# Structure and Physical Properties of Seed Proteins. I. Glass Transition and Crystallization of Zein Protein from Corn

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**SYNOPSIS**

Thermal behavior of a seed protein, zein from corn, was studied by differential scanning calorimetry (DSC), thermogravimetry (TG), thermal expansion, X-ray diffraction, and infrared spectroscopy. Zein film cast from aqueous ethanol is amorphous in the random-coil conformation. Water in the specimen is lost by evaporation at about 100°C. The glass transition is observed at 165°C. The amorphous zein crystallize to $\beta$-crystals at about 210°C accompanied by the random-coil → $\beta$-form conformational transition. The thermal degradation of zein occurs at about 320°C. Steam treatment of zein film results in the conformational change to the $\alpha$- and $\beta$-forms, simultaneously, irrespective of treating temperature. © 1992 John Wiley & Sons, Inc.

## INTRODUCTION

The stored proteins are very important as a stable food for humans all over the world. However, very few scientific studies have been reported on seed proteins,[1] especially ones directly concerned with the structure and physical properties of these proteins.

The seed of corn contains several kinds of proteins. These proteins are classified into four components according to their solubility[2]: albumin (soluble in water), globulin (soluble in saline), prolamine (soluble in alcohols), and glutelin (soluble in aqueous acid and alkali solutions). Prolamine, the alcohol-soluble fraction, is designated as zein. In the present study, the thermal behavior such as glass transition and crystallization of zein films was examined in detail.

## EXPERIMENTAL

### Material

Zein protein was isolated from maize seed of Kyosen No. 6. Corn meal was ground in a mill and then by pestle in a mortal. The corn meal was stored without prior defatting with petroleum ether. The corn meal was extracted with either 60 or 70% (by weight) aqueous ethanol[5] at room temperature for 12 h.[3] The extract was dialyzed against distilled water. The precipitated protein was centrifuged and lyophilized. The protein prepared was a white yellow granular solid.[4] The protein was again dissolved in 70% ethanol and washed repeatedly with dichloroethane or petroleum ether until all color was removed. After concentrated by removal of a part of ethanol by vacuum distillation, the solution was poured into a large volume of 1% sodium chloride solution. The gummy substance obtained was washed with water to remove sodium chloride and then freeze-dried after the remaining ethanol was allowed to dry in the air. The film specimen was obtained by casting the protein solution in aqueous ethanol on a glass plate at 20°C.

### Measurement

Differential scanning calorimetry (DSC) scans were recorded on a Seiko DSC-100 at a heating rate of 10 K/min. Thermogravimetry was done with a Rigaku TG-DSC 8085E1. Linear thermal expansion was measured using a Rigaku TMA type CN 8095 by recording the change in length of the film specimen under constant tension at a heating rate of 10 K/min. X-ray diffraction patterns were obtained with a Rigaku D3F X-ray diffraction apparatus. Ni-

