Rapid Communication

Aggregate formation of zein and its structural inversion in aqueous ethanol

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Abstract

Zein is a prolamine of maize. Conventionally, 70–90% aqueous ethanol is used to dissolve zein. When the hydrodynamic radii of zein molecules in aqueous ethanol were monitored with a dynamic light scattering instrument, it was found that zein aggregates in the solvent and the degree of aggregation depends on the composition of the solvent mixture. As the ethanol content of solvent increased from 70% to 90%, the aggregation number of zein molecules decreased from 10,000 until it reached a minimum. The aggregation number then increased abruptly to greater than 10,000 as the ethanol content of the solvent mixture increased to 92%. Since zein has amphiphilic characteristics, this behavior was interpreted as the formation of a micelle-like structure in its solution whereby ca. 90% ethanol that showed minimum aggregation number is regarded as a structural inversion point. This point of view was supported by a simple experiment that showed selective interaction of zein molecules with hydrophilic or hydrophobic particles.

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Zein is the major storage protein of corn. As revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), zein is mainly composed of two distinct bands, with major molecular weights of 19 and 22 kDa (Sessa et al., 2003). The high proportion of non-polar amino acid residues in zein is responsible for its solubility behavior. The major structure is a helical wheel conformation in which nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds (Argos et al., 1982). Zein can form tough, glossy, hydrophobic coatings and has anti-bacterial activity, which has been used in the food industry (Shukla and Cheryan, 2001).

Conventionally, aqueous ethanol has been used as a solvent for zein in many experiments (Dickey et al., 2003; Fu and Weller, 1999; Guo et al., 2005; Kim et al., 2004; Lawton, 2002; Parris and Coffin, 1997). It is one of the good solvents for zein (Manley and Evans, 1943). Although it is known that 60–95% ethanol content is adequate for solubilization of zein, the effect of variation of alcohol content on the behavior of individual zein molecules is not known.

Fu and Weller (1999) reported on the rheology of zein solutions in aqueous ethanol. Zein (2–12%) in 50%, 60%, 70%, 80%, and 90% aqueous ethanol was investigated. Regardless of zein concentration, the overall trend showed that the viscosity of the solution decreased as the concentration of ethanol increased from 50% to 90%. This means that the apparent molecular weight of zein decreases at a higher ethanol content.

Yamada et al. (1996) reported that zein films prepared from 80% (v/v) aqueous ethanol did not show enough waterproof property compared with those prepared from 70% (v/v) aqueous acetone. In addition, aggregate formation was observed in both films. Dong et al. (2004) reported
that SEM revealed that the zein film prepared from 70% ethanol was composed of particles of diameter 100–500 nm. These particles were agglomerated to form a film. The concentration of zein had no significant effect on particle size. Guo et al. (2005) used atomic force microscopy (AFM) for the visualization of zein globules formed from 70% (v/v) aqueous ethanol on the surface of mica. Depending on the concentration of zein in the solution, the diameter of globules ranged between 150 and 550 nm, or between 60 and 120 nm. If we compare zein with other proteins of similar MW, the diameter of a zein molecule is expected to be ca. 5 nm (Tanford, 1961). Therefore, the data from previous researches clearly indicate that zein forms aggregates with a very large aggregation number in aqueous ethanol. However, previous data were obtained from two-dimensional films assuming that the aggregation number does not change during the evaporation of solvents. In this report, we used a dynamic light scattering (DLS) technique to measure the aggregation number of zein in solution.

In order to find the best composition and the difference in the solubilization capability, the transmittance of zein solutions was measured at several zein concentrations with a custom-built turbidimeter that is composed of a He–Ne laser, temperature-controlled sample block, stirrer, neutral density filter, and a laser power meter interfaced with a laptop computer. The 633 nm beam from the He–Ne laser passes through the sample solution in a scintillation vial that is surrounded by a temperature-controlled copper block. The diameter of the scintillation vial is 2.5 cm. During the transmittance measurement, the sample solution is continuously stirred with a magnetic spin bar. The intensity of the transmitted laser beam was monitored with a laser power meter, which was interfaced with a laptop computer. Zein solutions of 0.2%, 0.5%, 1%, and 2% (w/w) were examined with a turbidimeter to find the best solvent composition. The experimental results are shown in Fig. 1. The data show a peak of transmittance at around 90% aqueous ethanol but the transmittance dropped with a higher concentration of ethanol. However, although there was variation in the transmittance in the range of 70–90%, no precipitation was observed. It is an indication that there is a size variation of zein. At greater than 90% aqueous ethanol, precipitation was observed and time-dependent transmittance was observed.

For the measurement of the degree of aggregation of zein in various ethanol/water mixtures, a DLS experiment was performed with a BI-9000AT autocorrelator and a BI-200SM goniometer (Brookhaven Instruments Corp., Holtsville, NY) equipped with an argon laser (λ = 514.5 nm, Lexel Laser, Inc., Fremont, CA). All experiments were carried out with 0.1% (w/w) zein solution at 25.0 ± 0.2 °C. A 10 mW He–Ne laser (λ = 633 nm) was used as a light source.

