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 separated into water-soluble and insoluble fractions 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 50%, 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.

RESULTS AND DISCUSSION

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 Gin-Gin 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 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-sheet, and unordered structure based on the three reference structures of poly-γ-benzyl-L-glutamate (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.

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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compared with the observed 51% in Table 1. 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.

LITERATURE CITED


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