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β-L-ARABINOSIDASE FROM *CAJANUS INDICUS*: A NEW ENZYME

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SUMMARY

A new enzyme, β-L-arabinosidase, has been detected in the dormant seeds of *Cajanus indicus*. This enzyme is different from α-galactosidase and does not hydrolyse an α-D-galactoside or an α-D-fucoside.

INTRODUCTION

α-Galactosidases are known to hydrolyse α-D-galactoside, α-D-fucoside and β-L-arabinoside[1–6]. Two factors are similar in these substrates, namely, the glycosidic linkage and the orientation of the hydrogen and hydroxyl groups at C-2, C-3 and C-4 of the pyranoid glycon residues. A similar type of specificity is displayed by the anomeric enzyme β-galactosidase[7–10]. In some cases the β-galactosidase activity has been separated from that of β-fucosidase[11–12]. However, there is no report of the existence of a specific β-L-arabinosidase.

In this paper, the separation and partial characterization of a β-L-arabinosidase are reported.

MATERIALS AND METHODS

Enzyme assays

p-Nitrophenyl-D-galactosides were prepared as described earlier[13] and *p*-nitrophenyl β-L-arabinoside was synthesized by the method of Feier and Westphal[14]. *p*-Nitrophenyl α-D-fucoside was a gift from Professor G. A. Levvy, Rowett Research Institute, Bucksburn, Aberdeen. All other reagents were of analytical grade.

The enzyme activities were assayed by adding appropriately diluted enzyme solution (0.3 ml) to a mixture of McIlvaine buffer, pH 5.5 (0.2 ml) and the respective substrate (0.5 ml of 10 mM solution in buffer) pre-incubated at 30 °C. After incubation for 15 min at this temperature, the reaction was stopped with 5 ml 0.1 M Na_2CO_3. The alkaline solution developed a yellow colour due to *p*-nitrophenol which was liberated during the enzymic reaction. The absorbance was measured at 405 nm

on an Unicam SP 500 spectrophotometer. Under these assay conditions a linear relationship was observed between product formation and amount of enzyme or time of incubation; the substrate was also found to be stable.

A unit of enzyme activity is defined as the amount that hydrolyses one μmole of the substrate per min under the specified conditions. The specific activity was expressed as enzyme munits/mg of protein. Protein was determined by the method of Lowry *et al.*[15].

Sephadex-gel filtration

A Sephadex G-100 column (2.4 cm × 100 cm) was prepared as recommended by Andrews[16] using McIlvaine buffer, pH 5.5, and containing 0.1 M KCl. All enzyme preparations were equilibrated against this buffer by dialysis, before passing through the Sephadex column. The column was maintained at 4 °C and the same buffer was used for elution at a flow rate of 30 ml/h; 3-ml fractions were collected. The column was then calibrated by determining the elution volumes of a number of reference proteins of known molecular weight. The results are expressed in terms of R_{SA} values (*i.e.* elution volume of bovine serum albumin/elution volume of the protein).

RESULTS AND DISCUSSION

Detection and purification of the enzyme

All purification steps were carried out at approximately 4 °C. Powdered *Cajanus indicus* seeds were washed with acetone (at −15 °C) prior to use. In a typical enzyme extraction experiment, 500 g of the acetone powder was suspended in 1 l McIlvaine buffer, pH 5.5, for 24 h after which the extract was separated by centrifugation. The existence of a specific β-L-arabinosidase was first observed when the crude enzyme

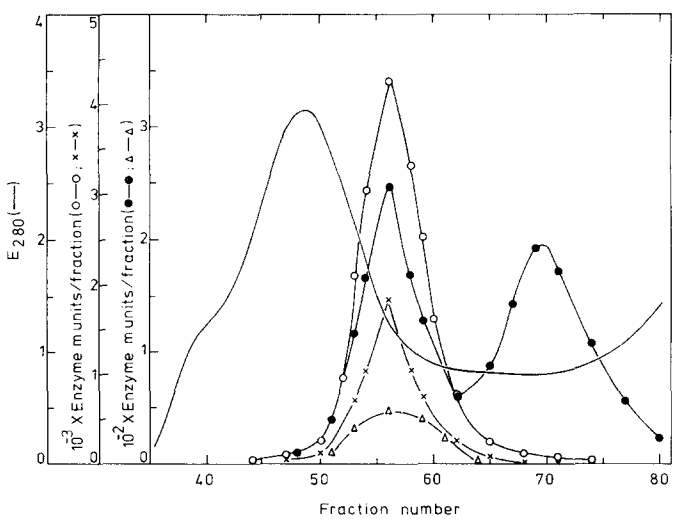
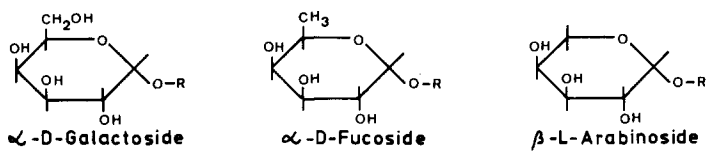


