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# STUDIES ON THE DISTRIBUTION OF α-GALACTOSIDASES IN SEEDS

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**Abstract**—Dormant seeds from various higher plant species have been examined for multiple forms of α-galactosidase. The properties of corresponding forms from *Pisum sativum* and *Vicia faba* have been compared. The high molecular form (I) from *P. sativum* has been purified 2770-fold.

## INTRODUCTION

α-GALACTOSIDASES (α-D-galactoside galactohydrolase, E.C. 3.2.1.22) have been shown to be widely distributed in nature[1,2] and it is generally believed that they are involved in the degradation of galactose-containing oligosaccharide and polysaccharide reserves.[1,3–5] Only in a few cases have the α-galactosides been purified and their properties investigated in detail.[2,5–13] The dormant seeds of *Vicia faba* contain at least two molecular forms of α-galactosidase which are separable by Sephadex gel filtration.[8,9] We have now surveyed the occurrence of multiple forms of α-galactosidase in various seeds and a comparison of *P. sativum* and *V. faba* enzymes has been made. One *P. sativum* enzyme has been purified to a high specific activity.

## RESULTS AND DISCUSSION

### *Molecular Forms of α-Galactosidases*

α-Galactosidase activity was detected in the crude extracts of all the dormant seeds listed in Table 1 and in five leguminous and two other species two molecular forms, I and II, of the enzyme were observed. The levels of I and II in each species are compared in Fig. 1 which shows that in all cases, except *Helianthus*, the level of the high molecular weight form (I) is greater than that of the low molecular weight form (II). Seeds which are not fully mature and/or have too high a water content may not exhibit this difference and in the

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TABLE 1. NUMBER OF MOLECULAR FORMS AND MOLECULAR WEIGHTS OF α-GALACTOSIDASES FROM DORMANT SEEDS

| Source | | Molecular weight* |
|---|---|---|
| Aceraceae | | |
| *Acer pseudoplatanus* | I | 167,000 |
| | II | 50,000 |
| Compositae | | |
| *Helianthus annuus* | I | 159,000 |
| | II | 23,000 |
| Leguminosae | | |
| *Phaseolus aureus* | I | 209,000 |
| | II | 38,000 |
| *Phaseolus vulgaris* | I | 125,900 |
| | II | 39,800 |
| *Pisum sativum* | I | 121,600 |
| | II | 32,300 |
| *Vicia dumetorum* | I | 195,000 |
| | II | 57,000 |
| *Vicia faba* | I | 209,000 |
| | II | 38,000 |
| *Vicia sativa* | I | 166,000 |
| | II | 77,000 |
| *Cajanus indicus* | | 87,000 |
| *Medicago sativa* | | 38,000 |
| Rosaceae | | |
| *Prunus amygdalus* | | 32,400 |
| Rubiaceae | | |
| *Coffea* sp. | | 26,300 |

* By gel filtration.

extreme case, e.g. green *V. faba* seeds, form I may be absent. In addition, purification procedures may alter the ratio of the two forms; hence it would always seem advisable, when making these semiquantitative comparisons, to examine only simple buffered extracts of tissues.[14] Petek *et al.*[13] have shown that germinating *V. sativa* seeds contain a single α-galactosidase with an apparent molecular weight of 30,000. Our own studies with *V. faba*[14] indicate that the high molecular weight enzyme I diminishes rapidly during the early stages of germination with a concomitant increase in the level of II (or of an enzyme with similar gel-filtration properties). Presumably a similar phenomenon occurs in *V. sativa*. It should be noted, however, that the molecular weight quoted by Petek and his associates is quite different from our value for dormant *V. sativa* enzyme II. The method used for molecular weight determination and the state of purity of the protein may in part account for this difference or, possibly, during germination of this *Vicia* sp. enzyme II might be replaced by a third form of approximately half the molecular weight. In the case of the *V. faba* enzymes, we are now investigating the changes occurring during germination.

An α-galactosidase has also been isolated from germinating seeds of *Phaseolus vulgaris* by Agrawal and Bahl.[15] Here again, it must be assumed that this was form II; no mention of multiple forms was made by the authors. Preliminary evidence suggests that with *V. faba* other minor multiple forms of α-galactosidase may also exist.[14]

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The properties of *V. faba* α-galactosidases I and II have been studied in detail by Dey and Pridham;[9,10] some of these properties may now be compared with forms I and II from *P. sativum*. The molecular weights of *P. sativum* II and *V. faba* II are of the same order but *V. faba* I would appear to be a much larger macromolecule than *P. sativum* I. The two forms of the *Pisum* enzyme obtained by a single Sephadex gel fractionation of a crude seed extract, exhibited multiple pH optima the general profiles being somewhat similar to those for the corresponding *V. faba* enzymes (Fig. 2). The $K_m$ values for *Pisum* I (specific activity 163 munits/mg protein), *Vicia* I, *Pisum* II (specific activity 59 munits/mg protein) and *Vicia* II were 0·26 mM, 1·14 mM, 0·3 mM and 0·69 mM, respectively, and the $V_{max}$ values (μmoles *p*-nitrophenyl α-D-galactoside hydrolysed/min/mg protein) were 215·6, 42·1, 63·0 and 2·8, respectively. In both species, therefore, the high molecular form I has a greater catalytic activity than II.

