DEXTRAN-GEL FILTRATION OF RAT LIVER α-GLUCOSIDASES

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At least two enzymes are responsible for the hydrolysis of α-glucosyl disaccharides in mammalian liver: the acid and the neutral α-glucosidases[1-3]. The first enzyme is active at acid pH, is present in lysosomes and seems to be able to degrade glycogen; the other is active at neutral pH, is localized for the most part in the microsomes and degrades oligosaccharides like maltose and maltotriose but, at least in some mammals, not glycogen[3].

At present the functions of these enzymes are not completely clear. The neutral enzyme probably degrades oligosaccharides such as those yielded by the action of liver α-amylase. A function for the acid glucosidase may be inferred from a consideration of Pompe's disease. The only known enzymic defect associated with this glycogen storage disease is the lack of an acid glucosidase[4]. Thus this enzyme might function by degrading glycogen from its outer chain ends to free glucose, so that besides the degradation of glycogen *via* phosphorolysis and α-amylolysis, it is now supposed that degradation *via* glucamylolysis in mammalian tissues can also occur[5].

The three pathways of glycogen degradation and the possible role of the two glucosidases are briefly represented in Fig. 1.

Fig. 1. Pathways of glycogen degradation.

TORRES AND OLAVARRÍA were able to purify both α-glucosidases from dog liver[2]. The acid enzyme was purified about 30 times with a recovery of 2.6%.

As the acid enzyme has a weak dextranase activity[5] the behaviour of the two α-glucosidases on dextran gel was studied. An interaction between the acid glucosidase and dextran gel (for instance of the enzyme-substrate type) was possible, and would be expected to "retard" the elution of the acid glucosidase from the gel column, thus separating the acid from the neutral glucosidase and from other proteins which are not adsorbed on dextran gel. The results show that it is possible to separate the neutral from the acid glucosidase by dextran-gel filtration. By using this tech-

nique the acid enzyme from rat liver was purified about 700 times with a recovery of 20% of the initial activity present in the crude extract.

MATERIALS AND METHODS

Glucose was obtained from Merck, Darmstadt, Germany; shell fish glycogen, peroxidase and glucose oxidase were from Sigma Chemical Co., St. Louis, Mo., U.S.A.; Tris was supplied by C.F. Boehringer und Soehne G.m.b.H., Mannheim, Germany; *o*-dianisidine by British Drug Houses Ltd. All other reagents were of analytical grade.

The α-glucosidase activity was measured by the formation of glucose from maltose. The incubation mixture contained 20 μmoles of buffer (acetic acid–sodium acetate at pH 3.6, imidazole–HCl at pH 7.5) and 4 mg of maltose per ml. The formation of glucose from glycogen was measured only at pH 3.6 (the glycogen concentration was 8 mg per ml of the reaction mixture). The reaction was run at 37° for 0.5–1 h and stopped by the addition of NaOH and $ZnSO_4$. The glucose in the protein-free solution was determined enzymatically *via* glucose oxidase and peroxidase according to HUGGET AND NIXON[6] with the exception that 0.5 *M* Tris–HCl, pH 7, was used instead of phosphate buffer. Protein was measured by the method of LOWRY and coworkers[7], the modified reagent B introduced by EGGSTEIN AND KREUTZ being used[8].

Gel filtration

Sephadex G-100 was suspended in a solution of 6 *M* urea, stirred with a magnetic stirrer for about 1 h and exhaustively washed with water. The smallest particles were removed by decantation and a suspension of the gel was deaerated and packed into columns (1.5 cm diameter × 67 cm long). Columns of gel were equilibrated with the desired solution, which was allowed to pass through until the height of the gel bed remained constant. The flow rates of the columns were approximately 15 ml/h.

RESULTS

Five or six Wistar male rats were starved for 30 h and then killed. The livers were perfused with ice-cold 1 m*M* EDTA–25 m*M* NaCl, pH 6.7. The same solution was used in every step of the experiment. The livers were homogenized for 3 min with an equal volume of liquid. The homogenate was frozen and thawed, and was then centrifuged at 105,000 × *g* for 60 min. The supernatant was concentrated by dialysis at reduced pressure. 7 or 8 ml of the sample, containing about 200 mg protein per ml, were pipetted on to the top of a gel column and filtered. When the sample was applied on the column a reduction of about 5 ml of the gel bed volume occurred.

