This is the author’s version of a work that was accepted for publication in **Colloids and Surfaces B: Biointerfaces 148 (2016) 238–248**.

Publisher’s version: http://dx.doi.org/10.1016/j.colsurfb.2016.08.058

**Effect of surfactants on surface activity and rheological properties of type I collagen at air/water interface**

Aleksandra Kezwońa, Ilona Górala, Tomasz Frączykb, Kamil Wojciechowskia,\*

aFaculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664, Warsaw, Poland

bInstitute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland

\*Corresponding author. E-mail address: kamil.wojciechowski@ch.pw.edu.pl (K. Wojciechowski).

Abstract

We describe the effect of three synthetic surfactants (anionic – sodium dodecyl sulfate (SDS), cationic – cetyltrimethylammonium bromide (CTAB) and nonionic – Triton X-100 (TX-100)) on surface properties of the type I calf skin collagen at the air/water interface in acidic solutions (pH 1.8). The protein concentration was fixed at 5 × 10-6 mol L-1 and the surfactant concentration was varied in the range 5 × 10-6 mol L-1 – 1 × 10-4 mol L-1, producing the protein/surfactant mixtures with molar ratios of 1:1, 1:2, 1:5, 1:10 and 1:20. An Axisymmetric Drop Shape Analysis (ADSA) method was used to determine the dynamic surface tension and surface dilatational moduli of the mixed adsorption layers. Two spectroscopic techniques: UV–vis spectroscopy and fluorimetry allowed us to determine the effect of the surfactants on the protein structure. The thermodynamic characteristic of the mixtures was studied using isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). Modification of the collagen structure by SDS at low surfactant/protein ratios has a positive effect on the mixture’s surface activity with only minor deterioration of the rheological properties of the adsorbed layers. The collagen/CTAB mixtures do not show that pronounced improvement in surface activity, while rheological properties are significantly deteriorated. The mixtures with non-ionic TX-100 do not show any synergistic effects in surface activity.

Keywords: Type I calf skin collagen, Surface tension, Surface dilatational rheology, Calorimetry, Spectrophotometry, Fluorimetry, SDS, CTAB, TRITON X-100

1. Introduction

Biosurfactants can be obtained from many renewable substrates, mainly, but not exclusively, from plants and animals [1,2]. This provides a paramount advantage over the synthetic surface active agents, produced largely from petroleum feedstock. Biosurfactants are usually characterized by low toxicity, high biodegradability, low critical micellization concentration (CMC) and high surface activity [3,4]. Moreover, they can be modified using genetic, biochemical or biological manipulation to develop tailored products for more specific requirements [5]. Because of the crucial importance of surface active agents in cosmetics, pharmaceutical and food industries, surface properties of biosurfactants are intensively investigated in both industry and academia. These studies are mainly driven by the potential of replacing the synthetic surfactants with their biological alternatives [1,6–8].

One of the most interesting groups of biocompatible surface active molecules of natural origin are proteins. Some of them, e.g., lysozyme [9–11], soy proteins, ovalbumin [12], casein [11,13,14] or whey protein isolate [12,15], are able to stabilize foams and emulsions. Despite a huge amount of data on surface properties of globular proteins, till now only a few papers focused on surface activity of fibrous proteins, e.g., collagen, keratin or elastin. In contrast to most of the globular proteins, the fibrous ones can be obtained from waste of meat and fabrics industries [16]. Collagen is one of the major representatives of fibrous proteins. Currently, the collagen superfamily comprises 28 members(including 5 classical fibrillar collagens: I, II, III, V, XI [17]) identified in vertebrates, differing in amino acid composition, length, structure, abundance and biological role [17–19]. A collagen molecule consists of three polypeptide chains, called chains, rolled into a right-handed triple helical domain. Each chain consists of about1000 amino acid residues. Besides the central helical region, a fibrillar collagen molecule may also contain non-helical domains at its N- and C-termini (called ‘non collagenous’ domains [19]). The characteristic feature of all collagens is a repeating Gly-Xaa-Yaa triplet unit, where Xaa and Yaa can be any amino acid, although proline (Pro) or hydroxyproline (Pro-OH) residues are the most often encountered [16,20–22]. The outer (solution facing) side of the triple helix is enriched with hydrophilic amino acids (arginine, lysine, aspartic acid, and glutamic acid). The triple helix is stabilized by a specific amino acid sequence (glycine as every third residue, together with the high content of proline and hydroxyproline) which is responsible for the formation of inter-chain hydrogen bonds and electrostatic interactions involving lysine and aspartate[23,24]. One of the most common representatives of the collagen superfamily is a type I collagen [25–27], heterotrimer comprising two identical 1-chains and one 2-chain with the triple helix accounting for 96% of the whole molecule [18].