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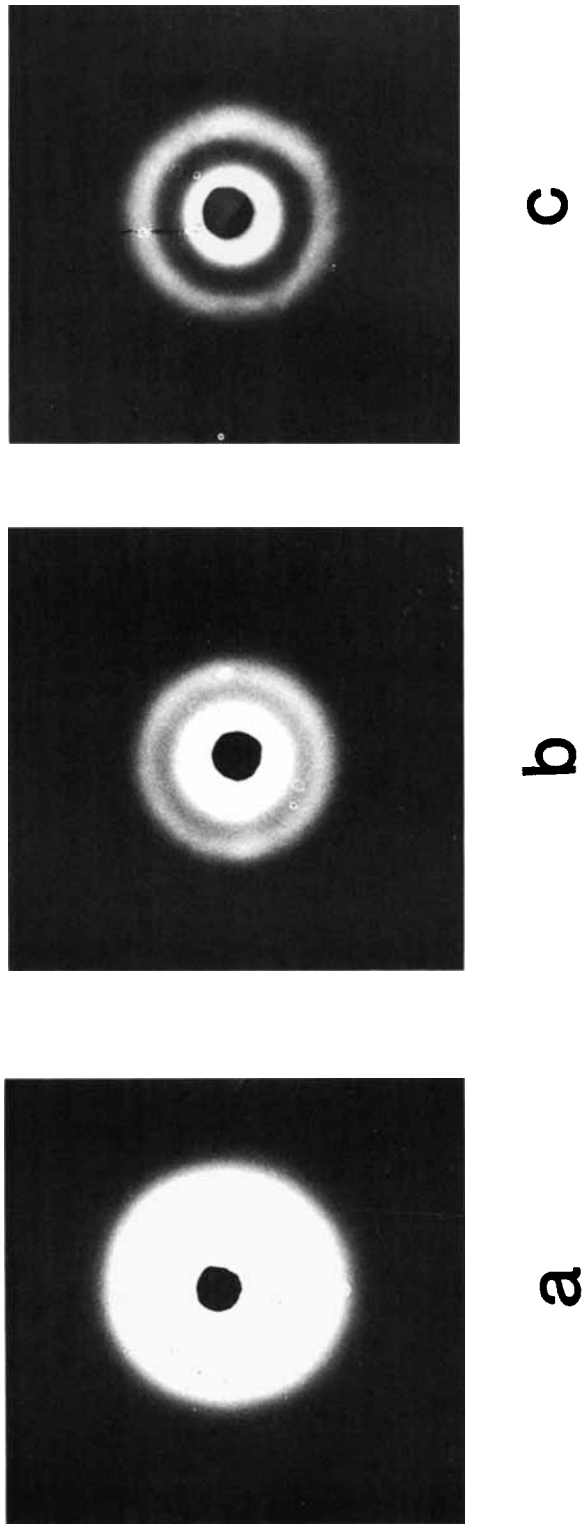


**Figure 1** X-ray diffraction patterns of zein films (a) untreated and (b) treated at 190°C for 10 min and (c) treated at 210°C for 30 min.

filtered CuK$\alpha$ radiation was used at 35 kV and 20 mA. Infrared spectra was recorded on a Nicolet Model 60SCR infrared spectrometer. The specimen placed between two NaCl plates was heated at 5 K/min with a Hitachi HPC-300 temperature programming controller. The temperature was measured by a thermocouple attached to the NaCl window. Band intensity was determined by the base-line method.

## RESULTS AND DISCUSSION

### Amino Acid Composition

The amino acid composition of zein was determined by an amino acid analyzer to consist of 28% glutamic acid, 23% valine, 14% lysine, 11% proline, and 9% alanine. Zein was separated into four fractions by SDS-PAGE according to molecular weight, whose molecular weights were 24 kD (strong), 27 kD (strong), 38 kD (weak), and 42 kD (weak), respectively.

### Conformation and Crystallinity

Figure 1 shows X-ray diffraction patterns of zein film cast from aqueous ethanol solution that annealed at 190°C for 10 min and at 210°C for 30 min.

