Conformation of Corn Zein and Glutelin Fractions with Unusual Amino Acid Sequence[1]

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ABSTRACT

The conformations of zein, reduced zein, alcohol-soluble reduced glutelin (ASG), water-soluble ASG, and water-insoluble ASG in 70% ethanol-0.5% sodium acetate (with and without mercaptoethanol) were studied by optical rotatory dispersion from 600 to 260 nm and circular dichroism from 240 to 204 nm. Zein has 45% α-helix in 70% ethanol and almost the same α-helical content when disulfide bonds are broken. The α-helical contents of water-soluble ASG and water-insoluble ASG in 70% ethanol-0.1*M* mercaptoethanol are near 25%, but ASG has 51% α-helix. The water-soluble ASG has 25 mole percent proline and a -Pro-Pro-Pro-Val-His-Leu- sequence tandemly repeated six to eight times near the N-terminal. Apparently, the portion of water-soluble ASG that is relatively less rich in proline allows α-helix formation.

The most abundant protein in corn endosperm is zein, the prolamin of corn. Some glutelin proteins become soluble in alcohol when a disulfide-reducing agent is present. Alcohol-soluble reduced glutelin (ASG) extracted by 70% ethanol (EtOH) containing 0.5% sodium acetate (NaAc) and 0.1*M* β-mercaptoethanol (ME) was separated into water-soluble and insoluble fractions (Paulis and Wall 1977). The first 58 amino acid residues from the N-terminal end of water-soluble ASG contain six to eight tandem-repeating sequences of hexapeptide -Pro-Pro-Pro-Val-His-Leu- (Esen et al 1982). The complete amino acid sequence of zein shows that the dipeptide repeats Ala-Ala, Leu-Leu, and Gln-Gln comprise about 30% of the residues, and that seven to nine tandem repetitions of a highly conserved repeating units of 20 amino acids are present (Argos et al 1982, Geraghty et al 1981). Kretschmer (1957), Danzer et al (1975), and Argos et al (1982) reported the conformation of zein from optical rotatory dispersion and circular dichroism measurements. The structure of reduced zein, ASG, water-soluble ASG, and water-insoluble ASG, however, have not appeared in print based on these techniques. This paper reports the conformations of zein, reduced zein, ASG, water-soluble ASG, and water-insoluble ASG by optical rotatory dispersion (ORD) and circular dichroism (CD) measurements.

MATERIALS AND METHODS

Protein Isolation

Defatted corn endosperm meal was extracted with 0.5*M* NaCl to remove albumins and globulins. The residue was next washed with water to remove salt. Zein was extracted from the meal residue with 70% ethanol containing 0.5% sodium acetate (Paulis and Wall 1971, Paulis et al 1975). Then ASG was extracted from the meal residue twice with 70% ethanol-0.5% sodium acetate-0.1*M* ME. The combined ASG extract was dialyzed against water at 4° C to separate the precipitated protein (water-insoluble ASG) from the supernatant (water-soluble ASG) (Paulis and Wall 1977).

Optical Rotatory Dispersion

Optical rotation measurements were made in a Cary 60 recording spectropolarimeter (Cary Instruments, Palo Alto, CA) at 25° C from 600 to around 320 nm. For some proteins, measurements were also made from around 340–260 nm. The concentration of protein solution varied between 0.2 and 0.32%, and the path lengths of the cells were 0.2–5 cm. The maximum absorbance of the solution in the wavelength range measured was less than 2, and a constant band pass of 1.5 nm was used throughout. The instrument was calibrated by a quartz control plate certified by the National Bureau of Standards. The observed rotation of the protein solution was corrected for solvent blank. A smooth curve of optical rotation vs wavelength was obtained for each protein without any cotton effect from 600 to 260 nm. Values of average residue weight, calculated from the amino acid composition, are 109, 108, 106, and 108, respectively, for zein, ASG, water-soluble ASG, and water-insoluble ASG. The wavelength of refractive-index measurement is 589 nm in a Bausch & Lomb refractometer at 25° C. The value of λ_o was 212 nm, which was found best for poly-γ-benzyl-L-glutamate in a variety of solvents (Moffitt and Yang 1956).

Circular Dichroism

The CD measurements were made in a Cary model 6001 CD accessory for the Cary 60 instrument in a 0.01-cm cell from 250 to around 205 nm at 25° C. No CD measurement was made between 250 and 350 nm because no cotton effect from ORD was observed from 600 to 260 nm. The instrument was standardized against 0.1% d-10-camphorsulfonic acid. An average of three runs was used. The solvent blank was subtracted from the observed ellipticity of the solution. The lowest wavelength attainable with 70% EtOH-0.5% NaAc is 200 nm in a 0.01-cm cell, whereas that with 70% EtOH-0.1*M* ME-0.5% NaAc is 206 nm.