*P. sativum* seeds are a good source of α-galactosidase I. The enzyme has been obtained with a high specific activity (2770-fold purification) by the following simple four step fractionation procedure.

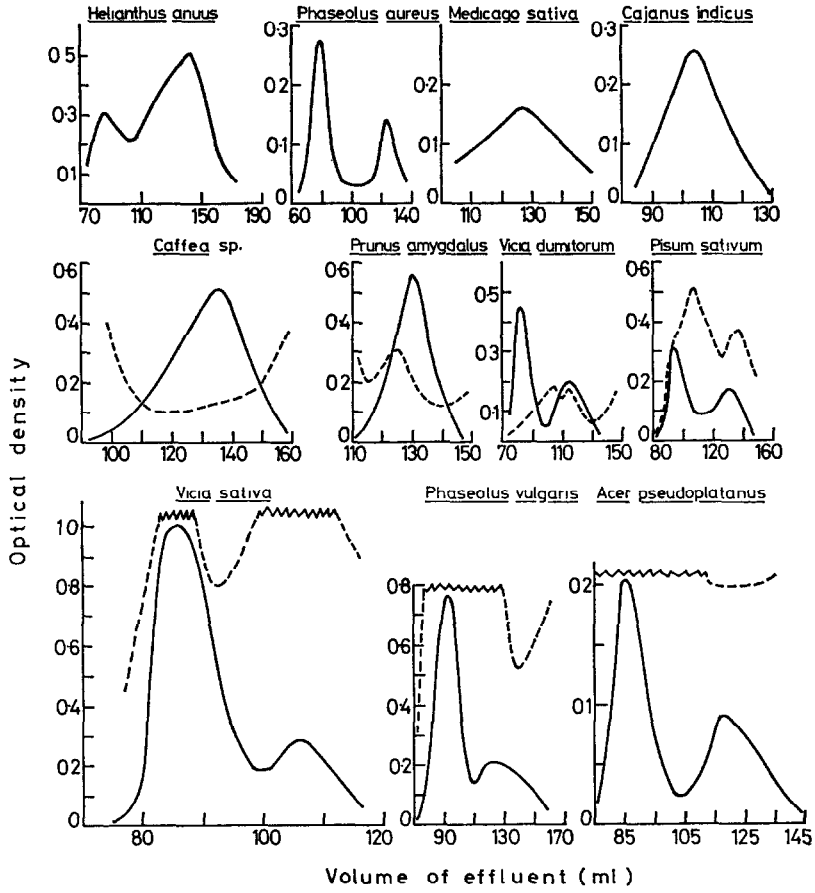


FIG. 1. GEL-FILTRATION ON SEPHADEX G-100 OF CRUDE SEED EXTRACTS OBTAINED FROM VARIOUS SOURCES. THE CONTINUOUS LINE REPRESENTS THE α-GALACTOSIDASE ACTIVITY (OPTICAL DENSITY AT 405 nm) AND THE BROKEN LINE, THE ABSORBANCE AT 280 nm.

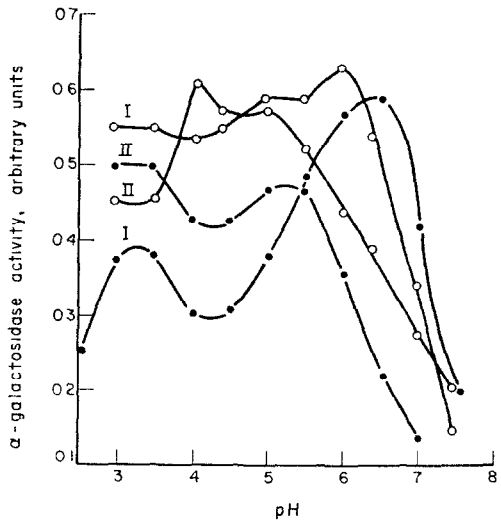


FIG. 2. THE pH PROFILES OF α-GALACTOSIDASES I AND II FROM *Pisum sativum* (○—○) AND *Vicia faba* (●—●) (DATA TAKEN FROM REF. 9) USING *p*-NITROPHENYL α-D-GALACTOSIDE AS THE SUBSTRATE.

