SPECTROSCOPY OF MAIZE PROTEINS

II. ALBUMINS

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The albumins of maize grain isolated by precipitation from a total salt extract have been investigated by IR, UV, derivative, and fluorescence spectroscopy. It has been shown that the variability of the spectral parameters is connected with a change in the association of the proteins with nonprotein substances according to the genotype of the maize.

The albumins of cereal grains are represented by a complex of functionally active proteins fulfilling an important role in the realization of genetic information [1]. Investigations performed by gel filtration, chromatography, and electrophoresis have shown the genotypic variability of the composition of the maize albumins [2, 3]. The available spectral characteristics of the maize albumins are limited to those obtained in a study of a water-soluble protein extract [4-6], on the basis of the optical parameters of which methods have been developed for selecting forms of maize differing in the quality of the grain [7, 8].

In view of this, in the present work we have made a spectral-fluorescent investigation of the proteins of the albumin complexes isolated from grains of the initial maize lines (+/+) and a maize line mutant in the opaque-2 gene (o2/o2) by precipitation from a total salt extract [9].

The IR spectra of the albumins under investigation showed the absorption bands characteristic for proteins. The amide I (1652-1660 cm^{-1}) and amide II (1536-1544 cm^{-1}) bands were well resolved for the albumins of both the ordinary and the mutant lines (Table 1). On the other hand, it has been shown previously [5] that an increase in the optical density of the amide II band relative to the amide I band and a decrease in the degree of resolution of these bands are characteristic for the fraction of water-soluble proteins of maize grains of mutant lines. This fact is connected with an excessive accumulation of free amino acids having the absorption characteristics of proteins in the grains of mutant lines. The parameters obtained for the IR spectra of the albumins under investigation therefore confirmed the efficacy of the procedure used for the purification of the protein preparations.

TABLE 1. Characteristics of the IR, UV, and First Derivative of the Spectrum of Maize Albumins

Genotype of the maize	IR spectrum, cm^{-1}		UV spectrum			First derivative of the spectrum
	amide I	amide II	λ_{max}, nm	$D_{\lambda_1}/D_{\lambda_2}$	$D_{\lambda_3}/D_{\lambda_4}$	$\frac{dD_{288-293}/d\lambda}{dD_{283-288}/d\lambda}$
A 204+/+	1656	1536	280	0,89	3,14	1,83
A 204 o2/o2	1652	1544	276	0,94	2,18	1,92
W 64 A+/+	1656	1544	279	0,75	3,98	2,12
W 64 A o2/o2	1656	1540	279	0,74	4,59	2,61
W 155+/+	1656	1544	289	0,75	2,55	2,93
W 155 o2/o2	1656	1540	279	0,80	2,51	2,91
Wf 9+/+	1656	1540	289	0,77	3,13	2,35
Wf 9 o2/o2	1660	1544	279	0,79	4,59	2,58

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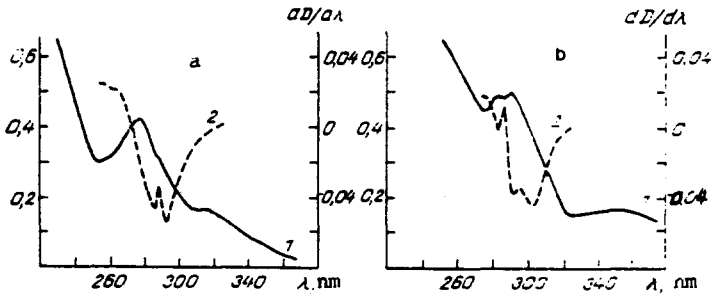

Fig. 1. UV spectrum (1) and first derivative of the spectrum (2) of a solution of maize albumins (from the line A 204 +/+) in water (a) and in 1 N caustic soda solution (b). For curve 1 the scale of ordinates is on the left and for curve 2 it is on the right.

The IR-spectroscopic results were also confirmed by a study of UV spectra. For the protein preparations under investigation a pronounced absorption maximum was observed at 276-280 nm (Table 1 and Fig. 1a), which agrees with information for albumins isolated from the seeds of cultivated plants by the precipitation of a total salt extract [9, 10]. Here the optical density ratios, D_{260}/D_{280}, which are used for evaluating the degree of purification of protein preparations from nucleic acids [4], were 0.70-0.94 (see Table 1). In contrast, a broad absorption maximum at 251-264 nm due to the combined absorption of proteins and nucleic acids was characteristic of the water-soluble maize grain proteins obtained previously, while the D_{260}/D_{280} values were between 1.11 and 1.21.

The parameters of the first and second derivatives of the spectrum permit the main absorption maximum of the albumins under investigation to be interpreted as a superposition of the specific contributions of tyrosine and tryptophan residues. Thus, the presence of two main negative maxima at 285-286 and 292-293 nm and also of a positive maximum at 288-289 nm was characteristic for the first derivative of the spectrum (see Fig. 1a). For the second derivative of the spectrum minima were observed at 290 and 283 nm and maxima at 294 and 287 nm. Here, while the positions of the maxima in the derivatives of the spectrum scarcely differed, the degree of their resolution changed according to the nature of the protein preparation, which may be connected not only with different quantitative ratios of tyrosine and tryptophan residues in the proteins but also with a broadening of the absorption maxima as a result of the action on the chromophoric systems of processes involving the interaction of the protein macromolecule with nonprotein substances [11]. This conclusion was confirmed by the variability of the ratio of the following differences in intensities: the maximum at 288 nm – the minimum at 293 nm, and the maximum at 288 nm – the minimum at 285 nm of the first derivatives of the spectra (see Table 1).