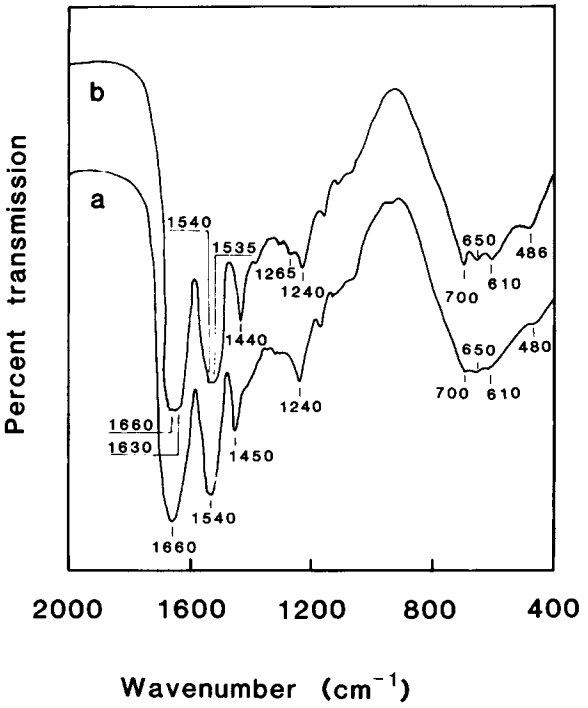


**Figure 2** Infrared spectra of zein films (a) untreated and (b) treated at 210°C for 30 min.

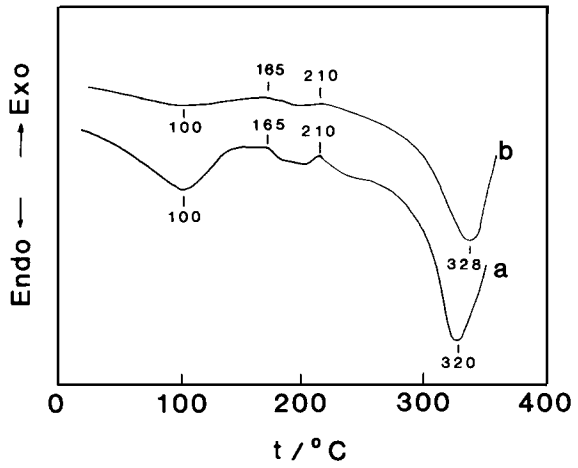


**Figure 3** DSC curves of zein films (a) untreated and (b) treated at 210°C for 30 min.

An amorphous halo is observed for the as-cast film, whereas diffraction rings appear by annealing films and these diffraction patterns are identified to be caused by $\beta$-form crystals.

In Figure 2 are shown the infrared spectra of zein film before and after heat treatment at 210°C for 30 min. The as-cast film shows absorption bands at 1660, 1540, 1240, and 650 cm$^{-1}$, which are assigned to amide I, II, III, and V bands for the random-coil conformation, respectively.[6]

By annealing the film at 210°C for 30 min, new absorption bands appear at 1630, 1535, 1265, and 700 cm$^{-1}$. They are assigned to amide I, II, III, and V bands for the $\beta$-form conformation, respectively.[6]