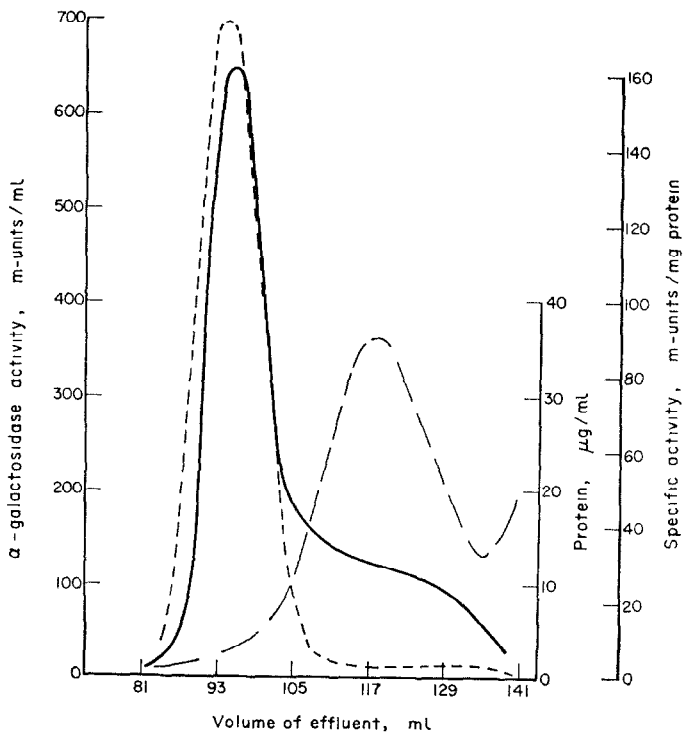


FIG. 3. GEL-FILTRATION ON SEPHADEX G-100 OF THE PARTIALLY PURIFIED α-GALACTOSIDASE PREPARATION (40–60% AMMONIUM SULPHATE FRACTION, SEE TABLE 3) FROM THE DORMANT SEEDS OF *Pisum sativum*. (———) ENZYME ACTIVITY; (– – –) PROTEIN; (· · · · ·) SPECIFIC ACTIVITY.

The pH of a McIlvaine buffered extract (pH 5·0; 49 ml) of powdered seeds (20 g) was lowered to 3·0 by slow addition of citric acid solution (1 M, 3 ml): constant stirring was essential during this operation. The resulting precipitate was removed by centrifugation and discarded and the pH of the active supernatant solution then adjusted to 5·0. Ammonium sulphate fractionation was carried out and the protein fractions were dissolved in minimum volumes of McIlvaine buffer (pH 5·0) and the specific activities then determined. The enzyme preparation obtained at 40–60% ammonium sulphate saturation had the highest specific activity and was passed through a Sephadex G-100 column. The fractions containing enzyme I were pooled (see Fig. 3).

Dormant seeds of *Cajanus*, *Medicago*, *Coffea* and *Amygdalus* spp. appeared to contain a single form of α-galactosidase, each with a relatively low molecular weight as judged by gel-filtration. Two forms of *Coffea* α-galactosidase have been reported by Petek and To Dong;[16] these were separated by alumina column chromatography and they appeared to possess similar kinetic properties and had pH optima of 5·3 and 6·0, respectively. We have found that crude coffee extracts, after passage through Sephadex, yield a single enzyme peak with a sharp pH optimum at 6·3.

## EXPERIMENTAL

All the chemicals used were of A.R. grade. *Acer* and *Helianthus* seeds were collected from our College grounds and *Vicia dumitorum* and *Vicia sativa* seeds were obtained from the London University Botanical Supply Unit. All other seeds were supplied by the local merchants.

*Extraction.* This was effected by suspending finely powdered dry seeds (10 g) overnight in McIlvaine buffer (60 ml; pH 5·0); the resulting slurry was centrifuged (1000 *g* for 20 min) and the enzymically active supernatant solution retained. With *Medicago sativa* the powdered seeds were treated with acetone followed by ether (both at −20°) and then dried under reduced pressure prior to extraction with the buffer; this removed excess lipid which interferes with gel-filtration.

*Enzyme assay.* α-Galactosidase was assayed by following the initial rate of hydrolysis of *p*-nitrophenyl α-D-galactoside.[17] The appropriately diluted enzyme solution (0·4 ml) and McIlvaine buffer[18] (pH 5·0; 0·3 ml) were incubated at 30° for 15 min with the substrate (2 m M,0·3 ml). $Na_2CO_3$ (0·1 M; 5 ml) was then added and the absorbance was measured at 405 nm. Appropriate controls were included using boiled enzyme. The substrate concentration was kept low because α-galactosidase is generally inhibited at high substrate concentrations.[6,9]

One unit of enzyme activity is defined as the amount of enzyme required to hydrolyse 1 μmole of substrate per min under the assay conditions described. Specific activity is defined as munits of enzyme activity per mg of protein. Protein was determined by the method of Lowry *et al.*[19]

*Sephadex gel-filtration.* A column (2 × 45 cm) of Sephadex G-100 equilibrated with McIlvaine buffer, pH 5·0 containing 0·1 M KCl was prepared according to Andrews.[20] The enzyme solution (4 ml) was applied to the column and elution carried out with the same buffer–KCl mixture. Flow rate was maintained at 30 ml/hr and 3 ml fractions were collected. The column was calibrated with proteins of known molecular weight for molecular weight determinations. Sephadex G-200 was used for α-galactosidases with molecular weights greater than $10^5$.

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