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Characterization of Albumins from *Lupinus luteus* L. Cotyledons

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Abstract. The cotyledons of yellow lupin seeds contain 15.4 mg of the albumins, water soluble proteins, per 100 mg of dry matter. These proteins were resolved by SDS gel electrophoresis into 13 polypeptides with relative molecular mass, Mr, between 16 000 and 84 000. Isoelectrofocusing analyses of the albumins showed that they were composed of six components with isoelectric points between 4.1 and 5.5. These results have indicated that the albumins are acid proteins and exhibited molecular and charge heterogeneity.

Albumins, water soluble proteins (OSBORNE 1924) besides globulins are principal proteins in the cotyledons of legume seeds. The most prominent feature of the albumin fraction is their enzymatic activity (KONOPSKA 1984). The albumins of the *Leguminosae* are also characterized by their ability to bind sugars and, to some extent, by an agglutinating activity upon erythrocytes and by mitotic activities in animal cell cultures (WEBER and NEUMANN 1980). Moreover, the albumins are regarded to be important storage proteins (YOULE and HUANG 1978). They are deposited within protein bodies of castor bean seeds (YOULE and HUANG 1978) and in these cell structures of pea cotyledons (KONOPSKA 1978). Despite several functions of albumins which were detected in legume seeds, composition of these proteins has been very little investigated (KONOPSKA 1983).

The present paper deals with the composition and biochemical characterization of albumins prepared from mature cotyledons of yellow lupin seeds.

MATERIAL AND METHODS

Cotyledons were separated by hand from mature yellow lupin (*Lupinus luteus* L. cv. Afus) seeds and then pulverized in an electric grinder. 5 g samples of meal were stirred with 50 ml of 1 M NaCl in 0.05 M sodium phosphate buffer (pH 7.5) at about 4 °C for 1 h, and centrifuged. Extraction was repeated, the supernatants pooled and subjected to dialysis against running tap water for about 16 h and then redistilled water for about 30 h. Further dialysis of the extracts did not affect precipitation of proteins. The water-soluble fraction was designated as albumin and subjected to SDS gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF).

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SDS-PAGE in 7.5 % gel was performed according to WEBER and OSBORN (1969) and WEBER *et al.* (1972). The albumin samples were dissociated in 0.01 M sodium phosphate buffer, pH 7.0 containing 1 % SDS and 1 % 2-mercaptoethanol at 100 °C for 5 min. The mixture of 5 μl 0.05 % bromophenol blue, a drop of glycerol, 5 μl 2-mercaptoethanol and the solution

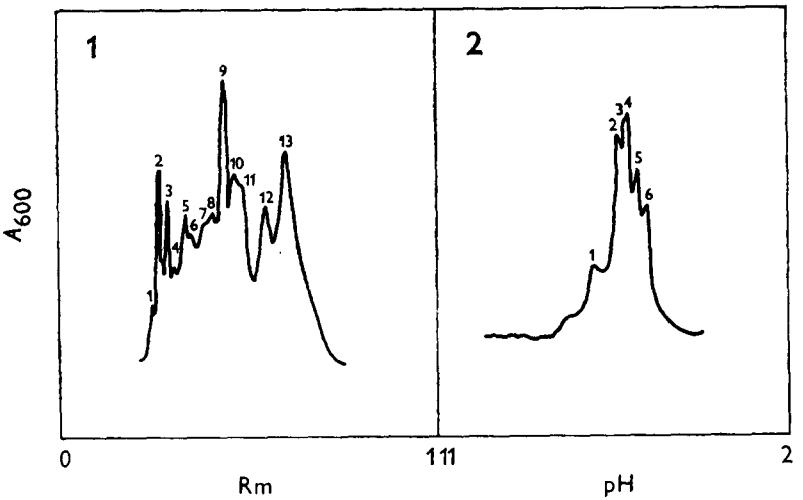

Fig. 1. Absorbance scans of the stained albumin prepared from lupin cotyledons and separated by SDS-PAGE.
Fig. 2. Absorbance scans of the stained albumin prepared from lupin cotyledons and separated by IEF.

containing about 20 μg of the dissociated protein were applied per gel. The electrophoresis was run at 6 mA/tube at room temperature for about 5 h. The gels were stained in 0.1 % Coomassie Blue R-250 at 65 °C for 20 min, and destained in a solution of ethanol, water and acetic acid (100 : 260 : 32). The bands were scanned at 600 nm. An electrophoresis calibration kit of Serva was used for mol. m. marker proteins.

