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METABOLIC REGULATION OF β-GLUCOSIDASE IN THE GUT CONTENT OF THE SNAIL *ACHATINA ACHATINA*

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Summary

An energy-dependent polymerization-depolymerization phenomenon in the β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) in the gut contents of the giant African snail *Achatina achatina* has been observed. The formation of the octamer, tetramer and dimer from the monomer (mol. wt. approx. 41 000) and vice versa are catalysed by proteins also present in the gut contents.

It has been noted that β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) obtained from a number of sources differ in molecular weight [1]. These various forms of β-glucosidase appear to be multiples of the form with a molecular weight of about 40 000 found in the urine of rats in which kidney damage was chemically induced [2]. Multiple enzymically active forms of β-glucosidase (mol. wt. approx. 40 000, 80 000, 160 000 and 320 000) have been found in culture filtrates of *Botryodiplodia theobromae* Pat. [1]. The behaviour of the enzyme from *B. theobromae* suggested that the multiplicity of β-glucosidase sometimes observed in preparations from the same source [3,4] may be due to varying degrees of aggregation of the monomers rather than to complex formation between a single enzyme and some polysaccharides as reported for the aryl β-glucosidase of *Stachybotrys atra* [5]. That the multiplicity of β-glucosidase is due to varying degrees of aggregation, at least in some instances, is shown by the results presented here.

In these studies, the gut contents of the giant African snail *Achatina achatina* were drained, centrifuged at 10 000 × *g* for 15 min at 4°C and the supernatant solution was used as source of β-glucosidase. Gel filtration of the gut contents obtained from active snails revealed the presence of two forms of β-glucosidase with molecular weights of about 82 000 (species C) and 41 000 (species D) estimated by their behaviour during gel filtration [6]. The elution profile is shown in Fig. 1a. When aestivation was induced by

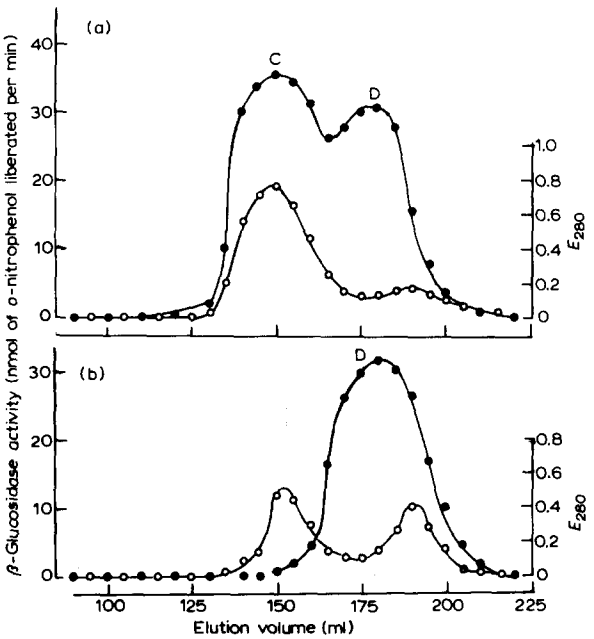

Fig.1. Gel filtration of the gut contents of (a) active snails and (b) aestivating snails on a column of Sephadex G-100 (88 × 2.5 cm) equilibrated with the elution buffer (0.1 M citric acid/0.2 M Na_2HPO_4 buffer containing 0.1 M NaCl, pH 5.0). Total protein was estimated by absorbance at 280 nm (○). The distribution of β-glucosidase activity (●) was determined by measuring the amount of *o*-nitrophenol liberated/min at 40°C in a reaction mixture containing 2 ml of 1.0 mM *o*-nitrophenyl β-D-glucopyranoside in 0.05 M sodium acetate buffer (pH 5.0) and 0.5 ml eluate.

starvation and the gut contents from these aestivating snails was used for gel filtration, only the lower molecular weight form (species D) was found (Fig. 1b). When aestivating snails were reactivated by supplying plenty of water and leaves and maintained in the activated state for 6—7 days, β-glucosidase species C and D were found in their gut contents. Since the disappearance and re-appearance of species C in the gut contents may be due to depolymerization and polymerization, respectively, induced by the difference in the nutritional states of the snails, the gut contents of active snails were made 10 mM with respect to ATP and $MgSO_4$ and used for gel filtration after incubation at 30°C for 45 min. Fig. 2a shows the elution profile of the β-glucosidase components on a column of Sephadex G-200 (superfine) of the Mg^{2+}-ATP-treated gut contents. Fig. 2b shows the elution profile obtained when the gut contents of aestivating snails were similarly treated. The molecular weights of the β-glucosidase components found were estimated by gel filtration to be 329 000 (species A) and 165 000 (species B). All four species of β-glucosidase (A—D) were found to differ in properties; Michaelis constants