Members of the collagen superfamily find numerous applications in the fields of regenerative medicine, tissue repair and engineering, as well as in cosmetic surgery [28]. Nevertheless, the most useful types of collagen in biomedical application are the fibrillar collagens I and II, and the nonfibrillar collagen type IV [22]. A number of reports on potential applications of collagen in medicine focused on functionalization of its surface [29] or immobilization at different surfaces [30,31]. To the best of our knowledge, studies of surface activity of collagen proteins have been limited to the fibrillar type I collagen. For this purpose, the protein has been modified using enzymatic hydrolysis with either collagen-specific enzyme – collagenase [16] or non-specific one – alcalase [32]. Other modifications include: pH variation (in the range 1.8–9.0) [16], temperature treatment (in the range 21–90.C) [20] and chemical acetylation using lauryl chloride or succinic anhydride [33,34].

Interaction between surfactants and proteins may result in either synergistic or antagonistic effects [35–39]. The ionic character of the surfactant seems to be a prerequisite for a synergistic interaction between globular proteins and synthetic surfactants.

Non-ionic surfactants tend to hydrophilize the surface of globular proteins due to a hydrophobic interaction between the alkyl chains of the surfactant and the nonpolar fragments of the protein molecule, rendering the complex less surface active than the original protein. In the case of ionic surfactants, at low surfactant/globular protein ratios, the interaction is dominated by electrostatic forces, typically producing complexes with surface activity higher than the starting protein. On the other hand, when all ionic sites capable of interaction with the surfactant are saturated, hydrophobic interactions start to set in, reducing the surface activity analogously to the case of non-ionic surfactants[11,14,35,36,38–40].

The general mechanism described above has been experimentally confirmed for many globular protein-surfactants complexes, but so far the evidence for other types of proteins (e.g. fibrous ones) is scarce. In this paper, the effect of three model synthetic surfactants (sodium dodecyl sulfate, cetyltrimethylammonium bromide and Triton X-100) on surface activity and surface dilatational rheology of the calf skin type I collagen is described. The tensiometric measurements are completed with spectroscopic and thermodynamic analysis in order to verify the validity of the proposed model for the mixtures of type I collagen with surfactants.

2. Experimental

2.1. Chemicals

Collagen from bovine calf skin (type I) was obtained from Biochrom AG (Collagen G, 4 g L-1, solution in 0.015 M HCl) and was used as received. Other chemicals: hydrochloric acid (84428), sodium dodecyl sulfate – SDS (71725), cetyltrimethylammoniumbromide – CTAB (52365) and Triton TX-100 (T9284) were purchased from Sigma Aldrich. Milli-Q water (Millipore) was used toprepare all solutions. Surface purity of water and HCl used in this work was verified by monitoring their dynamic surface tension for1 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.

2.2. Preparation of collagen and collagen/surfactant solutions

The procedure for preparation of collagen/surfactant solution was adapted from Ref. [41]. A 5.0 × 10-6M solution of collagen (pH1.8) was prepared by diluting the stock collagen solution (4 g mL-1in 0.015 mol L-1HCl). The collagen solution was mixed separately with SDS, CTAB and TX-100 to give mixtures of the following collagen/surfactant molar ratios: 1:1, 1:2, 1:5, 1:10 and 1:20. Collagen concentration was fixed in all mixtures at 5 × 10-6mol L-1andthe surfactant concentrations were: 5 × 10-6, 1 × 10-5, 2.5 × 10-5,5 × 10-5, 1 × 10-4mol L-1. The solutions were incubated for 24 h at room temperature (25.C).

2.3. Tensiometric measurements using axisymmetric drop shape analysis (ADSA)

The surface tension and surface dilatational rheology measurements were performed using a drop profile analysis tensiometerPAT-1 (Sinterface Technologies, Germany) as described in Ref. [42].

Each measurement was performed at constant temperature (21.C, controlled with a thermostatic bath). A drop of the tested solution was formed at the tip of a steel capillary immersed in a glass cuvette(20 mL) filled with air.

All experiments were performed during 5000 s and repeated at least three times (typically six times). In the first part of the measurement (0–3600 s) the drop volume was kept constant (18 μL), providing information about a dynamic surface tension measurement (i.e., surface tension vs. time). The equilibrium surface tension(…eq) was calculated by extrapolating the dynamic surface tension to the infinite time following the approach by Joos and Hansen[42], as an intercept with the ordinate in vs. t-1/2coordinateseq= -RT…2C0…4Dt, where eq is the equilibrium surface tension, is the dynamic surface tension, T is the temperature, D is the diffusion coefficient, c0 is the bulk concentration, R is the gas constant, t is the time.