IEF was carried out in 7.5 % acrylamide gels. The Ampholine commercially called Servalyt (Serva) in a 2.0 to 11.0 pH range was used. Approximately 150 μg of the albumins were focused in one tube. The lower electrode vessel contained 1 % H_3PO_4 at the anode, and the upper one 1 % ethylenediamine at the cathode. IEF was run in glass tubes for about 6 h. Low voltage (50 V) was used at the initial phase of focusing, then it was successively increased up to 200 V, and finally followed by 400 V for 5 min. The gels were stained and destained like those after SDS-PAGE. For determination of isoelectric points marker proteins of Serva were used and pH in 2 mm sections of gels measured.

RESULTS AND DISCUSSION

It has been found in this study that the cotyledons of yellow lupin seeds contain 15.4 mg of the albumins per 100 mg of their dry matter. Part of them are localized in aleurone grains (SOBOLEV *et al.* 1976) in which 12.58 % of their total proteins are the albumins (KONOPSKA 1984).

TABLE 1
Average values of rel. mol. m. (Mr) of the components separated by SDS gel electrophoresis of the albumins from lupin cotyledons

Component no.	Mr
1	84 000
2	81 000
3	73 000
4	65 000
5	58 000
6	54 000
7	45 000
8	41 000
9	36 000
10	31 000
11	28 000
12	21 000
13	16 000

SDS gel electrophoresis of the albumins prepared from the cotyledons indicated that these proteins were composed of 13 components (Fig. 1). Average values of mol. m. ranging from 16 000 to 84 000 are shown in Table 1. In the cotyledons the albumin polypeptides 2, 9, and 13 with mol. m. 81 000, 36 000, and 16 000 respectively, predominate. Moreover, the albumin bands 3, 5, 10, 11, and 12 with mol. m. 73 000, 58 000, 31 000, 28 000, and 21 000 respectively, at medium concentration and relatively small amount of components 1, 4, 6, 7, and 8 with mol. m. 84 000, 65 000, 54 000, 45 000, and 41 000 were found in lupin cotyledons. These results indicated molecular heterogeneity in the albumin fractions. Similarly, KULKA and GRZESIUK (1978) suggested that the total albumins of legume cotyledons were exceptionally complex.

The IEF banding patterns of the albumins are shown in Fig. 2. These proteins resolved into six heavily stained bands at the acid end of the IEF gel. The isoelectric points of the three major bands were 4.8, 4.6, and 4.5 and those of the three minor ones 5.5, 4.3, and 4.1 (Table 2). The isoelectric points of the albumins were between 4.1 and 5.5 indicating that the albumins

TABLE 2
Average values of isoelectric points of the components after isoelectrofocusing of the albumins from lupin cotyledons

Component no.	isoelectric point
1	5.5
2	4.8
3	4.6
4	4.5
5	4.3
6	4.1

of lupin cotyledons were acid proteins and that charge heterogeneity might occur.

RIGHETTI *et al.* (1977) reported that the charge heterogeneity of zein polypeptides was not an artifact caused by either extraction procedure, prosthetic groups (sugars or lipids), carbamylation, or Ampholine-protein interactions but was due to a combination of *in vivo* deamidation of glutamine and asparagine residues and spot mutations in zein genes. If the observed charge heterogeneity for albumin of the lupin cotyledons is not artifactual, then each charge variant may be the product of slightly different structural genes, the posttranscriptionally modified products of one or more identical genes, or a balance between these two possibilities.

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