From the infrared spectra and X-ray diffraction

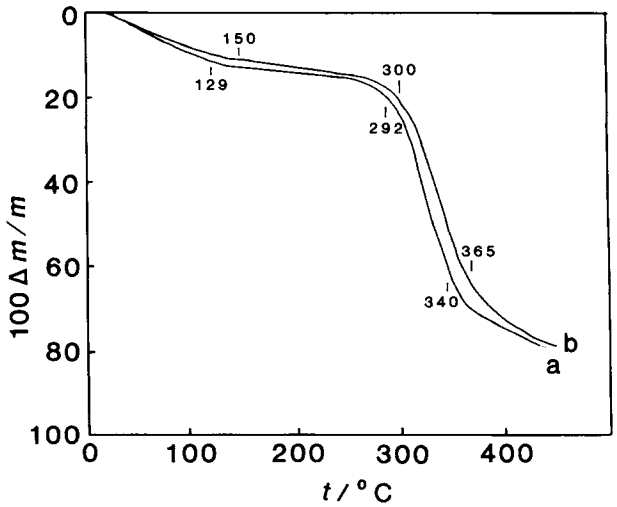


**Figure 4** TG curves of zein films (a) untreated and (b) treated at 210°C for 30 min.

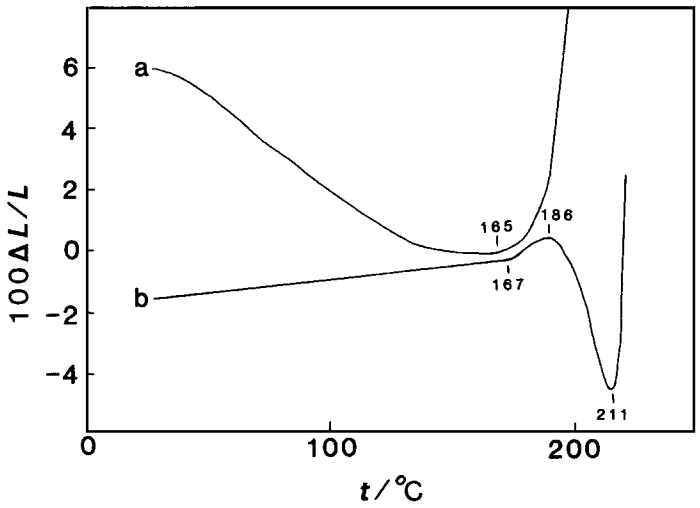


**Figure 5** Thermal expansion curves of zein films (a) untreated and (b) treated at 210°C for 30 min.

patterns, zein film cast from aqueous ethanol is amorphous and has random-coil conformation and crystallizes to β-crystal by heat treatment accompanied by the random-coil → β-form conformational transition.

## Glass Transition and Crystallization

Curve **a** of Figure 3 shows DSC curves of amorphous zein film in the random-coil conformation under nitrogen. Two endothermic peaks appear at 100 and 320°C. An exothermic peak at 210°C and an endothermic shift at 165°C are also observed. The endothermic peak at 320°C is prominent, suggesting the degradation of zein, since an abrupt weight loss is observed in the thermogravimetry (TG) curves of zein film at about 300°C under nitrogen (Fig. 4). The TG curve shifts to higher temperature by heat treatment, indicating the increase in thermal stability.

The broad endothermic peak observed at 100°C is attributed to the evaporation of water in the specimen, since the peak became smaller when the spec-

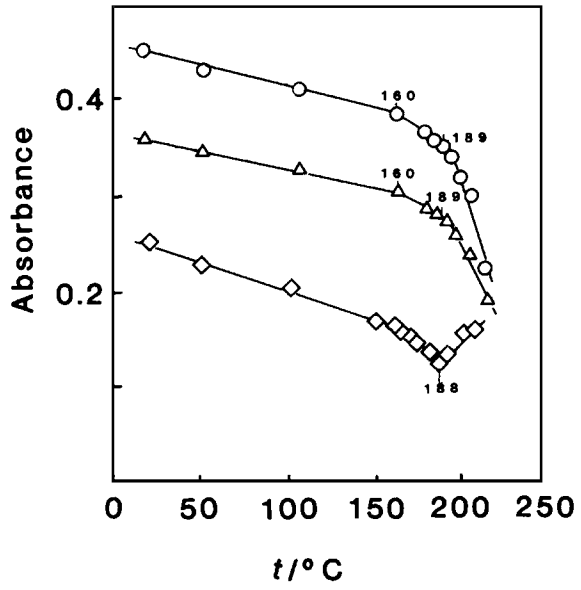


**Figure 6** Temperature dependence of infrared absorption bands of amorphous zein film. Random-coil bands at (○) 1660 and (△) 1540 $cm^{-1}$ and β-conformation band at (◇) 1535 $cm^{-1}$.