The eluted fractions were examined for extinction at 280 mμ, for maltase activity at pH 3.6 and 7.5, and for glucose formed from glycogen at pH 3.6. It should be noted that maltase activity in liver is due to the α-glucosidases and that if the acid α-glucosidase is a glucamylase it forms glucose from glycogen.

Fig. 2 shows the elution diagrams from a Sephadex G-100 column. (A) is the extinction at 280 mμ, (B) the maltase activity at pH 7.5, (C) the maltase activity at pH 3.6, and (D) the glucose formed from glycogen at pH 3.6.

Curve B shows that the neutral maltase activity is eluted with most of the protein.

Graph C shows the elution profile of acid maltase activity; at least two peaks are recognizable. The first peak corresponds to the peak of neutral maltase activity and it is possible that it is due to the activity of the neutral α-glucosidase at this pH. The second peak, eluted after the total volume of the gel column, is a peak of activity which has clearly been retarded. There are three interesting facts to emphasize at this point: (1) the negligible extinction at 280 mμ, corresponding to the second peak of acid maltase activity, (2) the negligible neutral maltase activity, and (3) a peak of glucose production from glycogen at acid pH visible from the elution diagram presented in D. The fact that the second peak of acid maltase activity and the single peak of glucose formed from glycogen have the same elution volume from the gel column, and that both activities are retarded, supports the claim of several authors that both activities are due to the same enzyme, the acid α-glucosidase.

The specific enzymic activity of the fraction corresponding to the descending part of the elution profile of the second peak of acid maltase activity, where the neutral activity is absent, was measured. The acid maltase activity was found to have been purified 700 times after the gel filtration with a recovery of 20 % of the initial activity in crude extract.

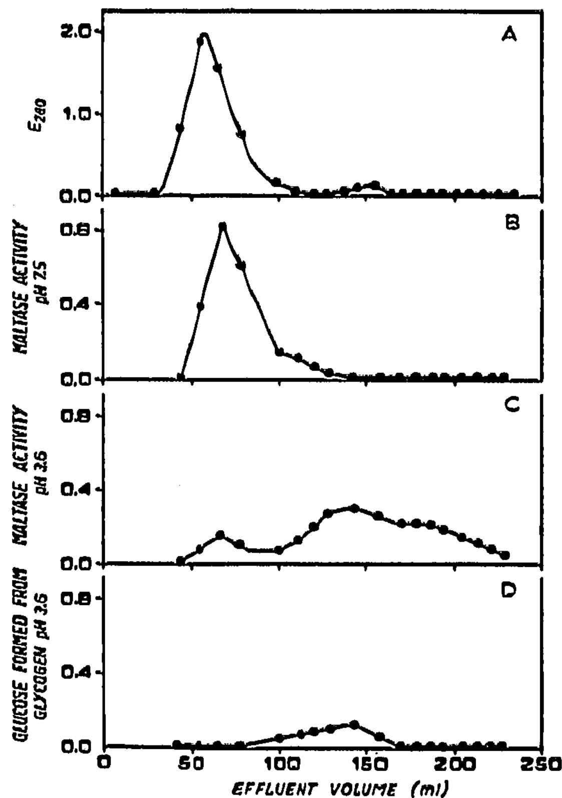

Fig. 2. Elution diagrams from a Sephadex G-100 column (1.5 cm × 67 cm) of (A) extinction at 280 mμ, (B) maltase activity at pH 7.5, (C) maltase activity at pH 3.6, and (D) glucose formed from glycogen at pH 3.6.

SUMMARY

The behaviour of the acid and neutral α-glucosidases from rat liver on dextran gel was studied.

As the acid enzyme has dextranase activity an interaction between the acid α-glucosidase and dextran gel was possible and would be expected to "retard" the elution of the enzyme from the gel column, thus separating the acid α-glucosidase from proteins which are not adsorbed on dextran gel. By using dextran gel filtration, the acid α-glucosidase was purified about 700 times with a recovery of 20% of the initial activity in crude extract.

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