Fig. 1. Elution pattern of various enzyme activities from Sephadex G-100 column. 5 ml of the crude enzyme preparation was applied. Experimental conditions are given in the text. ———, absorbance at 280 nm using a 10 mm cell; ○—○, α-galactosidase activity; ●—●, β-L-arabinosidase activity; ×—×, α-fucosidase activity; △—△, β-galactosidase activity.

preparation was passed through a Sephadex G-100 column. The fractions were separately assayed for the activities of α-galactosidase, α-fucosidase, β-L-arabinosidase and β-galactosidase. The elution pattern (Fig. 1) shows that a major protein peak appeared well before the enzyme activities were eluted. All four enzyme activities were eluted in a single peak. At a higher V_e value, a second peak of β-L-arabinosidase activity appeared; this protein apparently has a lower molecular weight. In contrast to the first enzyme peak, there were no other associated enzyme activities in the area of this second peak. It is, therefore, apparent that the second peak consisted of a specific β-L-arabinosidase. Out of four enzyme activities of the first peak only β-galactosidase could be separated using a CM-cellulose column (Dey, P. M., unpublished). Attempts to separate rest of the three activities from one another using DEAE-cellulose, hydroxyapetite or Cγ alumina were not successful.

The parallel appearance of α-galactosidase, α-fucosidase and β-L-arabinosidase in the first peak, and their inseparable nature can be explained on the basis of known substrate specificity of α-galactosidase[4-6]. The structural similarity among the respective substrates can be seen as follows.



In analogy to β-galactosidase[10], all the above substrates were shown to be hydrolysed by a homogeneous α-galactosidase preparation[5,6].

The specific activity of β-L-arabinosidase in the crude extract was found to be 12. The first step of purification consisted of acid denaturation; the pH of the crude extract was lowered to 4.0 with 0.5 M citric acid and allowed to stand for 24 h. A thick precipitate of denatured proteins was separated by centrifugation and then

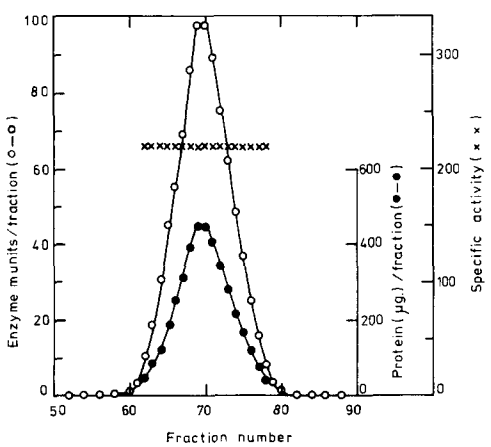


Fig. 2. Sephadex G-100 gel filtration of purified β-L-arabinosidase. Procedure and conditions were the same as described in Fig. 1. ○—○, β-L-arabinosidase activity; ●—●, protein; ×—× specific activity of the enzyme.

the pH of the clear supernatant was adjusted to 5.0 with 0.5 M Na_2HPO_4. As described earlier, the β-L-arabinosidase activity was determined only after separating the enzyme by Sephadex-gel filtration. The specific activity was 84 (a 7-fold increase) with a 80% recovery of the enzyme. The supernatant from this step was further purified by $(NH_4)_2SO_4$ fractionation. The 30–50% fraction contained 85% of the enzyme activity and showed a specific activity of 220 when analysed by gel filtration. The active fractions of the enzyme (obtained after the gel filtration) were concentrated by ultrafiltration and recycled through the same Sephadex column. The enzyme activity and the protein were eluted in single and symmetrical peaks parallel to one another (Fig. 2). The specific activity of the enzyme in the fractions was the same as that of the preparation applied to the column. The finally purified enzyme could be stored for two months without any loss of activity, in McIlvaine buffer, pH 5.0, at 4 °C.

Characterization of the enzyme

The ultraviolet spectrum of the enzyme showed a maximum absorption at 280 nm. The $E_{280\ nm}$ was 19 and the $E_{280\ nm}/E_{260\ nm}$ ratio was 2.0.

The apparent molecular weight of the enzyme was determined by Sephadex G-100 gel filtration[16]. The calibration curve of the Sephadex column is shown in Fig. 3 and the molecular weight of β-L-arabinosidase in relation to the marker proteins was found to be 25 900.

The enzyme reaction followed a linear course with respect to time and enzyme concentration, provided that the extinction of the final assay at 405 nm did not exceed 0.9. This was determined under the conditions described for enzyme assay.

