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Collagen-surfactant mixtures at fluid/fluid interfaces

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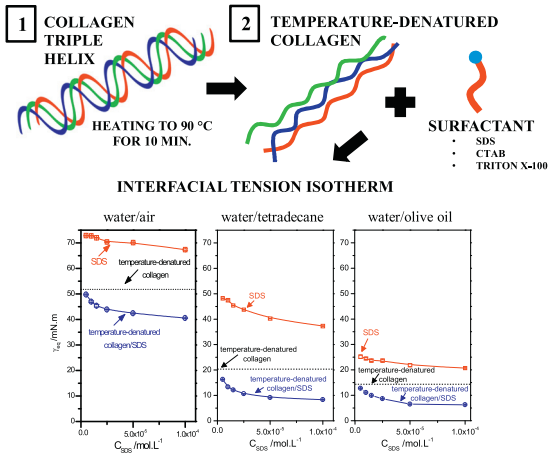
HIGHLIGHTS

- Type I collagen was temperature-denatured and mixed with surfactants at pH 4.5.
- SDS, CTAB, Triton X-100 were used as model surfactants.
- Water/air, water/tetradecane and water/olive oil interfaces were studied.
- Synergistic effects in surface tension reduction and foamability were observed.
- Emulsions showed extensive creaming, probably due to bridging between oil droplets.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of three synthetic surfactants on surface activity and surface dilatational rheology of temperature-denatured type I collagen at water/air interface is described. An anionic (sodium dodecyl sulfate, SDS), a cationic (cetyltrimethylammonium bromide, CTAB) and a nonionic (Triton X-100, TX-100) surfactants were employed at variable concentrations (5×10^{-6} mol L^{-1}–1×10^{-4} mol L^{-1}). With the protein concentration fixed at 5×10^{-6} mol L^{-1}, the protein/surfactant mixtures with molar ratios of 1:1, 1:2, 1:3, 1:5, 1:10 and 1:20 were obtained. An Axisymmetric Drop Shape Analysis (ADSA) method was used to determine the dynamic and equilibrium surface tension, as well as the surface dilatational moduli of the mixed adsorption layers formed at the water/air interface at pH 4.5. For the collagen-SDS mixtures, analogous studies were also performed at the water/tetradecane and water/olive oil interfaces. These results were complemented with the foam and emulsion formation ability tests, and the oil-in-water emulsions were characterized using Dynamic Light Scattering (DLS) and visual assessment.

The most beneficial effect on surface activity, mechanical properties of adsorbed layer and foaming ability was observed for the mixtures of temperature-denatured collagen with SDS. The rheological parameters of the adsorbed layers formed by the same mixtures at the water/olive oil and water/tetradecane interfaces worsened significantly (values of rheological parameters *E*' and *E*" are lower than values of *E*' and *E*" of the adsorbed layers formed at the water/air interface). The corresponding emulsions, despite very low interfacial tension values, phase-separated within hours. We hypothesize that the poor kinetic stability was caused by flocculation due to collagen bridging between the oil droplets, resulting in emulsion creaming.

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1. Introduction

Synthetic surfactants are present in a vast majority of cosmetic, food and pharmaceutical products, thus having a huge impact on human lives. The worldwide use of surfactants has grown significantly over the past few decades [1], stimulating the interest in sustainable surfactants from renewable resources. In this context, biosurfactants, surface active molecules produced by living organisms, are the subject of increasing scientific interest [2]. Biosurfactants can be obtained from both microorganisms (bacteria, fungi) and higher organisms (plants, animals) [3]. The main drawback of bacterial and fungal products is the still relatively low yield, not sufficient to reach a satisfactory economical level. The downstream processing necessary to recover and purify the microbial surfactants is time-consuming and generates high costs [4]. Even though purification is still an issue for animal-derived biosurfactants, the yield is no longer a problem, since the waste products from meat and leather industries can be used [5].

Biosurfactants derived from animal waste are mainly surface-active polypeptides and proteins. Their amphiphilicity stems from the presence of a polyamide backbone decorated with sidechains of different hydrophobic/hydrophilic nature, some of them also with acid–base properties [3,6]. Some proteins, e.g. casein [7–9], lysozyme [7,10,11] or whey protein isolate [12,13] are intrinsically surface active and are commonly used as foaming agents or emulsifiers. On the other hand, there are many proteins, e.g., collagen, keratin or elastin, with "hidden" amphiphilicity, which can be potentially triggered by modifications of the protein's higher-order structure or by interaction with other molecules.

Collagens are one of the most abundant proteins in mammals. In humans, they constitute 1/3 of the total protein content and account for 3/4 of the dry weight of skin [14]. The most characteristic feature of this group of proteins is their assembly into fibers that form the matrix of bones, skin, tendons, cornea, blood vessel walls and other connective tissues [15]. The collagen superfamily comprises 28 members numbered with Roman numerals [16]. The collagens differ in the amino acid composition, structure, length, biological role and abundance [17]. A characteristic feature of collagens is the amino acid sequence of polypeptide α chains involved in the formation of triple-helical structure. The sequence, called "collagenous domain" [18], consists of repeating Gly-X-Y triplets [19], where Gly – refers to glycine, and X and Y positions are often occupied by proline and 4-hydroxyproline, respectively. The amino acid sequence of collagens contains both hydrophobic (glicyne, proline, hydroxyproline or tyrosine) and hydrophilic (arginine, lysine, aspartic acid, and glutamic acid) amino acid groups. The latter are predominantly exposed on the outer side (solution facing) of the triple helix.

