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Study of Rice Glutelin Conformation by Using Circular Dichroism (CD) Spectroscopy

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Abstract: The conformation of rice glutelin under the influence of protein structure perturbants, sodium per chlorate, sodium chloride and pH were studied by Circular Dichroism (CD) spectroscopy. The estimated secondary structure fractions of rice glutelin from CD spectrum shows that rice glutelin mainly composed of β-form secondary structure (59.1%) followed by random coil (33.0 %) and α-helix (7.9 %). A negative minima occurred around at 209 nm accompanied by a weak shoulder at 222 nm. In the presence of some protein perturbants (SDS and EG), the helix content increased. The SDS also lead to increase random coil fractions and negative minima shifted to 207 nm and the shoulder to 217 nm indicating protein unfolding. In the presence of sodium per chlorate, the α-helix content increased in the expense of β-type structure during dissociation. Sodium chloride showed very little effect on secondary structure of glutelin. At extreme acidic condition (pH 3), there was decrease in α-helix and increase in random coil fraction indicating change in protein conformation but at extreme alkaline condition (pH 11), there was increase in both α-helix and random coil fractions indicating protein denaturation.

Key words: Rice glutelin; CD spectroscopy; protein conformation

INTRODUCTION

Rice glutelin, the major storage protein fractions in rice, is an oligomeric protein with a molecular structure similar to that of other legumin like protein(Zhao *et al.*, 1983). Glutelin sub-units have an approximate molecular weight of 60 kilodaltons(KD) and consists of an heterogeneous collection of disulfide linked polypeptides (Yamagata *et al.,* 1982; Zhao *et al.,* 1983). Upon reduction and under denaturing conditions, glutelin sub-units can be dissociated in to two major fractions, i.e.. the acidic or α-polypeptides and the basic or β-polypeptides. The acidic and basic polypeptides have isoelectric points between pH 6.5-7.5 and 9.4-10.3 and molecular weights ranging from 28.5-39 KD and 20-23 KD respectively(Yamagata *etal.*, 1982; Zhao *et al.,* 1983; Robert *et al.,* 1985; Wen and Luthe, 1985). A thorough understanding of the structure-function relationship in rice glutelin is important in predicting and controlling the functional performance of rice glutelin in manufactured foods. Studies of the conformation of proteins under the influence of different environmental conditions can provide crucial information for improving specific functional properties such as gelation and emulsification.

Rice glutelin has been extensively studied with respect to parameters such as size, amino acid composition and charge heterogeneity. There is, however, no report of CD study on rice glutelin. We have, therefore, undertaken such study on rice glutelin and have been able to get some insight in to the secondary structure of rice glutelin.

MATERIALS AND METHOD

Rice seeds (variety Xiang Mi) were collected from Thailand. They were dehulled in pin-mill and defatted by Soxhlet extraction with hexane. Rice glutelin was extracted from the defatted rice flour with 0.07 M NaCL(Osborne *et al.,* 1914). The purity and homogeneity of rice glutelin were checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE) according to Laemmli, (1970).

Circular dichroism spectroscopy (Far-UV CD): Circular dichroism(CD) spectroscopy is an optical technique that allows the detection and quantitation of the chirality of molecular structures. It also provides information about the secondary and tertiary structures of proteins. The CD spectra of proteins can give insights in to a number of aspects of protein structure and when in combination with other structural techniques can play an important role in examining the processes of folding and unfolding of proteins. CD is far more sensitive to changes in orientation, conformation and chromophore micro movement as compared to absorbance measurement.

Rice glutelin dispersions (0.1%) were prepared in different solvents. For the control, protein was dispersed in 10 mM phosphate buffer (pH 7.4) containing 0.5 M

NaCL. The buffer used in this study was filtered and degassed before use. All chemicals used in this study were of highest purity (Sigma Ultra). Effect of protein perturbants including sodium dodecyl sulfate, ethylene glycol, sodium per chlorate, NaCL and effects of pH were studied. The protein dispersions were stirred for 30 minutes in room temperature prior to CD measurement and insoluble materials were removed by centrifugation in a bench top centrifuge (5000X rpm, 5 min., room temperature).

