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Role of surfactant in the formation of zein/Tween-20 nanoparticles studied by fluorescence and circular dichroism

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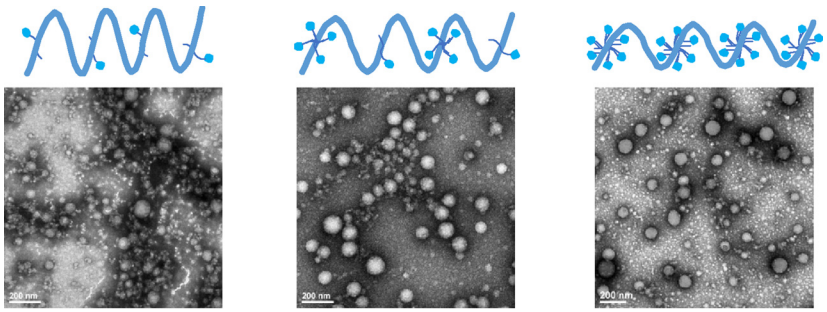
GRAPHICAL ABSTRACT



$C_{Tween\text{-}20}$=0-0.2 g/L $C_{Tween\text{-}20}$=0.2-2.8 g/L $C_{Tween\text{-}20}$=2.8-4 g/L

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ABSTRACT

The formation of zein/Tween-20 nanoparticles through the antisolvent precipitation method at various Tween-20 concentrations ($C_{Tween\text{-}20}$) has been investigated using dynamic light scattering, surface tension, fluorescence, and circular dichroism. When $C_{Tween\text{-}20}$ increases from 0 to 0.2 g/L, the particle sizes of zein/Tween-20 nanoparticles significantly decrease from 140 to 95 nm, followed by nearly constant values of 90–92 nm at $C_{Tween\text{-}20}$ = 0.2–4 g/L. Compared to Tween-20, zein/Tween-20 nanoparticles give smaller values of surface tension at $C_{Tween\text{-}20}$ = 0–2.8 g/L, and almost the same values at $C_{Tween\text{-}20}$ higher than 2.8 g/L. The values of fluorescence maximum intensity and α-helix content of zein molecules in zein/Tween-20 nanoparticles show pronouncedly different changes at $C_{Tween\text{-}20}$ = 0–0.2, 0.2–2.8, and 2.8–4 g/L. The formation of zein/Tween-20 nanoparticles is proposed to be induced by the aggregation of zein/Tween-20 complexes mainly driven by the hydrophobic force. Zein/Tween-20 complexes exhibit different structural properties at low, intermediate, and high Tween-20 concentrations.

1. Introduction

Nanoparticles constructed from proteins are gaining considerable attention in the field of nanoscience, especially in food, cosmetic, and pharmaceutical areas [1–3]. Compared to the proteins derived from animals, plant proteins are inexpensive, less allergenic, more biocompatible and biodegradable [4]. As the major storage protein in maize kernels, zein has a variety of unique characteristics and is generally recognized as safe by the US Food and Drug Administration [5]. More than 50% of the amino acid residues in zein are hydrophobic, which makes zein insoluble in water but soluble in 60–90% ethanol/water mixed solution. Due to the highly inherent hydrophobicity, zein

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can be easily constructed into zein nanoparticles, which have been used as the delivery systems to encapsulate drugs and bioactive molecules [6–8].

One common approach to prepare zein nanoparticles is the antisolvent precipitation method, by simply adding an ethanol solution containing dissolved zein into water [9,10]. When preparing zein nanoparticles, the stabilizer such as polymer and surfactant is often dissolved in water phase to prevent the extensive aggregation, improve the stability, and control the particle size of zein nanoparticles. According to the studies from Patel et al. [9] and McClements et al. [10,11], the physical stability of zein nanoparticles is generally thought as a result of the formation of polymer or surfactant layer around the core of zein nanoparticles, which brings about the reduced hydrophobic attraction and increased electrostatic/steric stabilizations. However, this kind of stability effect is based on the assumption that the aggregation of zein molecules into nanoparticles is occurred before the binding of stabilizer molecules. This assumption is possibly reasonable for the formation of zein nanoparticles stabilized by polymer and protein-type of surfactant, which have limited solubility and slow diffusion in water [12]. However, when small molecule surfactant with high water solubility and rapid diffusion is used as the stabilizer, surfactant molecules may preferentially bind with zein molecules to form zein/surfactant complexes driven by the forces of hydrogen bonding, electrostatic and hydrophobic interactions. In fact, during the studies of the solubilization of zein, Somasundaran et al. [13] and Mehta et al. [14] found that small molecule surfactant can bind with zein to form zein/surfactant complexes in water. Meanwhile, the complex formation between protein and surfactant can prevent the aggregation of protein, as reported by McGuire et al. [15] Besides the modulation of protein aggregation, the binding of surfactant with protein may also cause the changes of the physicochemical properties, such as the surface activity and spectrometric characteristics of protein [14,16]. These kinds of studies can be used to deeply understand the formation mechanism of zein nanoparticles stabilized by the surfactant with high water solubility and rapid diffusion.

