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Surface activity and safety of deamidated zein peptides

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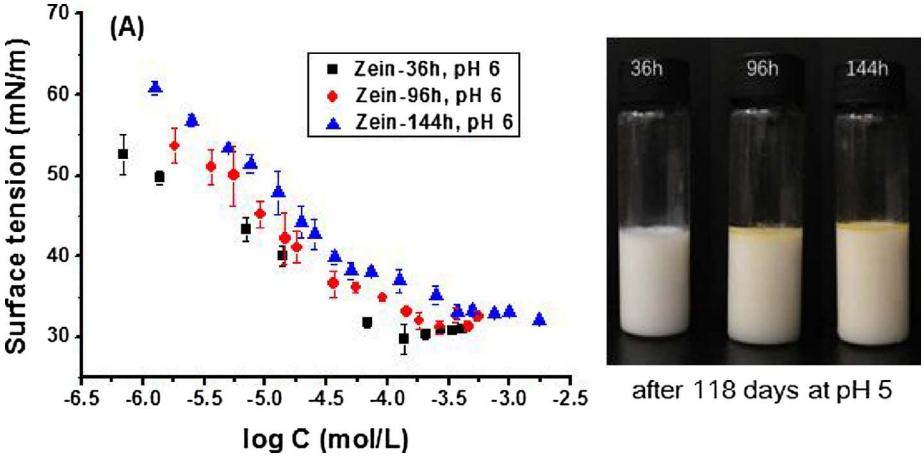
GRAPHICAL ABSTRACT



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ABSTRACT

Hydrolysis of food proteins is a convenient and feasible way to produce amphipathic and low cost peptides. In this study, three deamidated zein peptides were produced from zein via 36, 96 and 144 h alkaline hydrolysis reaction at 37 °C. Longer hydrolysis reaction produced smaller peptides with more carboxyl groups. Changing aqueous solution pH from 4.0 to 8.0, the peptides carried more negative charges that reduced the aggregation of the peptides. In vitro cytotoxicity study verified that the peptides were much safer than sodium dodecyl sulfate (SDS). In pH 4.0–8.0 aqueous solutions, the peptides presented better surface activities than SDS. The apparent critical micelle concentration (CMC) values of the peptides were much smaller than the CMC value of SDS, the pC_{20} values (ability to reduce surface tension of 20 mN/m) of the peptides were much larger than the pC_{20} value of SDS, and the surface pressure π_{CMC} values of the peptides were similar to the value of SDS. In addition, the peptides had non-foaming property, good emulsifying capacity and emulsion stability at acidic condition. This study demonstrates that renewable, safe, degradable and low cost peptides produced by hydrolysis of food protein have excellent surface activities. Deamidated zein peptides with adjustable hydrophilicity/hydrophobicity are promising alternatives to chemical surfactants in food industry.

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1. Introduction

Surfactants are a group of amphiphilic organic compounds that help to lower the surface tension of a liquid or interfacial tension between two liquids. Surfactants have several important properties, such as wetting, penetration, emulsification, dispersion, solubilization and foaming [1]. Various surfactants, which have different charges, different structures, thus have different surface activities, can be obtained by changing the hydrophilic and hydrophobic moieties chemically [2]. The disadvantages of chemical surfactants are their potential toxicity [3,4] and issues with relation to biodegradation [5,6]. There is a rising interest in finding safe and green surfactants [5,7–11]. Amino acid/protein-based surfactants are ecological, biocompatible and renewable [12]. Many food proteins, which are nourishing, nontoxic and low cost, possess excellent emulsifying capacity [13–15]. However, proteins diffuse slowly to the interfaces and their surface activities are difficult to adjust because of their large molecular weights and complex structures [16,17]. Peptides possess smaller molecular weights and simpler structures compared with proteins. Peptides and their derivatives with desired amphipathy, component and structure can be synthesized chemically and biologically [18]. However, the synthesis and purification of peptides are costly and time-consuming, which limit their applications as surfactants in food industry. Hydrolysis of food proteins is a convenient and feasible way to obtain low cost peptides [9,19,20]. Amphipathic peptides can be obtained by hydrolysis of amphipathic food proteins [21]. However, there are two problems for the peptides produced by hydrolysis of food proteins: (1) each protein hydrolysate is a mixture containing several different protein fragments, and (2) the hydrophilic and hydrophobic regions of the peptides are not clearly separated. Due to their complexity, by now, the surface activities of such peptides have not been well studied [21]. Furthermore, for the reported peptides produced by hydrolysis of food protein, to the best of our knowledge, their capacity to reduce surface tension is not as good as the capacity of chemical surfactants that blocks their applications as surfactants.