The far-UV CD measurements were carried out using a Jasco J-720 spectrometer (Japan spectroscopic C. Ltd., Tokyo, Japan), calibrated at 290.5 nm with ammonium d-10-camphorsulphonate. The CD spectrum was recorded from 250 to 200 nm in 0.1 cm quartz cell with resolution of 0.2 nm, scan speed of 20 nm min^{-1}, time constant of 2.0 sec., 1.0 nm band width and sensitivity of 20 mdeg. The measured CD curves are a superposition of the individual spectra of α-helix, β-sheet, β-turn and randomly coiled conformations. Secondary structure estimation of protein was calculated using the Jasco SSE-338 Protein Secondary Structure Estimation Program (Japan spectroscopic Co. Ltd., Tokyo, Japan) which was based on the reference CD spectra of Yang (1986).

RESULTS AND DISCUSSION

Figure 1 shows the CD spectrum of rice glutelin between 200 nm and 250 nm. The spectrum exhibited a negative minima centered at around 209 nm and a shoulder near 222 nm. The weak shoulder at 222 nm suggested very little helical structure.

The quantitative estimation of the relative amounts of α-helical, β-form and random coil secondary structure fractions of rice glutelin control and different buffer conditions have been presented in Table 1. According to the CD data, the rice glutelin mainly composed of β-form secondary structure (59.1%) followed by random coil (33.0 %) and α-helix (7.9 %).

Mawal *et al.* (1990) also reported that rice glutelin consists of high number of random coil fraction with a low level of α-helical structure.

Effect of protein structure perturbants

Sodium dodecyl sulphate (SDS): The effect of SDS on the Far-UV CD spectral characteristics of rice glutelin is shown in Fig. 2. In the presence of 10 and 20 mM SDS the helix content increases, indicating change in conformation. SDS is known to increase the amount of helical structures in proteins of low helix content an effect that primarily depends upon the nature of protein (Su and Jirgensons, 1977). The "necklace model" proposed by

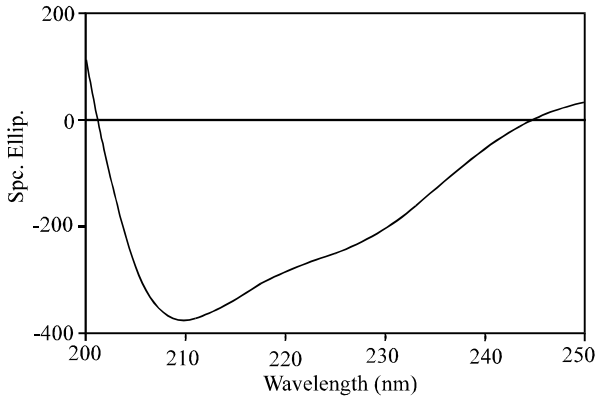

Fig. 1: Far-UVCD spectrum of rice glutelin control (0.1% protein in 10 mM phosphate buffer, pH 7.4)

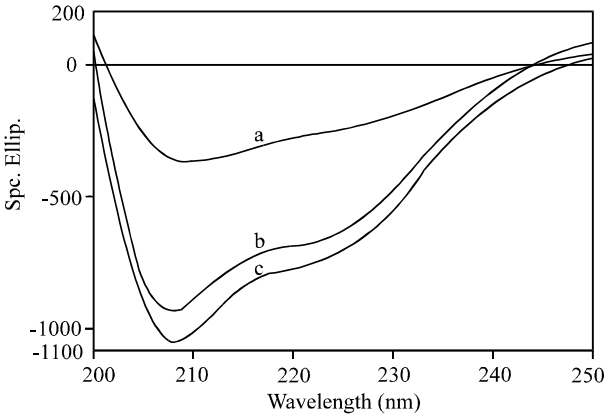

Fig. 2: Far-UVCD spectrum of rice glutelin. a: control; b: 10 mM SDS; c: 20 mM SDS

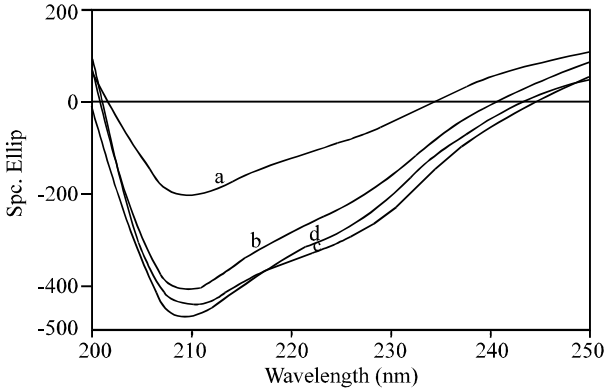

Fig. 3: Far-UVCD spectrum of rice glutelin. a: control; b: 0.5 M $NaClO_4$; c: 1.0 M $NaClO_4$; d: 1.5 M $NaClO_4$