A distinguishing feature of the UV spectra of the albumins under investigation was the presence of absorption at ~320 nm appearing, as a rule, in the form of a shoulder on the main band. The intensity of the long-wave band was considerably lower than that of the main band (276-280 nm) and varied according to the genotype of the maize, which was confirmed by changes in the density ratio D_{280}/D_{320} (see Table 1). It must be mentioned that for the fractions of water-soluble proteins of maize [4] and of wheat maize [12] investigated previously the intensity of absorption in the long-wave region was comparable with that of the main maximum.

On the basis of the known spectral parameters of proteins [11], it may be concluded that the long-wave maximum is connected with an aromatic chromophore in proteins but is a spectral manifestation of accompanying nonprotein substances as a result of modificational changes of the proteins during biosynthesis or isolation from the plant material, and its hypochromic effect is therefore due to an increased degree of purification of the protein preparation.

As a result of chromatographic separation using sorbents of different polarities, it has been shown previously [3, 13] that albumins are complex combinations of proteins and nonprotein components (nucleic acids and carbohydrates). At the same time, the nature of the long-wave maximum of the albumins at ~320 nm cannot be explained by the presence of these components in the protein preparation since nuclei acids have a well-defined maximum at 260

TABLE 2. Parameters of the Fluorescence of the Maize Albumins

Maize genotype	λ_{max}, nm	φ	r
A 204+/+	334	0,066	0,068
A 204 o2/o2	334	0,057	0,069
W 64 A +/+	335	0,069	0,054
W 64A o2/o2	336	0,088	0,057
W 155+/+	334	0,134	0,042
W 155 o2/o2	335	0,088	0,055
Wf 9+/+	335	0,074	0,046
Wf 9 o2/o2	335	0,089	0,046

nm [14], while carbohydrates do not absorb in the ultraviolet region because of the absence of conjugated chromophores. On the basis of an analysis of literature information it must be assumed that the absorption at ~320 nm is the spectral manifestation of the flavonoids that participate in processes of modifying plant proteins through covalent or noncovalent binding [15].

It is known [16] that the spectra of flavonoids (flavones and flavonols) have two absorption bands, at 240-270 and 320-380 nm the spectral parameters of which are sensitive to a change in the acid-base properties of the solution. In the light of this fact we studied the UV spectra of solutions of albumins at various alkaline pH values. It must be mentioned that with a rise in the pH to 8.4, together with the maximum at 320 nm, an additional long-wave maximum arose at ~350 nm for which a hyperchromic effect was observed with a further rise in the pH to 9.3. At the pH values of 8.4 and 9.3 the position and nature of the manifestation of the main absorption maximum of the albumins at ~280 nm did not change, as was also confirmed by an investigation of the first derivative of the spectrum.

The results obtained showed the presence as components of the albumins of compounds possessing an increased capacity for ionization in an alkaline medium at low pH values. On the other hand, in 1 N caustic soda solution (Fig. 1b), together with the clear appearance of a bathochromic shift of the long-wave maximum to 350 nm, there were considerable changes in the main band of protein absorption that were characterized by the appearance of two resolved maxima at 276 and 290 nm (similarly to wheat albumins [12]), connected with the ionization of tyrosine residues in the proteins against a background of the tryptophan chromophore. On passing to a 1 N solution of caustic soda the nature of the first derivative of the spectrum also changed sharply (see Fig. 1b); additional positive maxima were observed at 279, 288, and 297 nm and negative maxima were observed at 285, 294, and 304 nm, which are characteristic for proteins under the conditions of alkaline denaturation [11]. Thus, the results obtained show that the albumins include two groups of chromophores differing in their degrees of ionization.

The fluorescence characteristics of the proteins under investigation were determined by the tryptophan fluorophore (Table 2). At an excitation wavelength of 290 nm the fluorescence of the albumins was characterized by a maximum at 334-336 nm, while the values of the quantum yield (φ) and the anisotropy of fluorescence (r) were close to those found previously for the trypsin inhibitor isolated from maize [17].

Thus, as the result of a comparative study, the spectral-fluorescent characteristics of the albumins have been determined and a dependence of the associative properties of the proteins on the genotype of the maize has been found.

EXPERIMENTAL

In this work we used preparations of albumins isolated by ammonium sulfate precipitation from a total salt extract after the separation of the globular proteins by the method of [9]. Isolation was carried out from defatted flours of the initial maize lines (+/+) and of lines mutant with respect to the opaque-2 gene (o2/o2) (lines A 204, W 64 A, W 155, and Wf 9).

IR spectra were investigated on a Specord M80 spectrophotometer in the 1100-1400 cm^{-1} region. The samples were prepared in the form of thin films by depositing solutions (5 mg of substance in 1 ml of water) on fluorite glass, followed by drying in vacuum.

The measurement of UV spectra and optical densities at the analytical wavelengths was conducted on a Specord M40 spectrophotometer in a 1-cm cell. The concentration of the solution was 1.25 mg/ml. The first and second derivatives of the spectra were obtained on a DU-7HS instrument in a thermostated cell at 20°C. The measurements at different basicities of the medium were carried out after the addition of equal volumes of 3 N caustic soda solution to the solution under investigation and a control solution and determining the pH.

Fluorescence spectra were recorded on a MPF-4 spectrofluorimeter in 1-cm cells at an excitation wavelength of 290 nm. The parameters of the fluorescence were determined as described in [17]. The calculation of the spectral characteristics and the statistical treatment of the results were carried out on an IBM PC/XT personal computer. The Tables give the mean results of three measurements, the error of measurement not exceeding 5%.

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