Phytochemistry, 1970, Vol. 9, pp. 1167 to 1172. Pergamon Press. in England.

PURIFICATION AND PROPERTIES OF A *TRITICUM AESTIVUM* SPECIFIC ALBUMIN

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(*Received* 13 *May* 1969)

Abstract—A specific albumin has been isolated from wheat seeds (*Triticum aestivum*) in sufficient yield and purity (approx. 95%) to carry out characterization studies. The protein has an electrophoretic mobility of 0·19 (referred to that of bromophenol blue taken as 1) in polyacrylamide disks in glycine-tris, pH 9·5, and an isoelectric point of approximately 7·3. The molecular weight, calculated by amino acid composition data, was 20,200, in good agreement with the value of 23,800 computed from ultracentrifugal studies.

INTRODUCTION

IT HAS been shown by many authors[1-8] that the "soluble" proteins from seeds of *Triticum aestivum* are different from those of *T. durum*. Yet, largely because these workers used varying conditions of extraction and fractionation, it is impossible to compare their results and thus establish how many and which protein fractions are characteristic for each species. In view of the importance of "specific" proteins for the solution of genetical[4, 9-10] and analytical[7, 11-14] problems, we considered it useful to isolate and characterize several such proteins.

In this paper we describe the isolation and properties of an albumin which is always present in albumin extracts[7-8, 14] of the seeds of fifty-two different varieties of *T. aestivum*, but is absent from numerous analysed varieties of *T. durum*. To our knowledge only one paper[15] on the isolation and characterization of "soluble" proteins from soft wheat has so far been published.

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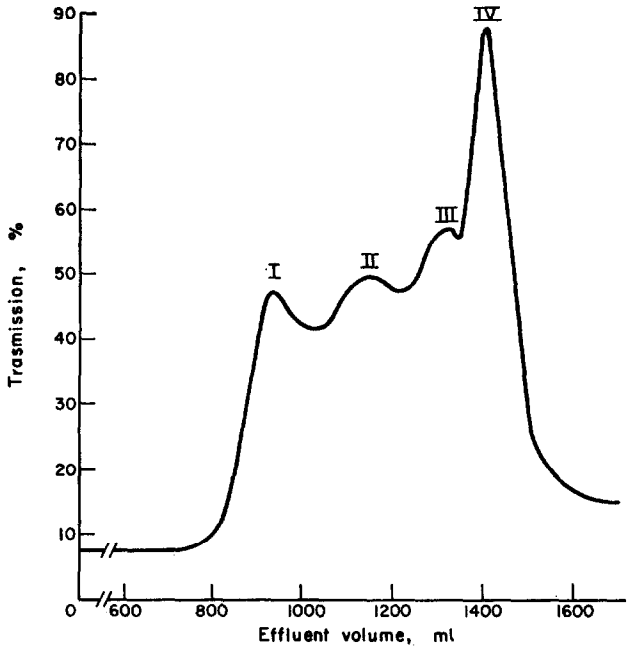

FIG. 1. WHEAT ALBUMIN FRACTIONATION BY DEAE-CELLULOSE COLUMN CHROMATOGRAPHY.

1 g of albumin in 100 ml of phosphate buffer, pH 7·8 (3·35 mM Na_2HPO_4 and 0·367 mM KH_2PO_4), applied to 5·57 ft (i.d. 4·0 cm) column of DEAE-cellulose. Flow rate of effluent was 1·3 ml/min.

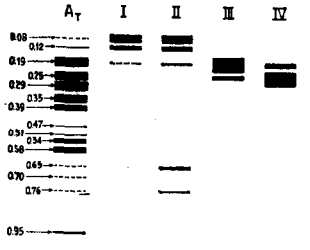

FIG. 2. DISK-ELECTROPHORETIC PATTERNS OF ALBUMIN FRACTIONS OBTAINED WITH DEAE-CELLULOSE COLUMN CHROMATOGRAPHY.

Disk-electrophoresis on polyacrylamide gel was carried out in a glycine-tris buffer system, pH 9·5.

A_T, unfractionated albumins; I–IV, peaks as in Fig. 1.