In the second part of the measurement, in time range3600 s–5000 s the sinusoidal oscillations of the volume of the drop were applied, with the relative amplitude of 4.4% and the following frequencies of oscillations: 0.005, 0.01, 0.02, 0.05, 0.1 Hz. The analysis of the surface tension response to the drop area oscillations provided the imaginary (loss, E”) and real (storage, E’) partsof the complex surface dilatational viscoelastic modulus, E [43], E =ddlnA= E…+ iE……, where … is the interfacial tension, A is the area subjected to deformation, and i (-1)1/2.

2.4. UV–vis spectroscopy

The UV–vis spectra were recorded at 21.C on a Cary 60 UV–vis(Agilent Technologies, USA), over the spectral range of 190–400 nm with 1 nm s-1speed scan. A quartz cuvette with the path length of 1 cm was used for all the spectroscopic measurements. The absorption spectra of the studied solutions were recorded with a0.015 mol L-1HCl solution as a reference. The absorbance vs. surfactant concentration curves were constructed for the wavelength of a maximum absorbance (… = 267 nm) for collagen, surfactants and collagen/surfactant mixtures. The average values of at least three measurements are reported.

2.5. Fluorimetry

The fluorescence spectra of collagen, surfactants and their mixtures were recorded on a Cary Varian fluorescence spectrophotometer (Agilent Technologies, USA) with a 100 μL quartz cuvette of 1 cm path length at 21.C. The excitation and emission slits width were fixed at 5 nm. The excitation wavelength was set at 275 nm to selectively excite tyrosine (Tyr). The emission spectra were monitored in the wavelength range 280–500 nm using 2 nm s-1speedscans. Each measurement was repeated at least three times. The fluorescence intensity vs. surfactant concentration was plotted for the wavelength of a maximum fluorescence emission intensity of tyrosine (lambda = 303 nm). The average values of at least three measurements are reported.

2.6. Differential scanning calorimetry (DSC)

DSC thermograms of collagen and collagen/surfactant solutions with molar ratio 1:20 incubated for 24 h at room temperature (25.C) were recorded on a Nano-DSC differential scanning calorimeter (TA Instruments, USA) using a sample volume of 300 μL. Each solution was thoroughly degassed prior to loading.

The samples were scanned at 1.C min-1in a range 5–60.C and using a 0.015 mol L-1HCl solution as a reference. Each experiment was repeated at least three times. The total denaturation enthalpy(…Hd) and entropy (…Sd) were estimated by measuring the area under the DSC thermograms, while denaturation temperature Td was defined as a local maximum temperature at the DSC thermograms. The data were analyzed by NanoAnalyze Software.

2.7. Isothermal titration calorimetry (ITC)

ITC experiments were carried out on a Nano-ITC isothermal titration calorimeter (TA Instruments, USA) at 21.C and under constant stirring speed of 250 rpm. All of the used solutions were thoroughly degassed prior to loading. A concentrated solution of surfactants was placed in a 250 μL syringe and titrated into 950 μL of collagen or hydrochloric acid solution. Titrations were made by stepwise injection of 16 μL of the surfactant solution to the collagen/HCl solutions. The injections were added from an automatic microsyringe at an interval of 1000 s for SDS, 1200 s for CTAB and 800 s for TX-100, respectively. Each experiment consisted of 16 injections in the surfactant/collagen molar ratio range 0.7–12.8 and was repeated three-four times. The heat of dilution derived from titration of the surfactant to hydrochloric acid (without the protein) was subtracted from that obtained from the titration into the protein, in order to obtain the net heat of protein/surfactant interaction. The enthalpy change (…H) and association constant (Ka) were calculated by iterative curve fitting of the binding isotherms. The Gibbs free energy change (G) was derived from the equation …G = -RT × lnKa. The entropy change (…S) was calculated from the equation …G = …H – T..S. Data analysis was carried out using NanoAnalyze software.

3. Results

3.1. Surface tension of collagen, surfactants and collagen/surfactant mixtures

Native collagen can only be solubilized in acidic solutions. Therefore, first the stability of SDS, CTAB and TX-100 in acidic solutions (pH 1.8) was tested [44]. For this purpose the surface tension of their concentrated solutions (10-4mol L-1) after 24 h incubation at 25.C was measured repeatedly. The results for all the surfactants obtained at pH 1.8 did not differ significantly from those reported in the literature in neutral solutions [9,45–49]. Thus, any changes observed in the mixed collagen/surfactant solutions will result from the interaction between the two components and not from hydrolysis of the surfactant at low pH.