Takagi *et al.* (1975) in which SDS binds to the protein chain forming micelle-like clusters due to the extended hydrophobic environment of the bound SDS molecules, suggests localized formation of small amounts of α-helical structures. At the same time the presence of SDS, random coil fractions increases suggesting protein denaturation

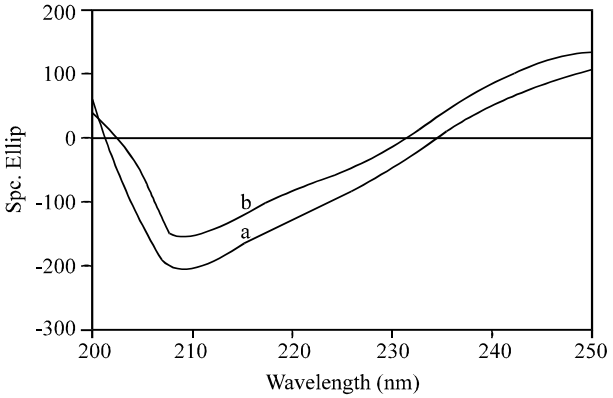

Fig. 4: Far-UVCD spectra of rice glutelin in 10 mM phosphate buffer. a: control; b: 1.0 M NaCl

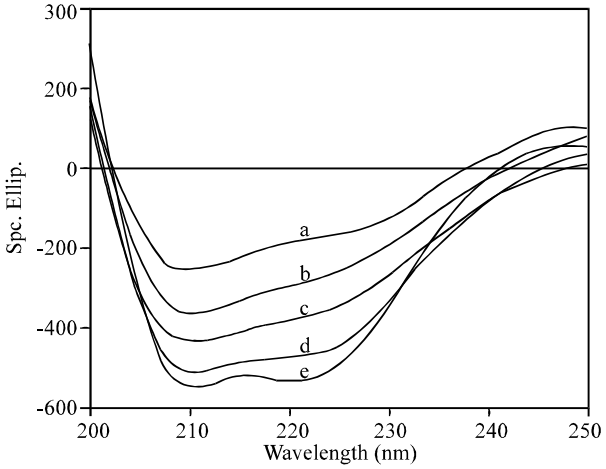

Fig. 5: Far-UVCD spectra of rice glutelin. a: control; b: 20% EG; c: 40% EG; d: 60% EG; e: 80% EG

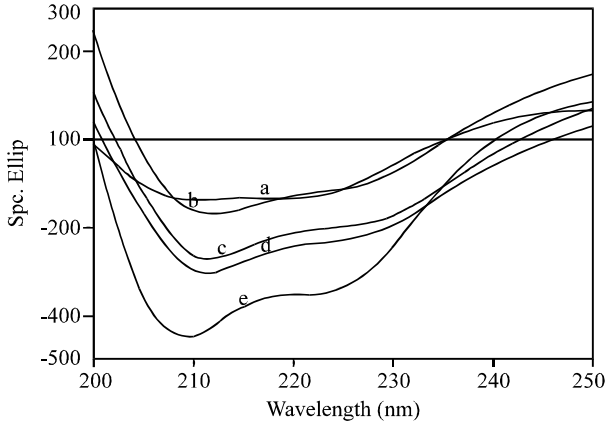

Fig. 6: Far-UVCD spectra of rice glutelin. a: pH 3; b: pH 5; c: pH 9; d: pH 7; e: pH 11

and the β-type fraction decreases. In the presence of SDS, the negative minima shifted to around 207 nm and the shoulder appeared at around 217 nm.