The CD curve of each protein was fitted by a linear combination of an α-helix, β-pleated sheet, and unordered structure based on the three reference structures of poly-L-lysine (Greenfield and Fasman 1969), as well as the three reference conformations of five proteins obtained by X-ray diffraction studies (Chen et al 1972) between 207 and 240 nm by a least square method. $[\Theta] = f_H[\Theta]_H + f_\beta[\Theta]_\beta + f_R[\Theta]_R$, $[\Theta]$ is mean residue ellipticity at any wavelength. The fs are the fractions of helix, β, and unordered form, the sum of the fractions is equal to unity, and each f is greater than or equal to zero.

Concentration determination of protein was made from micro-Kjeldahl nitrogen analysis, and a factor of 6.25 times nitrogen was used to convert nitrogen to protein. Commercially available chemicals of the highest purity were used without further purification.

RESULTS AND DISCUSSION

A number of investigators have used CD of polypeptides or proteins in theoretically known conformations as basis spectra to predict the secondary structure of a protein from its CD spectrum. Greenfield and Fasman (1969) and Chen et al (1972) calculated the amount of helix, β, and unordered form of protein, whereas Hennessey and Johnson (1981) included five independent "superstructures" by extending CD spectra into the vacuum ultraviolet to 178 nm. Siegel et al (1980) showed that the CD of 16

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proteins between 210 and 240 nm correlates only with the amount of α helix. Since the strong absorption of the solvent limited our CD data to above 200 nm even in a 0.01-cm cell, only helix content will be emphasized. β and unordered structure are also included in the calculation, however.

The solvent chosen for this study was 70% ethanol for zein and 70% ethanol plus ME for the glutelins. The use of alcohol was necessary to make zein soluble, and alcohol plus ME were required to dissolve the glutelin fractions. Water-soluble ASG gave a turbid solution in water, and centrifugation of this solution reduced the turbidity as well as protein concentration. We were not able to obtain reliable data for water-soluble ASG in water either in the original turbid solution or in the centrifuged solution. Zein is also insoluble in water. Although the conformations of the corn proteins in alcohol-water solution may be different than those in a purely aqueous environment, such a comparison cannot be made here because of the solubility problem mentioned.

Figure 1 listed the ellipticity of five corn proteins, and Table I showed the percent helix, β, and unordered form of the proteins based on b_o value from ORD as well as CD values from Fig. 1. The helix values for zein and reduced zein in Table I calculated from CD and ORD data agree well. However, larger differences were observed for the helix values for the remaining three proteins. The large difference of helix content for ASG from CD and ORD values is unexpected, and we do not have a good explanation for this difference. The helix values calculated from CD data with polypeptide and with proteins as reference agree well except for water-soluble ASG. Values for β and unordered structure from CD data showed larger difference than helix values. The average value for helix, β, and unordered form for each protein will be used for discussion purposes.

Kretschmer (1957) found that zein in 80% ethanol has a helical content of 50% based on ORD measurement. Danzer et al (1975) used a number of solvents and determined from ORD data that the helix content of zein ranged from 9% in trifluoroacetic acid to 42% in 2-chloroethanol and in *N*-methylacetamide. Argos et al (1982) used CD measurement and found that zein in 70% methanol had 44% α-helix, 5% β-strand, and 51% irregular or turn by the method of Chen et al (1974), whereas the Greenfield and Fasman (1969) application resulted in 59% α-helix, no β-structure, and 41% turn. Our data in Table I for zein, allowing for different solvents used, agree reasonably well with those values reported in the literature.

Zein has 45% α-helix in 70% ethanol-0.5% sodium acetate (Table I), and it has 10.8 mole percent proline (Paulis and Wall 1977). Since proline residues do not usually fit well into α-helix, except in N-terminal positions of helixes (Argos and Palau 1982, Argos et al 1982) the high level of α-helix is somewhat surprising. The proline residue, however, is not uniformly distributed in zein (Geraghty et al 1981). This would allow comparatively long segments of α-helix to form in the regions relatively free of proline as well as relatively short α-helices in regions more abundant in proline. The relatively high α-helix content for zein is reasonable, because sorghum prolamin has 10% proline and 40-47% α-helix in 60% tert-BuOH from ORD measurements comparable to zein (Wu et al 1971). When the disulfide bonds in zein are broken by the addition of mercaptoethanol, the α-helical content remains essentially constant. Disulfide bonds in zein are therefore not important to maintain α-helical structure, at least in 70% ethanol-0.5% sodium acetate.