The measured hydrodynamic radii of zein are shown in Fig. 2(a). At around 90% ethanol, a sharp decrease and increase in the hydrodynamic radius of zein was observed. Although it is not practically possible to obtain the data for the hydrodynamic radii for all ethanol/water compositions, it was presumed that each zein molecule exists as a freely moving individual particle at around 90% ethanol. With this speculation, by using the molecular weight of zein obtained from the SDS-PAGE experiment (Cabra et al., 2005; Sessa et al., 2003), the most probable hydrodynamic radius of a zein molecule was assumed at 89.7% ethanol. Including this conjectured data point, the overall trend of the size of aggregates is shown by the dotted lines in Fig. 2(a). The volume of each aggregate and the degree of aggregation were calculated by assuming the hydrodynamic radii for all ethanol/water compositions, it was presumed that each zein molecule exists as a freely moving individual particle at around 90% ethanol. With this speculation, by using the molecular weight of zein obtained from the SDS-PAGE experiment (Cabra et al., 2005; Sessa et al., 2003), the most probable hydrodynamic radius of a zein molecule was assumed. In this report, we used a dynamic light scattering (DLS) technique to measure the aggregation number of zein in solution.

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Hydrodynamic radii of zein aggregates were obtained as follows. The DLS experiment yields a correlation function given as

\[ g^{(1)}(t) = \exp(-\Gamma t), \]

where \( \Gamma = D_q q^2 \) (Johnson and Gabriel, 1994). From the slope of \( q^2 \) vs. \( \Gamma \) plot, we obtain a diffusion coefficient of a solute molecule, \( D_t \). Here, \( q \) is the scattering wave vector. The hydrodynamic radii of aggregates can be calculated by the Stokes–Einstein relationship

\[ R_h = \frac{(kT)/(6\pi\eta D_t)}{}, \]

where \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( D_t \) is the diffusion coefficient.

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This means there is a dramatic growth of aggregates in this regime. On the other hand, as the ethanol concentration was decreased from 90% to 70%, the solution became more and more turbid, indicating that the zein aggregates formed a larger colloidal suspension in the solution. At an even lower ethanol concentration, the aggregates precipitated, but the precipitation rate was not as fast as in the case where the ethanol concentration was increased from 90% to greater than 90% (Fu and Weller, 1999).

From the fact that the radii of aggregates decrease as the ethanol content increases from 70% to 90%, and increases again at greater than 90% ethanol, it is believed that there is a structural inversion at that solvent composition. In other words, zein aggregates form a micelle-like structure in which the hydrophilic moiety is oriented toward the solvent medium at lower than 90% ethanol and oriented toward the center of each aggregate at greater than 90% ethanol. The amphiphilic nature of the zein molecule was previously studied by Wang et al. (2004a, b). If each zein molecule behaves as an amphiphile, it is expected that zein molecules form a macromolecular micelle—globular aggregates with the hydrophilic moiety exposed to the surface and hydrophobic moieties clumped together—in the solution. As the solvent medium turns hydrophobic the orientation of each molecule will be reversed. In other words, micellar inversion is expected depending on the condition of the solvent medium. The structural inversion of zein molecules or aggregates had been suggested by Yamada et al. (1996) when they observed an odd behavior of zein when films were prepared from two different types of solvent systems. The aforementioned observations by other researchers support our view that the inversion of micelle-like structure of zein aggregates occurs at around 90% ethanol. The micelle-like structures formed in lower than 90% ethanol have hydrophilic moieties that are oriented toward the solvent medium. In this case, the surface charges of particles repel each other whereby they form a turbid solution. On the other hand, the micelle-like structures formed in greater than 90% ethanol have hydrophobic moieties that are oriented toward the solvent medium. This surface, the micelle formation of zein in aqueous ethanol is schematically illustrated in the top row of Fig. 3 (no particles). It is expected that zein molecules would surround hydrophilic or hydrophobic particles depending on the concentration of ethanol in the solution. The schematic presentation of this behavior is shown in the bottom row of Fig. 3 (with particles). This viewpoint is supported by a simple experiment as follows.

As shown in Fig. 2(b), addition of ethanol or water to a zein solution in 90% ethanol induces larger aggregates. For the verification of the inversion idea, 10% zein in 90% ethanol mixed with hydrophilic particles (glass spheres, o.d. = ca. 10 μm) and hydrophobic particles (toner, o.d. = ca. 10 μm) was prepared. When ethanol was gradually added to the solution, zein surrounded the glass spheres and formed a large agglomerate while the toner particles were not affected. As the agglomerate was
compressed in the cylindrical mold, a glass sphere composite was produced (Fig. 4). On the other hand, when water was added to the solution, zein surrounded the toner particles and formed small agglomerates while the glass spheres were not affected. The reason for forming smaller agglomerates instead of a large one must be because of the surface charges of particles that repel each other. A toner composite was produced as the agglomerate was compressed in the cylindrical mold (Fig. 4). In this experiment, the behavior of zein in the latter case is similar to that of detergents in a washer. Toner particles are non-polar and insoluble in water. When detergents and hydrophobic particles (e.g., toner or oily dirt) are mixed in water, the hydrophobic moiety of detergent molecules are attracted to the surface of hydrophobic particles. The micelle-like structures formed by this mechanism contain hydrophobic particles in the center. By the same reasoning, if some amphiphiles (e.g., zein) and hydrophilic particles (e.g., glass spheres) are dispersed in a hydrophobic medium, the hydrophilic moiety of zein molecules will be attracted to the surface of hydrophilic particles. In this way, the inversion of the micelle-like structures at ca. 90% ethanol is understood.

As stated above, addition of more ethanol to a zein solution in 90% ethanol induces large aggregates. It is the basis of the procedure developed in our lab for the production of polymer composite materials. Detailed procedure and characterization of the products will be presented in upcoming reports.

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**References**