Type I collagen is by far the most abundant protein in all vertebrates [15,19]. In contrast to the majority of other collagens, which are homotrimeric, the type I collagen is a heterotrimer, consisting of two identical α1 chains and one α2: $[\alpha1(I)]_{2\alpha}2(I)$. Some aspects of surface activity of the modified type I collagen have already been investigated in the past [5,20–22]. The beneficial effect of temperature denaturation on surface activity of collagen type I at different pH has been described in our previous report [5]. We also recently studied the effect of low-molecular-weight surfactants on the native type I collagen at low pH (1.8) [23]. The study showed that under highly acidic conditions the modification of the native collagen structure by SDS at low surfactant/protein molar ratios has a positive effect on the mixture's surface activity, with only minor deterioration of the rheological properties (lowering of surface dilational rheology modulus) of the adsorbed layers. The native collagen/CTAB mixtures did not show that pronounced improvement in surface activity, while the surface dilational rheology modulus was significantly reduced. On the other hand the mixtures with non-ionic TX-100 did not show any synergistic effects in surface activity at low pH.

In the present paper, the effect of low-molecular-weight surfactants on the surface activity and surface dilatational rheology of temperature-denatured collagen at different interfaces under milder conditions (pH 4.5) is described. It will help to broaden the knowledge on behavior of temperature-denatured collagen and its mixtures with surfactants at different interfaces mimicking those typically encountered in real-life applications of protein-surfactant mixtures. Furthermore the results are complemented with foam and emulsion formation ability tests and provide basis for further studies on potential applicability of type I collagen as a biosurfactant. The present study provides new possibilities of obtaining protein-surfactants mixtures from easily available (often as a waste) biological material at much higher pH than previously described.

2. Experimental

2.1. Chemicals

Collagen from calf skin (type I) was obtained from Biochrom AG (Collagen G, 4 g L^{-1}, solution in 0.015 M hydrochloric acid) and was used as received. Other chemicals: HCl (cat. No. 84428), $NaH_2PO_4{\cdot}2H_2O$ (cat. No. 71505) and $Na_2HPO_4{\cdot}2H_2O$ (cat. No. 71645), sodium dodecyl sulfate – SDS (cat. No. 71725), cetyltrimethylammonium bromide – CTAB (cat. No. 52365) and Triton X-100 – TX-100 (cat. No. T9284), tetradecane (99%, cat. No. 172456), olive oil (highly refined, low acidity, cat. No. O1514) were purchased from Sigma Aldrich. Milli-Q water (Millipore) was used to prepare all solutions. Phosphate buffer (pH 7.5) consisted of 0.01 mol L^{-1} $NaH_2PO_4{\cdot}2H_2O$ and 0.01 mol L^{-1} $Na_2HPO_4{\cdot}2H_2O$ solutions.

Surface purity of water, phosphate buffer and HCl used in this work was verified by monitoring their dynamic surface tension for 1 h. Similar tests were run for all glassware, by measuring surface tension of the last water used for rinsing the glassware. All glassware was cleaned with Hellmanex II solution (Hellma, Worldwide) and acetone prior to rinsing with Milli-Q water.

Tetradecane and olive oil were purified by shaking with Florisil® (60–100 mesh, Sigma Aldrich, cat. No. 288691) followed by centrifugation at 3000 rpm during 60 min and filtering through Whatman's filter paper (No. 1). For olive oil, the procedure was repeated twice, while for tetradecane four cycles were necessary. The lack of changes of the corresponding (water/tetradecane and water/olive oil) interfacial tension during at least 3600 s against pure water and phosphate buffer/HCl solution was chosen as a criterion for the surface purity of the oil phase.

2.2. Preparation of temperature-denatured collagen and temperature-denatured collagen/surfactant solutions

A 5.0×10^{-6} M solution of collagen (pH 1.8) was prepared by diluting the stock collagen solution (4 g ml^{-1} in 0.015 mol L^{-1} HCl). The collagen solution was heated to 90 °C for 10 min. After cooling down to room temperature, the appropriate volumes of the phosphate buffer solution and water were added to reach pH 4.5. In the last step, the temperature-denatured collagen solution was mixed separately with SDS, CTAB and TX-100 to give mixtures with the following collagen/surfactant molar ratios: 1:1, 1:2, 1:3, 1:5, 1:10 and 1:20. Collagen concentration was fixed in all mixtures at 5×10^{-6} mol L^{-1} and the surfactant concentrations were: 5×10^{-6}, 1×10^{-5}, 1.5×10^{-5}, 2.5×10^{-5}, 5×10^{-5}, 1×10^{-4} mol L^{-1}. The solutions were incubated for 24 h at room temperature (25 °C) prior to all measurements.