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SHORT COMMUNICATION

MULTIPLE FORMS OF α-GALACTOSIDASE IN *VICIA FABA* SEEDS

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Abstract—Two forms of α-galactosidase have been isolated from *Vicia faba* seeds and their physical and chemical properties compared.

THE PRESENCE of α-galactosidase (α-D-galactoside galactohydrolase; E.C. 3.2.1.22) in *Vicia faba* seeds was first demonstrated by Pridham and Walter.[1] We now report purification and separation of two proteins with α-galactosidase activity, from the dormant broad bean seeds.

Courtois and Petek[2] published the results of their separation on an alumina column of two α-galactosidases from coffee but apparently these enzymes possessed quite similar properties. In the present case, the two forms from beans show different properties as illustrated in Table 1.

TABLE 1.

Properties	α-Galactosidase I	α-Galactosidase II
Molecular weight*	209,000	38,000
Carbohydrate content†	25%	2·8%
Inactivation at 60°, pH 4·0 (30 min)	42%	80·6%
pH optimum (raffinose as substrate)	3·5–5·5	4·0
K_m‡	$1{\cdot}14\times10^{-3}$ M	$6{\cdot}9\times10^{-4}$ M
V_{max}‡	42·1	2·8
Energy of activation§	15·3 kcal/mole	27·2 kcal/mole

* Determined by the method of Andrews.[3]

† Glucose equivalents (phenol–H_2SO_4 method[4]).

‡ *o*-Nitrophenyl-α-D-galactoside was used as substrate and activity assayed at pH 4·0. V_{max} expressed as μmole substrate hydrolysed per min per mg enzyme protein at 30°.

§ Hydrolysis of *p*-nitrophenyl-α-D-galactoside was followed at pH 4·0, in the temperature range 30–45°.

[1] J. B. PRIDHAM and M. W. WALTER, *Biochem. J.* **92**, 20P (1964).
[2] J. E. COURTOIS and F. PETEK, *Meth. Enzymol.* **8**, 565 (1966).
[3] P. ANDREWS, *Biochem. J.* **91**, 222 (1964).
[4] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS and F. SMITH, *Anal. Chem.* **28**, 350 (1956).

Purification of the enzymes involved the following steps: (1) extraction of the powdered seeds with McIlvaine buffer, pH 4·8; (2) lowering the pH of the extract to approximately 3·0 with citric acid and discarding the residue; (3) removal of nucleic acids from the supernatant solution with protamine sulphate; (4) acetone fractionation at −20°, pH 4·8. The protein fraction obtained at 30–60 per cent acetone was dissolved in buffer (pH 4·8) and dialysed against four changes of distilled water followed by McIlvaine buffer, pH 3·5 (diluted 1:5 with distilled water). This gave rise to a precipitate which was dissolved in buffer, (pH 4·8) and fractionated on a Sephadex G-100 column (2·4 × 50 cm; eluted with 0·1 M-KCl-McIlvaine buffer, pH 5·5). Two active α-galactosidase peaks (I and II; Fig. 1) were obtained.

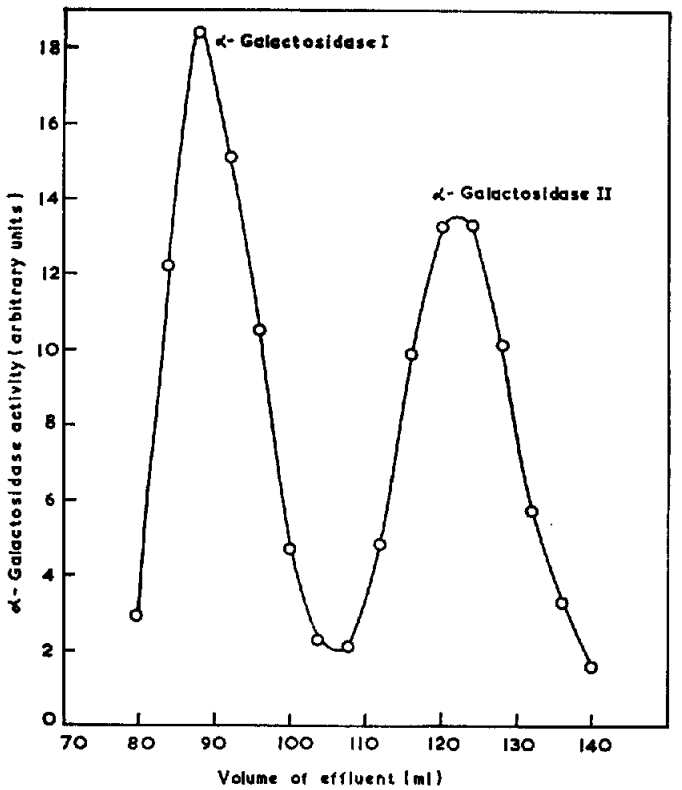

FIG. 1.

In the last step, fractions I and II were re-cycled through the G-100 column to effect further purification. At this stage, the overall purification of α-galactosidases I and II were 3600- and 337-fold, respectively. Both enzyme preparations appeared to be homogeneous when examined by polyacrylamide gel electrophoresis and with an analytical ultracentrifuge.

An examination of changes in α-galactosidase in bean seeds soaked under anaerobic conditions showed that the total level of activity was unaffected for a period of 6 days. Normal germinations under aerobic conditions produced a 30 per cent increase in activity after 3 days. This phenomenon may explain the rapid degradation of galactose-containing oligosacharides during germination: this is inhibited in the absence of oxygen (cf. Shiroya[5]).

[5] T. SHIROYA, *Phytochem.* **2**, 33 (1963).

Various properties of the two enzyme forms are summarized in Table 1. Further studies on the mechanism of action, substrate specificity and physiological importance of these enzymes are in progress in this laboratory.

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