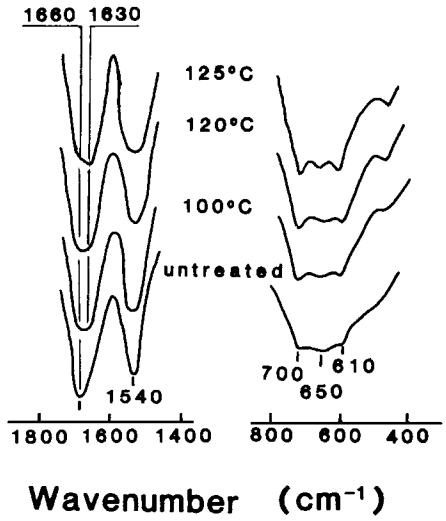


**Figure 7** Infrared spectra of zein films steam-treated at various temperatures for 15 min.

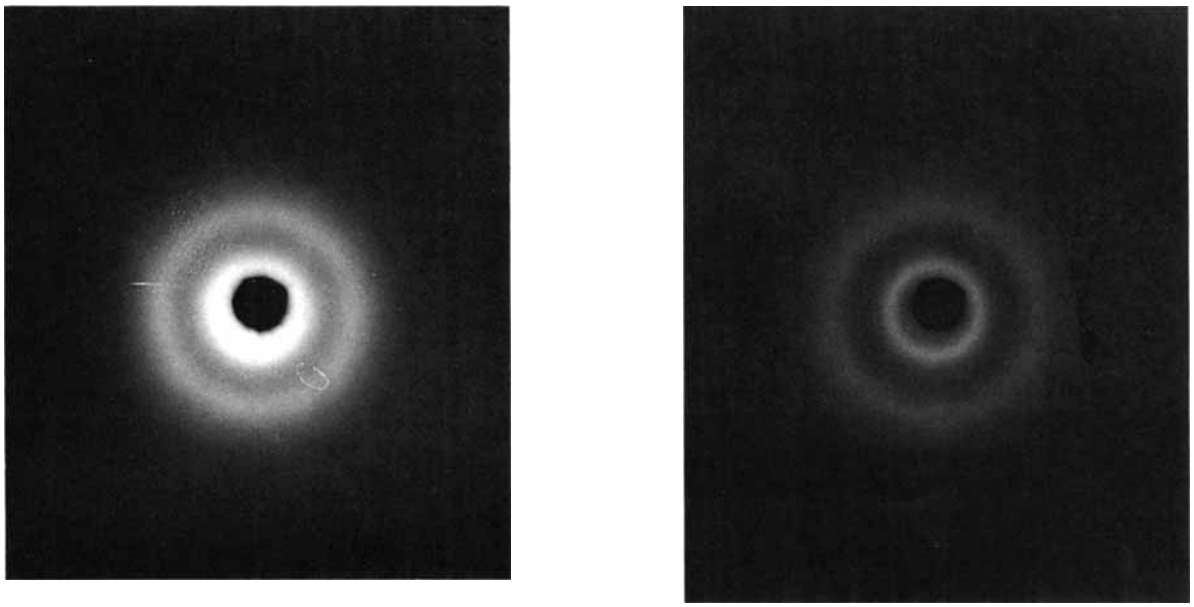


**Figure 8** X-ray diffraction patterns of zein film (a) untreated and (b) steam-treated at 120°C for 15 min.

imen was annealed at 210°C for 30 min (Fig. 3, curve **b**).

Figure 5 shows the linear thermal expansion curves of zein film. The as-cast specimen contracts slowly up to 130°C with increasing temperature, and at 165°C, the length of the specimen begins to increase abruptly (curve **a**). The initial contraction is attributed to evaporation of water, since prior drying at 100°C makes the contraction smaller.

The length of zein film heat-treated at 210°C starts to increase abruptly at 167°C and then the specimen started to contract at 186°C (curve **b**). Therefore, the endothermic shift of the DSC curves at 165°C is due to the glass transition of zein.

The exothermic peak at 210°C almost disappears by heat treating the specimen at 210°C for 30 min, as shown in curve **b** of Figure 3. Considering the results of X-ray diffraction and infrared spectroscopy mentioned above, this exothermic peak is attributed to the crystallization of amorphous zein to $\beta$-crystals accompanied by the random-coil → $\beta$-form conformational transition.

