containing 290 mg. of protein/ml. The eluate was examined for E_{280} (O), and for maltase activity at pH 4.5 in the absence of (Δ) and in the presence of ($\bullet$) 30 mM-turanose and at pH 7.5 (Δ).

Fig. 3. Elution diagram of human kidney acid α -glucosidase activity on a Sephadex G-100 column (1.5 cm. $\times$ 67 cm.) equilibrated with a solution of 1 mM-EDTA containing 25 mM-NaCl, pH 6.7. The sample applied to the column was 6 ml. containing 80 mg. of protein/ml. The eluate was examined for E_{280} (O) and for maltase activity at pH 3.6 ($\blacktriangle$).

Human kidney enzyme. Fig. 3 presents the elution profile of the acid-maltase activity from a human kidney sample. The homogenate hydrolysed 7 μ -

Fig. 4. pH-dependence of the hydrolysis of maltose (O) and glycogen (Δ) by rat liver (a) and human kidney (b) acid α -glucosidases purified by gel filtration.

moles of maltose/min./mg. of protein. The material eluted between 180 ml. and 300 ml. was pooled and further examined. The specific activity of the acid maltase was about 500-fold greater than that of the enzyme in the sample used for the gel filtration (which hydrolysed 10 μ moles of maltose/min./mg. of protein) and was recovered in a 20% yield. The maltase activity measured at pH 4.5 was 95% inhibited by 30 mM-turanose. The pH optima for the hydrolysis of maltose and of glycogen to glucose by the purified enzyme were 4.5 and 4.7 respectively (Fig. 4b).

DISCUSSION

Dextran-gel filtration is a very simple and efficient way to purify the acid α -(1 $\rightarrow$ 4)-glucosidase from different tissues. The purification and the recovery of the initial activity of the enzyme purified by means of this technique are higher than those obtained by other authors with a more laborious method (Torres & Olavarria, 1964).

The strong turanose inhibition of the second peak of the maltase activity measured at pH 4.5 (Fig. 2)

strongly suggests that this peak is due to the lysosomal α -glucosidase. This suggestion is further supported by the pH-dependence of the rates of hydrolysis of maltose and of glycogen by the purified enzyme (obtained by pooling the eluate corresponding to the descending part of the second peak of the acid maltase activity). The pH-dependence is very similar to that of the acid α -glucosidases purified from dog liver and from rat liver lysosomes (Torres & Olavarria, 1964).

The first peak of acid-maltase activity at pH 4.5 (Fig. 2) or pH 3.6 (Fig. 1b), eluted together with a peak of strong neutral-maltase activity, could, at least in part, be due to the activity of the neutral α -glucosidase at this pH, and its low turanose inhibition (Fig. 2) to the overlapping of this enzyme with the acid α -glucosidase.

The claims (Hers, 1963; Rosenfeld, 1964; Torres & Olavarria, 1964) that the acid α -glucosidase is responsible for the hydrolysis of both maltose and glycogen are now supported by two new lines of evidence: (a) the second peak of the acid-maltase activity and the single peak of glucoamylase activity at pH 3.6 have identical elution volume (Fig. 1b); (b) 30mM-turanose inhibits both the maltase and the glucoamylase activity at pH 4.5 to the same extent.

The acid- α -glucosidase activity purified from human kidney is very similar to that purified from rat liver with regard to the following properties: adsorption on dextran gel, pH optimum with maltose and glycogen as substrates and inhibition

by turanose of the acid-maltase activity. The acid α -glucosidases purified from different tissues of different mammals appear to be very similar to one another.

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