In the present work, the role of surfactant in the formation of zein nanoparticles through the antisolvent precipitation method has been investigated. The surfactant selected is Tween-20, which is one nonionic polyoxyethylene surfactant and widely used in many industries. At a fixed zein concentration of 0.4 g/L, zein/Tween-20 nanoparticles were prepared upon changing Tween-20 concentration from 0 to 4 g/L at pH 4. The morphology and size of zein/Tween-20 nanoparticles were characterized by transmission electron microscopy and dynamic light scattering, respectively. Surface tension measurement was carried out to monitor the surface activity of zein/Tween-20 nanoparticles with different Tween-20 concentrations. Following the studies of fluorescence and circular dichroism spectra of zein molecules, the formation mechanism of zein/Tween-20 nanoparticles has been proposed, which is induced by the aggregation of zein/Tween-20 complexes with different structural properties at low, intermediate, and high surfactant concentrations.

2. Materials and methods

2.1. Materials

Zein (Z3625, protein content ≥ 97%) and Tween-20 (44112, purity ≥ 99%) were purchased from Sigma-Aldrich Chemical Company. Absolute ethanol (purity ≥ 99.7%) was obtained from Shanghai Titan Scientific Company. All other chemical reagents used were of analytical grade, and water was double distilled.

2.2. Preparation of zein/Tween-20 nanoparticles

Zein/Tween-20 nanoparticles were prepared according to the procedure based on the antisolvent precipitation method [9,10]. Precisely, 1 g powdered zein was added into 50 ml 80% ethanol/water mixed solution and stirred for 30 min until completely dissolved. Under constant stirring, a syringe was used to rapidly drop 4 ml zein solution into 16 ml water containing various amounts of Tween-20. The solution of Tween-20 was adjusted to pH 4 with concentrated hydrochloric acid. The resulting dispersion was stirred for another 30 min, and then ethanol was evaporated using a rotary evaporator. To compensate for the lost ethanol, the same volume of water with pH 4 was added to get the solution of zein/Tween-20 nanoparticles finally. While the amount of zein was kept at 0.4 g/L, the concentration of Tween-20 in the final samples is varied from 0 to 4 g/L.

2.3. Transmission electron microscopy (TEM)

Samples of zein/Tween-20 nanoparticles were deposited onto a 400 mesh carbon-coated copper grid. After 1–2 min, the film was negatively stained with a solution of 2% (v/v) phosphotungstic acid for 20 s, and the excess phosphotungstic acid was removed using filter paper. The dried copper grid was imaged on a JEOL model JEM 1400 TEM at an operating voltage of 120 kV.

2.4. Particle size and zeta potential measurements

The particle size and zeta potential of zein/Tween-20 nanoparticles were determined using a Malvern Zetasizer Nano ZS (Malvern Instruments, London, England) at 25 °C. While the particle size was controlled in situ by dynamic light scattering, the zeta potential was determined from the electrophoretic mobility according to the Smoluchowski equation [17]. During the measurements, the samples were diluted with water of pH 4 and measured at least three times.

2.5. Surface tension measurement

The surface tensions of zein/Tween-20 nanoparticles and Tween-20 were measured by a Dataphysics model DCAT11 tensiometer (Sartorius, Goetingen, Germany) using the Wilhelmy plate method at 25 °C. The plate was first rinsed with double distilled water and then burned to red before each measurement. For comparability of equilibrium surface tension, the measurements were stopped when the standard deviation of the surface tension was less than 0.03 mN/m.

2.6. Steady-state fluorescence measurement

Steady-state fluorescence measurement for zein/Tween-20 nanoparticles was carried out on a Shimadzu RF-5301 spectrofluorophotometer at 25 °C. The intrinsic fluorescence of zein molecules in zein/Tween-20 nanoparticles was recorded from 285 to 400 nm with an excitation wavelength of 278 nm. The slit widths of excitation and emission were kept at 3 and 5 nm, respectively. Each fluorescence spectrum was the average of three runs.

2.7. Circular dichroism measurement

Circular dichroism (CD) spectra of zein molecules in zein/Tween-20 nanoparticles were measured on a Chirascan automatic recording spectrophotometer at 25 °C. The CD spectra were recorded at 1 mm pathlength in the range of 200–240 nm. The scan speed was 120 nm/min with a response time of 2 s. Each spectrum presented is the average of three consecutive scans. The results were expressed as ellipticity in millidegrees (mdeg). The α-helix content of zein molecules was calculated using CDNN, which is a deconvolution program for protein secodary structure analysis from CD data (Chirascan).

2.8. Statistical analysis

Data are presented as mean and standard deviations. For all