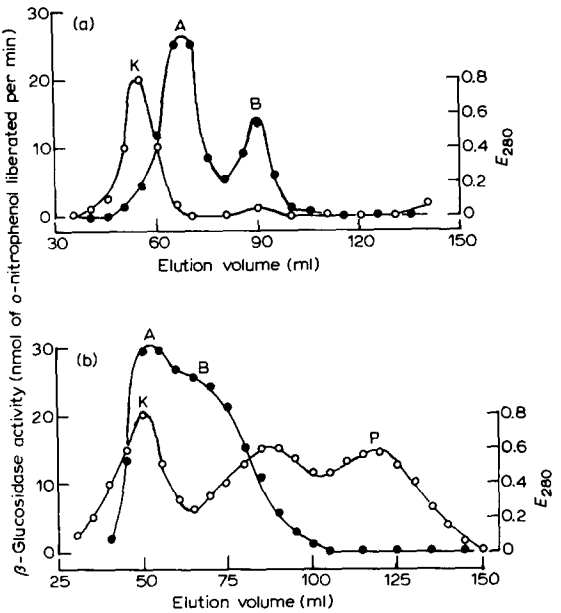

Fig. 2. Gel-filtration of (a) the gut content of active snails on a column of Sephadex G-200 (superfine) (33 × 2.5 cm) and of (b) the gut content of aestivating snails on a column of Sephadex G-100 (34 × 2.5 cm) after the gut contents had been incubated at 30°C for 45 min in the presence of 10 mM ATP and 10 mM $MgSO_4$. The columns were equilibrated with the eluting buffer (0.1 M citric acid/0.2 M Na_2HPO_4 buffer containing 0.1 M NaCl, pH 5.0). Total protein was estimated by absorbance at 280 nm (○). The distribution of β-glucosidase activity (●) was determined as shown in the legend to Fig. 1.

(K_m) calculated from double-reciprocal plots being 0.17 mM, 0.13 mM, 0.11 mM and 0.09 mM *o*-nitrophenyl β-D-glucopyranoside as substrate [7] for species A, B, C and D respectively. The calculated maximum velocities were 217.4 nmol, 96.9 nmol, 43.5 nmol and 34.8 nmol of *o*-nitrophenol liberated/min/mg protein for species A, B, C and D respectively. Thus, affinity for the substrate appears to decrease with increasing molecular complexity whereas catalytic rate appears to increase with increasing molecular complexity.

These results suggested that the interconvertibility of the various β-glucosidase forms which is indicated by the evidence available might have resulted from enzyme-catalysed polymerization and depolymerization since each β-glucosidase component was eluted at the expected position on re-chromatography. That this idea is correct was established by testing the main non-β-glucosidase protein components observed in the elution profiles of the gut contents for ability to induce polymerization or depolymerization of β-glucosidase components. Incubation at 40°C for 45 min of a mixture of β-glucosidase D and the protein peak marked K in Fig. 2a in the presence of

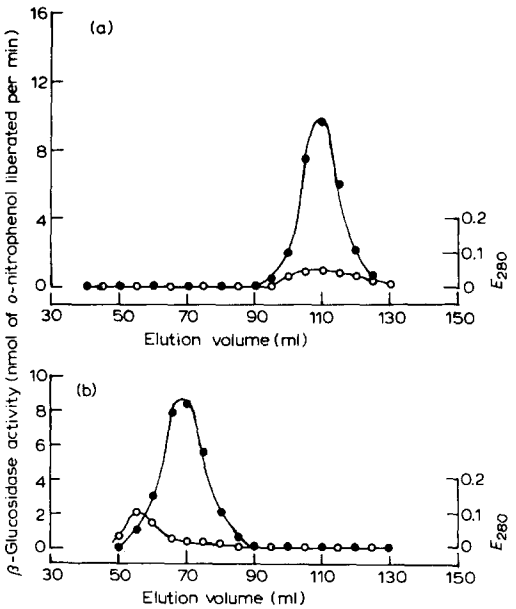

Fig.3. Gel filtration of β-glucosidase D (mol.wt. approx. 41 000) on (a) a column of Sephadex G-100 (34 × 2.5 cm) before and on (b) a column of Sephadex G-200 (superfine) (33 × 2.5 cm) after incubation at 40°C for 45 min in the presence an equal volume of protein peak K (2ml), 10 mM ATP and 10 mM $MgSO_4$. The columns were equilibrated with the eluting buffer (0.1 M citric acid/0.2 M Na_2HPO_4 buffer containing 0.1 M NaCl, pH 5.0). Total protein was estimated by absorbance at 280 nm (○). β-glucosidase distribution (●) was determined as shown in the legend to Fig.1. β-glucosidase D and protein peak K were obtained by re-chromatography of fractions obtained from separations similar to those shown in Figs. 1a and 2a, respectively.