To further examine the random-coil → $\beta$-form transition, three infrared bands of amorphous zein film were measured with stepwise increasing of temperature, as shown in Figure 6. The absorbance of the bands at 1660 and 1540 $cm^{-1}$, the amide I and II bands of the random-coil conformation, decreased linearly with increasing temperature, until an abrupt change in slope occurs at about 189°C.

The absorbance of the amide II band of the $\beta$-

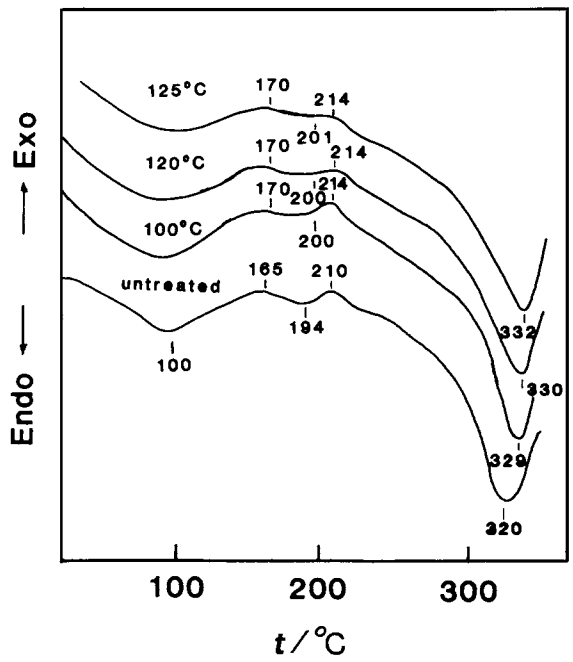


**Figure 9** DSC curves of zein films steam-treated at various temperatures for 1 h.

form at 1535 $cm^{-1}$ also decreases slowly with increasing temperature and begins to increase abruptly at 188°C. Therefore, the conformational change of random-coil to the $\beta$-form takes place above 188°C.

### Conformational Change by Steam Treatment

Figure 7 shows the change of infrared spectra in the range of 400–2000 $cm^{-1}$ of zein films steam-treated for 15 min at various temperatures. In addition to the amide I, II, and V bands at 1660, 1540, and 650 $cm^{-1}$ due to random-coil conformation, the amide I, II, and V bands at 1630, 1530, and 700 $cm^{-1}$ due to the $\beta$-form and the amide V band at 610 $cm^{-1}$ due to the $\alpha$-form appear by steam treatment irrespective of steam-treating temperature. The same results were obtained by extending the duration of steam treatment to 2 h.

By steam treatment, the random-coil conformation was converted to the $\alpha$- and $\beta$-forms simultaneously. Water molecules play an important role in the transformation. Presumably, water molecules cleave intra- and/or intermolecular hydrogen bonds of zein molecules, resulting in the transformation to the $\alpha$- and $\beta$-forms irrespective of treating temperature.

From the X-ray diffraction pattern (Fig. 8), the amorphous zein film was converted to the $\beta$-form crystals by steam treating at 120°C for 15 min.

Figure 9 shows DSC curves of the amorphous zein films before and after being steam-treated for 1 h at various temperatures. The endothermic shift at 165°C due to the glass transition slightly moved to higher temperatures by steam treatment and the exothermic peak due to thermal degradation also shifted to higher temperature. These results are induced by the partial crystallization of amorphous zein.

## CONCLUSIONS

Zein film cast from aqueous ethanol is amorphous in the random-coil conformation. Water in the specimen is lost by evaporation at about 100°C. The glass transition is observed at 165°C. The amorphous zein crystallizes to $\beta$-crystals at about 210°C accompanied by the random-coil → $\beta$-form conformational transition. The thermal degradation of zein occurs at about 320°C. Steam treatment of zein film results in the conformational change to the $\alpha$- and $\beta$-forms, simultaneously, irrespective of treating temperature.

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