The enzyme preparation showed a single protein band on polyacrylamide-gel electrophoresis at pH 8.3.

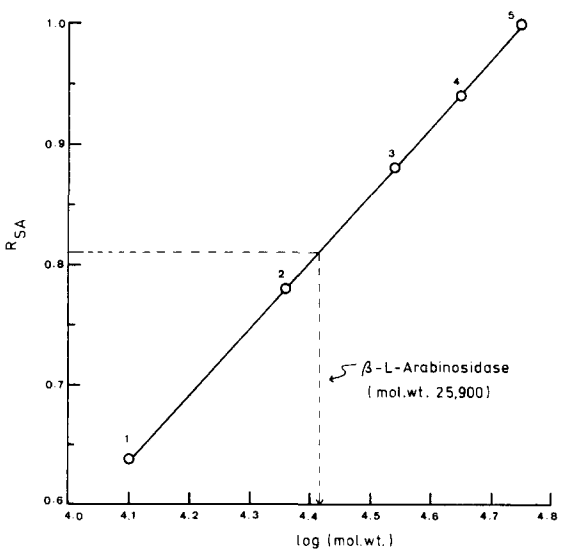


Fig. 3. Determination of the molecular weight of β-L-arabinosidase by Sephadex G-100 gel filtration. R_{SA} is defined in the text. Proteins used for calibration of the column were: 1, ribonuclease; 2, α-chymotrypsin; 3, pepsin; 4, ovalbumin; 5, serum albumin.

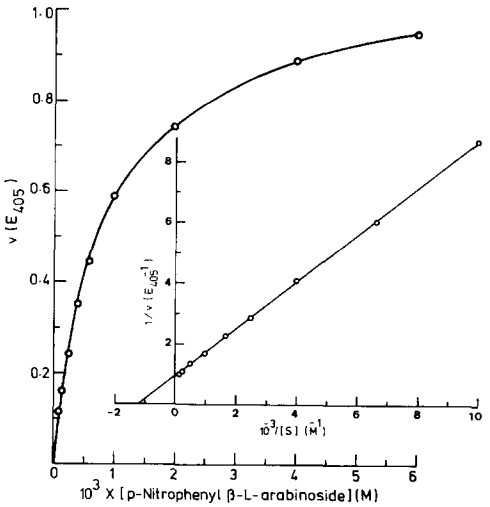


Fig. 4. Effect of substrate concentration on the initial rate of hydrolysis of *p*-nitrophenyl β-L-arabinoside in McIlvaine buffer, pH 4.0. β-L-Arabinosidase (32 μg) was incubated with varying amounts of substrate and the enzyme activity determined as described in the text. A Lineweaver–Burk double-reciprocal plot is given as an inset.

The effect of substrate concentration on the initial rate of enzyme activity is shown in Fig. 4; a simple Michaelis law was obeyed. The K_m and the V values as determined from the Lineweaver–Burk double-reciprocal plot[17] were found to be 0.83 mM and 780 munits/mg enzyme, respectively.

The pH–activity curve (Fig. 5) shows that the enzyme has a broad, acidic pH optimum (3–4.6). The enzyme was completely stable in the pH range 2–6.5 for 30 min at 30 °C.

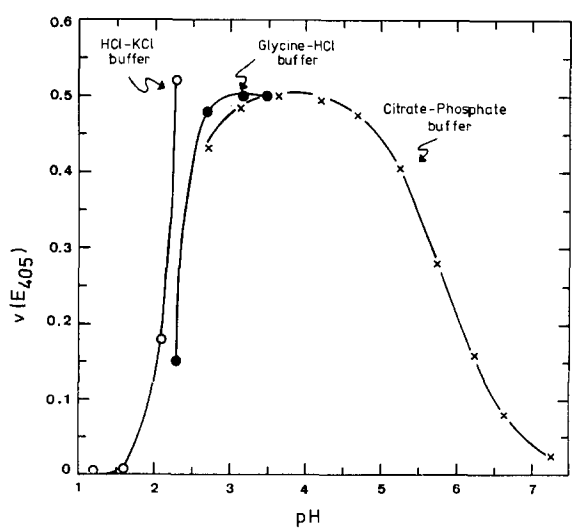


Fig. 5. Effect of pH on the activity of β-L-arabinosidase. Experimental conditions were the same as described for the enzyme assay except that different buffers were used.

The enzyme showed a high degree of substrate specificity. Under the normal assay conditions, but using 10-fold increased enzyme concentration, β-L-arabinosidase was unable to hydrolyse *p*-nitrophenyl α-D-galactoside, *p*-nitrophenyl α-D-fucoside or *p*-nitrophenyl β-D-galactoside.

ACKNOWLEDGEMENTS

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