RESULTS

Purification

In Fig. 1 the chromatographic fractionation on DEAE-cellulose column of the whole albumin fraction obtained by salting out from a 0·15 M NaCl extract of *Triticum aestivum* seeds is shown. Electrophoretic analysis of the fractions eluted by phosphate buffer, pH 7·8,

from the chromatographic column indicate that they are all heterogeneous and contain the albumin of M_b 0·19 which, as has been shown earlier,[8] is specific for soft wheat (Fig. 2). It should be noted that many of the bands present in the original extract A_T (Fig. 2) are not eluted from the column under our conditions. As can be seen the albumin M_b 0·19 is present in the highest state of purity in Fraction III, and, therefore, this fraction was used for further purification.

By preparative differential disk-electrophoresis on polyacrylamide gel lyophilized fraction III gave two sharply separated peaks (Fig. 3).

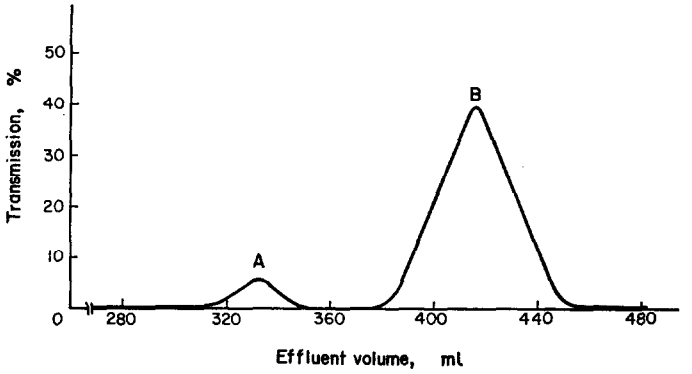

FIG. 3. EFFLUENT DIAGRAM OF ALBUMIN M_b 0·19 PURIFICATION WITH PREPARATIVE DIFFERENTIAL DISK-ELECTROPHORESIS ON POLYACRYLAMIDE GEL.

In all fractionations between 92–128 ml (approx) the presence of a peak due to elution of riboflavine was observed. This peak, therefore, must be considered an artifact of the technique.

The effective purification of the specific albumin of soft wheat by the method used is summarized in Fig. 4. This albumin constitutes approximately 24% (Fig. 4A) of the initial albumin extract, 75% (Fig. 4B) of the albumin fraction corresponding to the third peak (III) eluted from the DEAE-cellulose column (Fig. 1) and 95% (Fig. 4C) of the fraction corresponding to the second peak (B) eluted from the polyacrylamide gel column (Fig. 3).

Characterization

The isoelectric point of the specific albumin of soft wheat, determined by the technique of gel electrofocusing, was found to correspond approximately to a pH of 7·3. By this technique it was also possible to show that only one protein impurity, in negligible concentration, was present. This finding is in accordance with the results of the disk-electrophoresis on polyacrylamide gel (Fig. 4).

The sedimentation pattern of the purified protein fraction showed the presence of a single homogeneous peak with an apparently homogeneous boundary. The molecular weight, computed from the sedimentation velocity and by the diffusion method, resulted to be 23,800. In Table 1 the amino acid composition of the purified albumin is reported. From this latter data the calculated molecular weight, ignoring the tryptophan content,* was 20,200 in fairly good agreement with that from the ultracentrifuge experiments.

* Owing to lack of material it was not possible to determine the tryptophan content of this albumin, a tryptophan peak is, however, present in the chromatographic patterns of 24-hr acid hydrolysates.

