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α-GALACTOSIDASE FROM COCONUT KERNEL

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Abstract—The properties of two forms, A and B, of α-galactosidase from coconut kernel are described. They are interconvertible and in the absence of KCl the lower MW form, B, is favoured. When B is mixed with KCl and an enzymatically inactive protein fraction, C, from the kernel, there is almost complete conversion to A.

INTRODUCTION

α-Galactosidase (α-D-galactoside galactohydrolase, E.C. 3.2.1.22) occurs in multiple forms in various plant species [1]. We have recently detected this enzyme in coconut kernel [2] and the activity was resolved into two molecular forms by Sephadex gel-filtration. Previously it was reported that the two forms (A and B) had MWs of 123000 and 21400 respectively, and that they were interconvertible [2]. This paper describes the purification, interconversion and characterization of the α-galactosidases.

RESULTS AND DISCUSSION

Interconversion of enzymes

The partially purified α-galactosidase, P, had a sp. act. of 25.5 nkat/mg protein. Sephadex gel-filtration of this preparation in the presence of 0.1 M KCl resolved the α-galactosidase activity into two fractions, the major component A and B (sp. act. 35 and 38 nkat/mg protein, respectively). On repeating the gel-filtration of P in the absence of KCl in the column buffer, only a single peak of activity, corresponding to the lower MW enzyme, B, appeared: the sp. act. of this fraction was 47 nkat/mg protein and the total activity was equal to the sum of the activities of A and B. A protein peak, C, corresponding to the elution volume of A was also obtained from the column in the absence of KCl but it was devoid of α-galactosidase activity. It, therefore, appears that the conversion of A to the lower MW from B is inhibited by the presence of KCl. A single α-galactosidase peak (sp. act. 139 nkat/mg protein) corresponding to B and an inactive protein corresponding to C were also obtained if fraction P was passed through a Sephadex column in McIlvaine buffer containing 0.1 M NaCl in place of KCl. α-Galactosidase B obtained in this way was concentrated by ultrafiltration and stored at 4°. After 3 weeks some inactive protein was precipitated and this increased the sp. act. of the supernatant solution to 204 nkat/mg protein.

Conversion of B to an α-galactosidase with the same apparent MW as A was also observed. This occurred when enzyme B was mixed with the inactive protein fraction C and then placed on a Sephadex column which was eluted with McIlvaine buffer, pH 5.5, containing 0.1 M KCl. In the absence of C no conversion could be demonstrated.

Properties of α-galactosidases

At 55°, enzyme B was more stable than A. The preparations retained 38% and 16%, respectively, of their original activities after heating for 10 min despite the fact that in these experiments the protein concentration of the preparation A was higher than that of B. The reconstituted enzyme A which was obtained from B displayed a thermal stability similar to that of the original enzyme A. Enzyme A, reconstituted A and B were all anionic and showed identical electrophoretic mobilities on cellulose acetate at pH 5.5. The three forms showed similar biphasic pH/activity profiles using *p*-nitrophenyl α-D-galactoside as the substrate with the higher activities occurring, in all cases, at pH 5.6 (see Fig. 1).

All three enzymes were inhibited by concentrations of *p*-nitrophenyl α-D-galactoside exceeding 7.7×10^{-4} M.

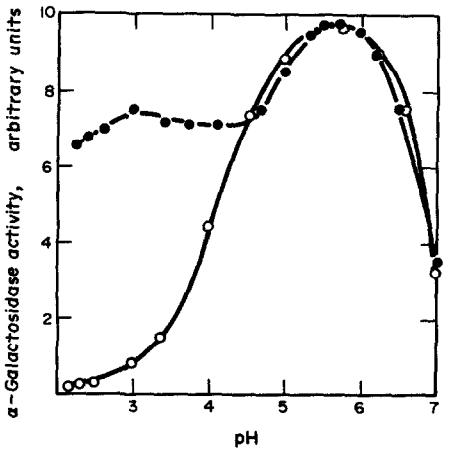

Fig. 1. Effect of pH on the coconut kernel α-galactosidase A —●— (the profiles for enzymes B and reconstituted A were identical); effect of 0.2 M glycine on the activity of α-galactosidase B —○—.

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The K_m values as determined by the Lineweaver–Burk plot [3] were the same for all the enzyme forms, i.e. 4.5×10^{-4} M. The V_{max} values for α-galactosidase A, reconstituted α-galactosidase A and α-galactosidase B were, 129, 70 and 332 nkat/mg protein, respectively.

The effect of glycine, citrate and phosphate (final concentrations 0.2 M) on the activities of enzymes A and B were examined at pH 3 and at 5.6. No significant inhibition by any of these compounds was observed at pH 5.6 but at the lower pH, glycine inhibited A and B by 74 and 80%, respectively. Phosphate produced 95% inhibition of both A and B: citrate had no inhibitory effect on either α-galactosidase. Glycine was also observed to change the pH/activity profile of enzyme B (Fig. 1): inhibition was shown to be noncompetitive with a K_i value of 5.3×10^{-2} M.

EXPERIMENTAL

Coconuts were purchased locally. Small pieces of coconut kernel were homogenised for 2 min at 4° (1 g kernel/1.5 ml McIlvaine buffer, pH 5.5). The homogenate was strained through cheesecloth and was centrifuged at 40000 *g* 20 min at 4°. The pH of the supernatant was lowered to 3.7 by addition of 0.1 M citric acid with continuous stirring and the ppt. was removed by centrifugation. The resulting supernatant (at 0° and stirred continuously) was adjusted to pH 5.5 with satd Na_2HPO_4. The ppt. obtained on adding $(NH_4)_2SO_4$ (50–75%) to this enzyme soln was dissolved in McIlvaine buffer, pH 5.5, and dialysed for 18 hr against this buffer to remove salt. This fraction was termed preparation P. Dilute enzyme solns were concentrated by ultrafiltration using a UM2 membrane.

α-Galactosidase activity was assayed as described in Ref. [4] and protein determined by the method of Ref. [5]. The sp. act. of the enzyme is recorded as nkat per mg protein under the specified assay conditions. The different molecular forms of α-galactosidase were separated on Sephadex G-100 columns (2.5 × 100 cm) equilibrated with McIlvaine buffer [6], pH 5.5, containing 0.1 M KCl (20 ml/hr, 3 ml/fraction). Protein was measured in each fraction by *A* at 280 nm.

Electrophoresis was affected on cellulose acetate using McIlvaine buffer, pH 5.5, diluted 1:1 with H_2O. Protein bands were detected with nigrosine and α-galactosidase activity with 4-methylumbelliferyl α-D-galactoside [7]. In studies on the effect of organic and inorganic ions on α-galactosidases A and B, control incubations were made in McIlvaine buffer and reactions were started by adding *p*-nitrophenyl α-D-galactoside. Inhibitor solns were prepared by dissolving the appropriate amounts of glycine, citric acid or Na_2HPO_4 in H_2O and then adjusting to the required pH with either HCl or NaOH. The final concns of these compounds in the incubation mixtures were 0.2 M.

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REFERENCES

1. Dey, P. M. and Pridham, J. B. (1972) *Adv. Enzymol.* **36**, 91.
2. Balasubramaniam, K., Dey, P. M. and Pridham, J. B. (1974) *Biochem. Soc. Trans.* **2**, 1128.
3. Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658.
4. Dey, P. M. and Pridham, J. B. (1969) *Biochem. J.* **113**, 49.
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
6. McIlvaine, T. C. (1921) *J. Biol. Chem.* **49**, 183.
7. Dey, P. M. and Wallenfels, K. (1974) *European J. Biochem.* **50**, 107.