10 mM ATP and $MgSO_4$ showed that this β-glucosidase species was quantitatively converted into β-glucosidase A (Fig. 3). Intermediate forms were obtained when the incubation was for shorter periods. Similarly, incubation at 40°C for 45 min of a mixture of β-glucosidase A and the protein peak marked P in Fig. 2b in the presence of 10 mM $MgSO_4$ resulted in the generation of lower-molecular-weight species of β-glucosidase (Fig. 4). When 2ml of a solution of β-glucosidase D (mol.wt. approx. 41 000) that had been purified by a method essentially similar to that used for the purification of β-glucosidase from *B. theobromae* [7] was mixed with an equal volume of protein peak K and incubated at 40°C it was found that an increasing amount of ADP was released with time. At the end of the reaction, 3 nmol of ADP was released per nmol of β-glucosidase D. Gel filtration of the excess reaction mixture revealed the presence of only β-glucosidase A. In this experiment, the amount of ADP released was determined enzymically by coupling the pyruvate kinase reaction to the lactate dehydrogenase reaction [8,9]. Protein

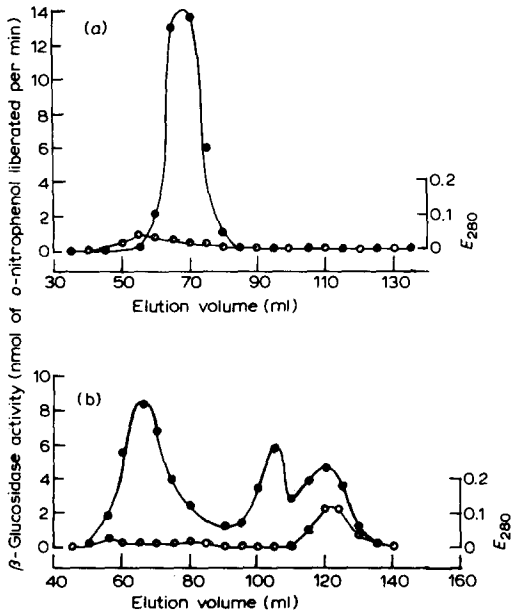

Fig.4.

Gel-filtration of β-glucosidase A (mol. wt. approx. 329 000) on (a) a column of Sephadex G-200 (Superfine) (33 × 2.5 cm) before, and on (b) a column of Sephadex G-100 (34 × 2.5 cm) after incubation at 40°C for 45 min in the presence of an equal volume of protein peak P (2 ml) and 10 mM $MgSO_4$. The columns were equilibrated with the eluting buffer (0.1 M citric acid/0.2 M Na_2HPO_4 buffer containing 0.1 M NaCl, pH 5.0). Total protein was estimated by absorbance at 280 nm (○). β-glucosidase activity of the eluates (●) was determined as shown in the legend to Fig.1. β-glucosidase A and protein peak were obtained by re-chromatography of fractions obtained from separations similar to those shown in Fig.2a and b respectively.

peak K thus appears to contain an enzyme capable of mediating a possible phosphorylation and consequent polymerization of lower molecular weight forms of β-glucosidase. On the other hand, the free phosphate content, determined by the method of Watts and Moreland [10], of a mixture containing 5 ml of protein peak P, β-glucosidase A and 10 mM $MgSO_4$ in a total volume of 10 ml was found to increase with time on incubation at 40°C. Although these results may suggest phosphorylation and dephosphorylation reactions, any other energy-requiring process, such as glycosylation, would result in ATP consumption.

Since gel filtration indicated that the high molecular weight polymerizing enzyme was virtually absent in crude gut contents of *A. achatina* but appeared after treatment with Mg^{2+}-ATP, it is possible that its activation involves enzyme-catalysed polymerization of a low molecular weight protein present in the untreated gut contents but not in those treated with Mg^{2+}-ATP (compare Figs. 1 and 2). The probable activating enzyme has not been detected.

It would appear that the modification of the structure and activity of the β-glucosidase in the gut contents of *A. achatina* may have some physiological significance in the light of changes in the environmental availability of food and water. Thus, the observed interconversion of β-glucosidase forms may be a means for regulating the rate of glucose production, after cellulase action, for glycolytic reactions in this herbivorous snail whose main source of carbohydrate is cellulose [11].

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