ISOLATION OF THE SPECIFIC ALBUMINS FROM PISUM SEEDS—

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An electrophoretic study of seed albumins of various Pisum forms revealed five distinct banding patterns that differed in the number and electrophoretic mobility of major bands designated "a"-"f" (1; see Fig. 1). Proteins corresponding to these bands were called "specific albumins".

The first attempt to isolate the specific albumins was separation from the rest of the water soluble proteins by fractionation on a Sephadex column (2). The separated fraction (fraction S2) was, however, heterogeneous in most of the Pisum forms analyzed: Its albumin spectra showed from one to three characteristic bands (2).

The present communication describes a simple fractionation procedure using an ion exchange chromatography technique that resulted in isolation of some specific pea seed albumins from different lines.

The following lines from the Weibullsholm Collection were investigated: P. sativum, WL 110 ('Kungsart'); P. humile, WL 936; P. cinereum, WL 1490; P. abyssinicum, WL 808; and P. fulvum, WL 1256. Albumin extracts were obtained from cotyledons of mature seeds with 0.15M acetate buffer, pH 4.6 (2). Disc electrophoresis was carried out according to Davis (3). Ion exchange chromatography was performed on Whatman DE-22 cellulose. A column was equilibrated with 5 mM phosphate, pH 7.5, and eluted with the same buffer until all unbound proteins were washed out. Elution was continued with 0.1 M phosphate, pH 7.0 and then with 0.1 M phosphate containing 0.4 M NaCl, pH 6.0 (4).

Elution profiles of seed albumins of all lines were similar; three main peaks were obtained. Electrophoretic spectra of the extracts and chromatographic fractions containing isolated specific albumins are shown in Fig. 1.

The specific albumins of P. abyssinicum and P. fulvum, corresponding to the bands "e" and "f", appeared in peak 1. At the trailing edge of this peak the specific albumins of P. sativum, P. humile, and P. cinereum, corresponding to the band "a", were eluted from the column. Albumins corresponding to bands "b" in P. sativum and "d" in P. cinereum were washed out with 0.1 M buffer at the trailing edge of peak 2. In P. fulvum the albumin corresponding to the band "b" was probably eluted with the starting buffer, at the trailing edge of peak 1 (data now shown). This albumin seems to differ from the albumins corresponding to the band "b" in other Pisum lines (5).

In comparison to Sephadex fractionation, DEAE-cellulose chromatography gave better results in three out of five lines studied. The best result was achieved in P. sativum; the specific albumins corresponding to the bands "a" and "b" were clearly separated from each other (Fig. 1). Similar separation of the major albumins of P. sativum was reported by Croy (6).

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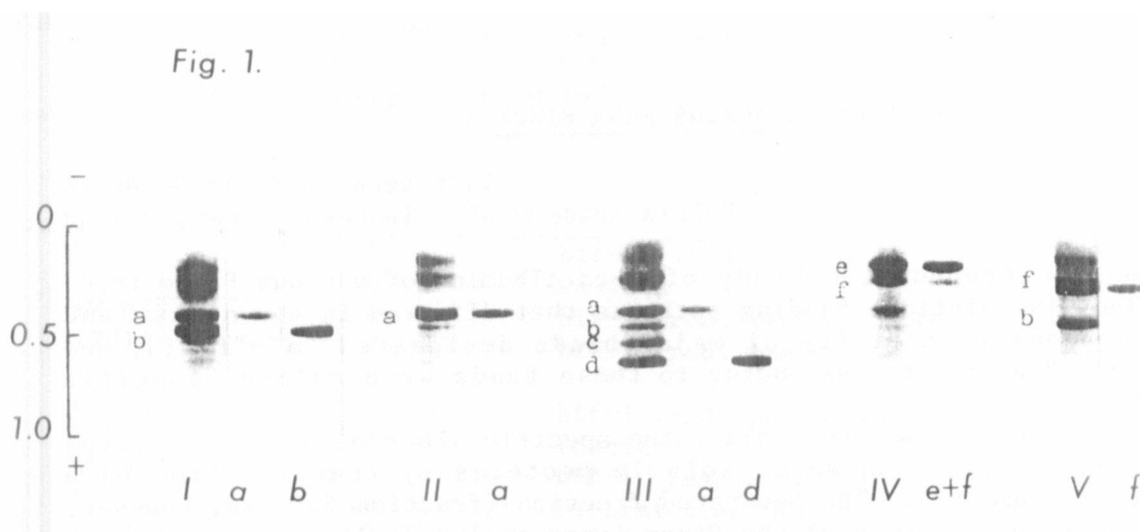

Fig. 1. Electrophoretic spectra of total albumin extracts (Roman numerals) and the specific albumins (a-f), isolated by DEAE-cellulose chromatography. The following Pisum lines were studied: I - P. sativum, WL 110; II - P. humile, WL 936; III - P. cinereum, WL 1490; IV - P. abyssinicum, WL 808; V - P. fulvum, WL 1256.

The results showed some resemblance between the specific albumins corresponding to the bands "e" and "f" in P. abyssinicum and P. fulvum and their distinction from the albumins corresponding to the bands "a"-"d" in the other three lines. The specific proteins of P. abyssinicum and P. fulvum were not retarded by the cellulose and were eluted in peak 1. The specific proteins of P. sativum, P. humile and P. cinereum were retarded or bound to the resin and were eluted at the trailing edge of peak 1 or 2.

1. Przybylska J., S. Blixt, J. Hurich, and Z. Zimniak-Przybylska. 1977. Genetlca Polonlca 18:27-38.
2. Jakubek M. and J. Przybylska. 1979. Genetlca Polonlca 20:369-380.
3. Davis B. J. 1964. Ann. New York Acad. Sci. 121:404-427.
4. Jakubek M. 1982. Doctor's thesis completed at the Institute of Plant Genetics, Polish Academy of Sciences, Poznan.
5. Przybylska, J., Z. Zimniak-Przybylska, E. Kozubek, and S. Blixt. 1983. Genetica Polonica. (In press).
6. Croy, R. 1984. Biochem. J. 218 (In press).