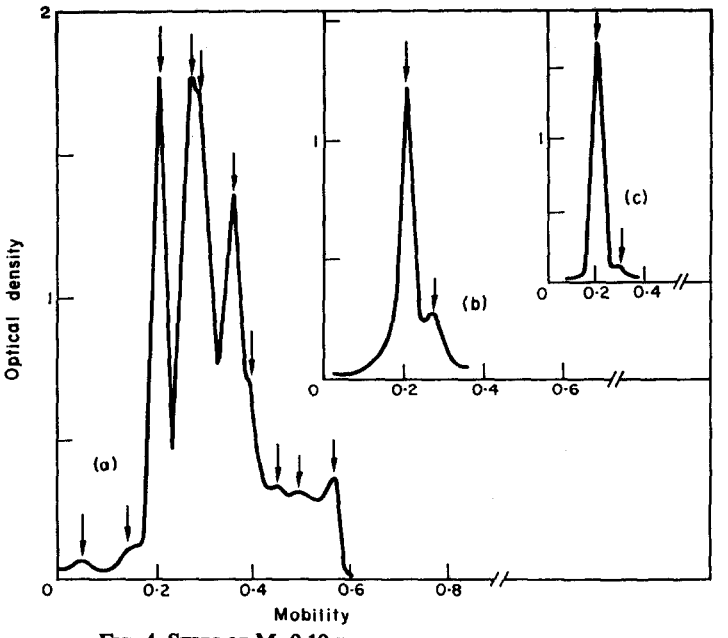

FIG. 4. STEPS OF M_b 0·19 WHEAT ALBUMIN PURIFICATION.

The densitometric recordings of disk-electrophoretic patterns are reported. A, Unfractionated albumins; B, Third peak eluted from DEAE-cellulose; C, Second peak eluted from polyacrylamide gel.

TABLE 1. AMINO ACID ANALYSES OF HYDROLYSATES OF M_b 0·19 ALBUMIN

Amino acid	Amino acid residues g/100 g ptotein	Calculated number of residues assuming His = 1	Number of residues to nearest integer assuming a mol. wt. 20,200 (threefold minimum mol. wt.)
Lys	2·78 ± 0·06‡	1·67	5
His	1·66 ± 0·01	1·00	3
Arg	5·96 ± 0·23	3·59	11
Asp	6·19 ± 0·08	3·73	11
Thr	3·04*	1·83*	5
Ser	6·66*	4·01*	12
Glu	13·49 ± 0.32	8·13	24
Pro	7·74 ± 0·31	4·66	14
Gly	8·84 ± 0·28	5·33	16
Ala	14·07 ± 0·36	8·48	25
Cys/2	6·45 ± 0·11	3·88	12
Val	7·85† ± 0·15	4·73†	14
Met	2·13 ± 0·04	1·28	4
Ile	2·59† ± 0·05	1·56†	5
Leu	8·81 ± 0·18	5·31	16
Tyr	3·51 ± 0·10	2·11	6
Phe	1·71 ± 0·01	1·03	3
Try	Not measured	—	—
Total	103·48		186

* Obtained by extrapolation to zero time.
† 48 and 72 hr values only.
‡ Standard error of the mean = $(Sy^2/n(n-1))^{1/2}$.

DISCUSSION

The data reported in this paper clearly demonstrate that preparative differential disk-electrophoresis on polyacrylamide gel is a very useful technique for the purification of cereal proteins.

The comparison of the molecular weights and the amino acid compositions of the specific albumin of soft wheat described in this paper and the protein isolated and characterized by Kelley[15] in 1964, clearly show that these are completely different, even if they have similar solubility characteristics and isoelectric point. The formation of a flocculent precipitate by adjusting the pH value of an approximate 2% (w/v) albumin solution to 6·2 and the subsequent decrease in the concentration of all albumin fractions present in the supernatant (unpublished data) suggest that the majority of these proteins have an isoelectric point of approximately 6·2. Although it is not possible to compare the albumin of M_b 0·19 described here with that isolated by Feillet *et al.*[16] and also described as specific for soft wheat, some analogies between the method of isolation described here and the one adopted by Feillet suggest that a comparison should be possible.

EXPERIMENTAL

Extraction and Purification of Albumin

160 g of *Triticum aestivum*, var. Mentana, wheat were grounded in a laboratory mill, Buhler (Uzwill, Switzerland), at stage 3, suspended in 240 ml 0·15 M NaCl according to Hall[17] and stirred for 180 min at laboratory temperature. The suspension was centrifuged at 18,000 rpm in a Martin Christ centrifuge, model Omikron, and the albumins were separated from the supernatant by "salting out" by 0·4–1·74 M $(NH_4)_2SO_4$, according to Pence *et al.*[1] The precipitate was collected by centrifugation at 18,000 rpm, redissolved in water and dialysed against H_2O for 48 hr at 4°. The albumin solution was then centrifuged at 18,000 rpm and freeze-dried. The lyophilized protein was stored at −20°.