Zein is a major byproduct of corn starch production, which is composed of α-zein (19 and 22 kDa), β-zein (14 kDa), γ-zein (16 and 27 kDa) and δ-zein (10 kDa) [22,23]. Zein possesses superior properties of film forming, antioxidation, biodegradability and biocompatibility [23–26]. Zein is insoluble in water and is soluble in 60 − 85% ethanol solution [26,27] because zein is rich in hydrophobic amino acid residues as well as deficient in basic and acidic amino acid residues [10,28–30]. More than 20% of the amino acid residues of zein are asparagine and glutamine, which can change into aspartic acid and glutamic acid residues via deamidation reaction [31]. The carboxyl groups of zein produced by deamidation reaction increase the solubility in water and emulsification ability of zein [28,29,32]. During the deamidation reaction, degradation reaction also happens because peptide bonds are also amido bonds, which produces deamidated zein peptides. Previously, we investigated alkaline hydrolysis of zein in a solution containing 0.5 M NaOH and 70% ethanol at 37 °C [10]. We found that longer hydrolysis reaction produced smaller peptides with more hydrophilicity due to more aspartic acid and glutamic acid residues produced. Furthermore, the hydrophilicity of the deamidated zein peptides increases with the solution pH. The peptides carry more negative charges at higher pH condition at which more carboxyl groups become deprotonated. Considering that the deamidated zein peptides have adjustable hydrophilicity/hydrophobicity and smaller molecular weight than protein, we speculated that deamidated zein peptides might have superior surface activity at certain pH condition. Herein, we produced three deamidated zein peptides via 36, 96 and 144 h of alkaline hydrolysis reaction and investigated their surface activities and cytotoxicity to prove that deamidated zein peptides are excellent surfactants.

2. Experimental

2.1. Materials

Zein from maize was purchased from Sigma (Shanghai, China). Medium chain triglyceride (MCT) for injection was from Avic (Tieling) Pharmaceutical Co., Ltd. (Tieling, Liaoning, China). DMEM cell culture medium and fetal bovine serum were from GIBCO BRL Life Technologies Inc. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was from Promega Co. (Beijing, China). Hela cell line was from American Type Culture Collection (ATCC, Manassas, Virginia, US). All other chemicals were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of deamidated zein peptides

Deamidated zein peptides were prepared as described previously [10]. Zein was dissolved in a solution containing 0.5 M NaOH and 70% (v/v) ethanol with zein concentration of 10 mg/mL. The solution was incubated at 37 °C under stirring for 36, 96 or 144 h. After the incubation, the ethanol in the solution was immediately removed by rotary evaporation under vacuum. The remaining solution was changed to pH 3.1 by addition of 5 M HCl and then the solution was kept at room temperature overnight to precipitate the deamidated zein peptide. The soluble components in pH 3.1 aqueous solutions were not used in this study. The precipitate was isolated and then was dissolved in an aqueous solution after addition of 2 M NaOH to reach the final pH of 9.0. The resultant peptide solution was freeze-dried to obtain deamidated zein peptide (Zein-36h, Zein-96h or Zein-144h) powder. The yields were about 64.5%, 52.5% and 50.6% after 36, 96 and 144 h of the hydrolysis reaction, respectively [10].

2.3. Surface tension (γ) characterization

Surface tensions were measured at 20 °C using Whilhemy hanging plate method on a surface tensiometer (BYZ-2, Shanghai Hengping Instrument, China). The peptide solutions with desired pH (pH 4.0, 6.0 and 8.0) and desired peptide concentrations (from 5.00×10^{-3} to 7.00 mg/mL) were prepared at least the day before the measurement. The Pt plate was cleaned through flaming; the glassware was rinsed sequentially with tap water, ultra-pure water and the peptide solution. Each measurement was performed for 30 min to ensure that the equilibrium value had reached. Each sample was measured repeatedly for three times. The apparent CMC (critical micelle concentration) was obtained by the intersection point of extrapolations above and below the break of the surface tension − concentration curve [33]. The surface tensions of sodium dodecyl sulfate (SDS) aqueous solutions with SDS concentrations of 1.00×10^{-1} to 6.00 mg/mL were measured for comparison.

2.4. Foaming characterization

The foaming properties of the peptide solutions were determined at 20 and 70 °C separately using Waring-Blender method as described in the literature [34]. After equilibrium at the predetermined temperature, the peptide solution of 100 mL with desired pH and concentration was stirred at 1200 rpm for 60 s, and then the maximum foam volume (V_{max}) and half-life of the foam ($t_{1/2}$) were recorded.

2.5. Emulsification characterization

The peptide was dissolved in deionized water with 10 mg/mL peptide concentration. NaN_3 with a final concentration of 0.02% was added to inhibit microbial growth. The peptide solution was adjusted to desired pH and then MCT with 10% volume fraction was added. For high-