Water-insoluble ASG has 24% α-helix, and the mole percent of proline is 13.9. The higher proline content of water-insoluble ASG is consistent with its lower α-helix contents compared with zein. Disulfide bonds are broken in the solvent used here and apparently do not contribute greatly to α-helix stability. The complete amino acid sequence of water-insoluble ASG is not known at present. There is one proline residue for every seven amino acid residues in this protein. This would indicate that the proline residues are not uniformly distributed along the protein chains or that the α-helix segments are relatively short.

Water-soluble ASG has 25 mole percent proline and 26% α-helix. The higher proline content of water-soluble ASG is consistent with its lower α-helix content compared with zein. The first 58 residues from the N-terminal end of water-soluble ASG contain six to eight tandem-repeating sequences of the hexapeptide -Pro-Pro-Pro-Val-His-Leu- (Esen et al 1982). Therefore, the rest of the water-soluble ASG molecule is relatively less rich in proline content and might allow α-helix formation. If the first 58 residues are subtracted from the overall amino acid composition of the protein, the remaining portion contains sufficient α-helix-forming amino acids (alanine, glutamic acid, leucine, lysine, methionine, phenylalanine, and tyrosine) to account for the amount of α-helix (Blout 1962).

Water-soluble ASG constitutes 33% of ASG. The calculated α-helix content of ASG based on the weight percentages and α-helix contents of water-soluble and water-insoluble ASG is 25%

TABLE I
Percent Helix, β, and Unordered Form of Corn Proteins

	Helix				β			Unordered		
	a[b]	b[c]	c[d]	av	b[c]	c[d]	av	b[c]	c[d]	av
Zein	46	45	43	45	9	20	15	46	37	42
Reduced zein	42	45	44	44	11	26	19	44	30	37
ASG[a]	33	60	61	51	6	21	14	34	18	26
H_2O-soluble ASG[a]	19	32	26	26	11	34	23	57	40	49
H_2O-insoluble ASG[a]	31	19	21	24	29	10	20	52	69	61

[a] Alcohol-soluble reduced glutelin.
[b] From optical rotatory dispersion data, percent helix is $-b_o/6.3$, calculated according to Moffitt and Yang (1956).
[c] From circular dichroism data, based on poly-L-lysine reference according to Greenfield and Fasman (1969).
[d] From circular dichroism data, based on five proteins reference according to Chen et al (1972).

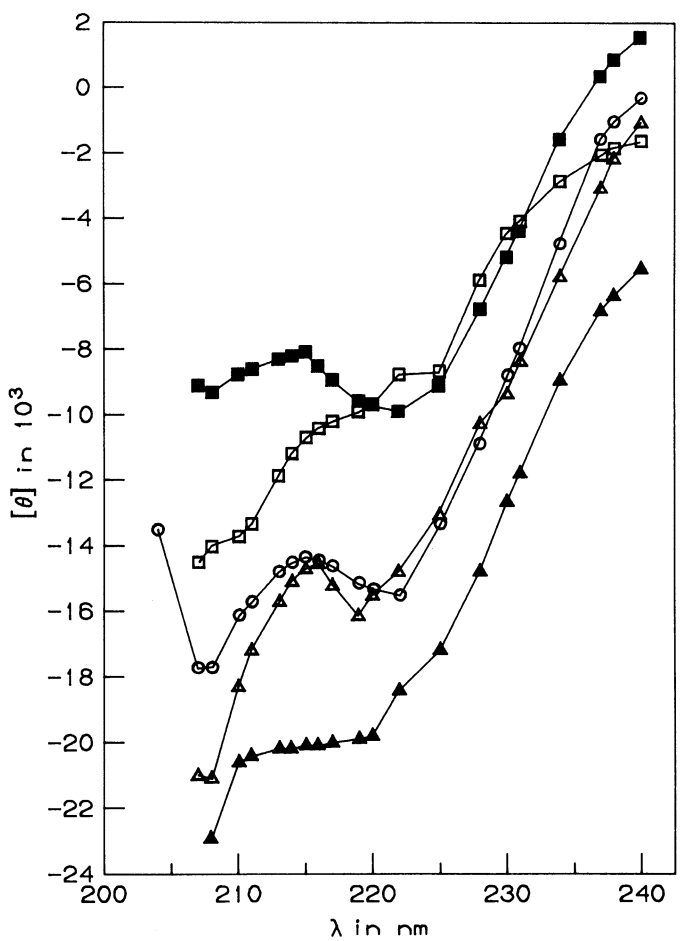

Fig. 1. Ellipticity of corn proteins at various wavelength. o = zein, △ = reduced zein, ▲ = ASG, □ = H_2O-soluble ASG, and ■ = H_2O-insoluble ASG.

compared with the observed 51% in Table I. It is possible that interaction between water-soluble and water-insoluble ASG in ASG results in a higher helix content than for the separated components.

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