Chromatography on DEAE-Cellulose

1 g of albumin was dissolved in 100 ml of phosphate buffer, pH 7·8 (3·35 mM Na_2HPO_4 and 0·367 mM KH_2PO_4), and passed through a DEAE cellulose (Whatman DE 52 pre-swollen) column of 1·7 m high (i.d. 4·0 cm). The fractionation was performed by elution with phosphate buffer, pH 7·8, at a constant flow rate of 1·3 ml/min. The absorbance of the eluate at 280 nm was measured continuously (Photocrom flow-analyser, Rastelli, Italy) and recorded (Kompensograph, Siemens, Germany). The eluted fractions were combined (Fig. 1), dialysed and dry frozen. The analysis of these fractions was performed by disk-electrophoresis on polyacrylamide gel.

Analytical Disk-Electrophoresis on Polyacrylamide Gel

The samples (20–120 μl of 1% protein (w/v) solution) were put on top of the gel in 11% (w/v) sucrose solution. The electrophoretic runs were performed according to Ornstein[18] and Davis.[19] The acrylamide concentration was 7·5% (w/v); the composition of the solution for gel and buffers is described in the information sheets of Shandon Scientific Company, London, for a gel system at pH 9·5.

The electrophoresis was performed in a vertical apparatus (Canal Industrial Corporation, Rockville, Maryland); 5 mA/channel were applied and the time for analysis was usually 75 min. At the end of the run, the gels were stained overnight with 0·5% (w/v) aniline blue-black in 7·5% (v/v) acetic acid. Excess stain was removed by electrophoretic de-staining in 7·5% (v/v) acetic acid, applying 12·5 mA/gel. The mobilities of protein bands are referred to that of bromophenol blue, used as tracking dye, which is taken as 1. The densitometric scanning was done in transmitted light by a microdensitometer Chromoscan (Joyce, Loeble & Co. Ltd., Gateshead).

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Differential Preparative Disk-Electrophoresis on Polyacrylamide Gel

A preparative disk-electrophoresis on polyacrylamide gel with zones of different porosity (differential preparative disk-electrophoresis) is the last step of the specific purification of albumin from soft wheat. This electrophoresis was performed in a vertical apparatus (Canal Industrial Corporation) using a PD 320 column. The separating gel "system" was prepared by allowing 6·6 ml of a 5% (w/v) acrylamide solution to polymerize first followed by addition of 26·4 ml of 10% (w/v) acrylamide. In this way a column of approximately 10-cm length was obtained in which the first 2 cm derived from the 5% acrylamide solution and the remaining 8 cm from 10% solution. On the top of the "separating gel system" 3·3 ml of "spacer gel" were added. Approximately 100 mg of lyophilized were finally put on the top of the column by means of a polymerization in a "sample gel". The composition of the solutions for the gels (separating, spacer and sample) and for the electrode buffers and of the eluting solutions are described in the information sheet of the Canal Industrial Corporation for the "Prep-Disc Electrophoresis". The flow of the elution buffer was approximately 0·2 ml/min. The densitometric and electrophoretic analysis of the eluted fractions was performed as described above. The fractions of similar composition were pooled, dialized against water at 4° for 48 hr and freeze-dried.

Polyacrylamide Gel Electrofocusing

The determination of the isoelectric point of the albumin was done by means of the gel electrofocusing technique.[20]

Acrylamide concentration was 7·5%, ampholine 1%, pH range was 3–10. Electrofocusing was performed in a vertical disk-electrophoretic apparatus (Canal Industrial Corporation, Rockville, Maryland) at 4°. Per twelve channels, approximately 80 V were applied during the first 90 min and 350 V during the successive 270 min.

Ultracentrifugal Studies

Sedimentation studies were made in a Spinco model E Ultracentrifuge, with schlieren optics, under the following conditions: rotor speed 50,740 rpm, temperature 4–6°, protein concentration 0·5%, NaCl 0·1 M buffer, pH 5. Sedimentation velocity and diffusion constant were measured in the conventional way.[21,22]

Amino Acid Analysis

The determination of the amino acid composition of the albumin studies was accomplished as described elsewhere[23] using the automatic chromatographic technique of Spackman *et al.*[24] Hydrolysis was performed for three different times, namely 24, 48 and 72 hr, in duplicate. The data reported in Table 1 represent the means of results obtained in each sample for each length of hydrolysis.

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