Table 1: CD spectroscopy secondary structure fractions of rice glutelin at different buffer conditions

	Structure fractions[a]		
Buffer conditions	α-helix	β-type structure	Random coil
Control	7.9 (±0.21)	59.1(±0.21)	33.0 (±0.21)
SDS 10 mM	9.0 (±0.07)	57.5(±0.07)	33.7 (±0.21)
SDS 20 mM	9.1 (±0.07)	57.1(±0.28)	33.9 (±0.07)
EG 20 %	8.2 (±0.07)	58.7(±0.14)	33.1 (±0.00)
EG 40 %	8.4 (±0.21)	58.4(±0.07)	33.2 (±0.21)
EG 60 %	8.8 (±0.14)	57.8(±0.14)	33.4 (±0.07)
EG 80 %	8.9 (±0.07)	58.2(±0.21)	32.9 (±0.07)
$NaClO_4$ 0.5 M	8.0 (±0.14)	58.9(±0.14)	33.2 (±0.07)
$NaClO_4$ 1.0 M	8.2 (±0.14)	58.5(±0.14)	33.3 (±0.14)
$NaClO_4$ 1.5 M	8.1 (±0.07)	58.8(±0.07)	33.1 (±0.21)
NaCl 1.0 M	7.4 (±0.14)	59.7(±0.21)	32.9 (±0.00)
pH 3	7.6 (±0.07)	59.1(±0.00)	33.3 (±0.07)
pH 5	8.0 (±0.28)	58.9(±0.28)	33.1 (±0.35)
pH 7	8.3 (±0.14)	58.2(±0.21)	33.5 (±0.07)
pH 9	8.1 (±0.00)	58.7(±0.07)	33.2 (±0.21)
PH 11	8.2 (±0.07)	58.3(±0.07)	33.5 (±0.21)

[a]Results are the mean values (±standard deviation) of two replications

SDS is an anionic detergent that can bind to protein by non covalent forces to increase the net charge and hence lead to ionic repulsion and unfolding of polypeptides (Steinhardt, 1975).

Ethylene glycol (EG): The effect of EG on the secondary structure fractions of rice glutelin are shown in Table 1 and Far-UV CD spectral characteristics are shown in Fig. 5. In the presence of EG, the helix content increases. There are also decrease in the β-type structure and increase in the random coil fractions suggesting protein denaturation. Ethylene glycol could lower the dielectric constant of water and weaken the non-polar interactions between protein molecules and thereby causing destabilization.

Effect of perchlorate: The Far-UV CD spectra of rice glutelin in solutions containing 0, 0.5, 1.0 and 1.5 M sodium perchlorate are shown in Fig. 3 and corresponding secondary structure composition calculated are presented in Table 1. It can be observed that the proportion of α-helix increased at the expense of β-type structure during dissociation. The negative minima remained unchanged. Similar observation was also noticed by Chambers *et al.* (1990) in case of pea legumin, where they found that the hexameric protein dissociate first to trimers and further to monomers in the presence of sodium perchlorate.

Effect of Sodium Chloride: The Far-UV CD spectra of rice glutelin in solutions containing 0 and 1.0 M sodium chloride are shown in Fig. 4 and corresponding secondary structure composition calculated are presented in Table 1. It reveals that no remarkable change occurred due to the effect of sodium chloride suggesting the stabilizing effect of NaCl on the secondary structure of glutelin. But at the same time α-helix content decreases slightly indicating a

little change in conformation. Y.H. Lee *et al.* (2000) also reported the Far-UV spectra of BP UreE showed that both salt and nickel stabilized the ordered structure of protein.

Effect of pH: The Far-UV CD spectra of rice glutelin at different pH values are presented in Fig. 6 and corresponding secondary structure fractions are in Table 1. The spectra of pH 7 and pH 9 are more or less similar to that of control. At highly alkaline condition (pH 11) the shoulder shifted to 218 nm from 222 nm, indicating change in conformation although the negative minima centered at around 209 nm. Other changes are the intensity of the spectrum increased.

At highly acidic condition (pH 3) the shoulder also shifted to 218 nm but at pH 9 the shoulder remained unchanged and negative minima centered at around 211 nm. The intensity of spectra was highly increased in highly alkaline condition (pH 11) than that of highly acidic condition (pH 3). This indicated that highly alkaline condition like pH 11 has more denaturing effect than that of highly acidic condition like pH 3. In highly acidic condition (pH 3), the α-helix content decreased indicating change in conformation. At the same time the random coil fractions increased at different pH conditions indicated denaturation of protein.

Most proteins are stable over a certain pH range normally near their isoelectric pH, where repulsive forces are low and the proteins remain in their native state. At pH far from the isoelectric point, large net charges are induced and proteins will be partially unfold due to intramolecular side-chain charge repulsion leading to rupture of hydrogen bonds and break up of hydrophobic interactions (Morrissey *et al.*, 1987).

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