In the second step, the surfactant/collagen mixtures (pH 1.8) were prepared as described in the experimental section and incubated for 24 h prior to the surface tension measurements. The comparison of dynamic surface tension results for bare surfactant(left panel) and its mixtures with type I collagen (right panel) during 3600 s is shown in Fig. 1. The curve for bare collagen solution was added to each panel to facilitate comparison.

In agreement with the previously reported data [20] the collagen solutions at 5 × 10-6mol L-1under the employed conditions lower the surface tension more slowly than the surfactants solutions. This is a consequence of a significantly higher molecular weight of the protein (300 kDa) as compared to the employed surfactants. Nevertheless, within one hour collagen is capable of reducing surface tension more than the most concentrated SDS solution used in this study (1 × 10-4mol L-1). On the other hand, CTAB and especially TX-100 in the same range of concentrations (5 × 10-6–1 × 10-4mol L-1) show higher surface activity and reduce surface tension more than the bare collagen solution.

Addition of collagen affects all the dynamic surface tension curves, but the changes are most pronounced for SDS. In the mixed collagen/SDS system the curves are shifted downward by between 23 mN·m-1(for the lowest SDS concentration, 5 × 10-6mol L-1) and 20 mN m-1(for the highest SDS concentration, 1 × 10-4mol L-1). For the collagen/CTAB mixtures, the difference is much smaller and varies between 11 mN m-1(for the lowest CTAB concentration, 5 × 10-6mol L-1) and 1 mN m-1(for the highest CTAB concentration, 1 × 10-4mol L-1). In the case of non-ionic TX-100, the presence of collagen has no significant effect on dynamic surface tension curves. It is worth mentioning that the analogous dynamic surface tension measurements performed on fresh solutions (without 24 h incubation at 25.C) were highly irreproducible, especially at the beginning of each measurement series.

The representative curves recorded 5 h after mixing the collagen with the surfactants presented in (Fig. S1 Supplementary materials) show that the highest differences between the fresh and incubated mixtures were observed for the lowest surfactant concentrations(for molar ratios 1:1 and 1:2). This suggests that the rate of collagen/SDS complex formation at the interface is limited by the availability of the surfactant.

Fig. 2 collects the surface tension isotherms obtained by extrapolating the dynamic surface tension data from Fig. 1 to infinite time for each studied system: bare collagen, SDS, CTAB, TX-100 and its mixtures after 24 h incubation at 25.C. The analogous isotherms for the mixtures without incubation (Fig. S2) differ slightly from those of Fig. 2, probably due to the incomplete surfactant/collagen complex formation.

In agreement with the dynamic curves, the effect of collagen on the surface tension isotherms is strongly dependent on the nature of the surfactant and decreases in order: SDS > CTAB > TX-100. The effect of collagen is the most pronounced at low surfactant concentrations, and the isotherms for the mixtures tend to plateau values more quickly than the ones for bare surfactants. Consequently, in the studied range of concentrations, the lower is the collagen/surfactant ratio the lower is the observed synergistic effect of the protein. For all SDS/collagen mixtures and in the presence of low amounts of CTAB, the equilibrium surface tension is always lower than any of the individual components. For example, at a concentration of 5 × 10-6mol L-1of SDS the surface tension is the same as that of pure water, and for the same concentration of collagen = 57 mN m-1, while their mixture can lower surface tension to reach = 47 mN m-1. The synergistic effect due to a complex formation accounts thus for about 10 mN m-1additional reduction of surface tension. At higher SDS concentrations (collagen/SDS ratio of 1:5 to 1:20) the gain in surface tension reduction is as high as 15 mN m-1. For CTAB and TX-100 the effect does not exceed 2 mN m-1 at the lowest surfactant concentration, but increases to about 6 mN m-1 for a collagen/CTAB ratio of 1:5.

…

Fig. 1. Dynamic surface tension for bare surfactants (left panel) and collagen/surfactants mixtures (right panel) at pH 1.8 after 24 h incubation at 25.C. Surfactants concentrations in the mixtures were as follows: 5 × 10-6mol L-1…

…

Fig. 2. Interfacial tension isotherms for the surfactants (

Surface dilatational rheological properties of collagen and collagen/surfactant mixtures.

The rheological parameters of the Gibbs layers spontaneously formed on the air/water surface of the collagen and collagen/surfactant solutions as a function of frequency of oscillation are shown in Fig. 3A. The data is expressed as the storage (E’) and loss (E”) moduli of the visco-elastic modulus, both describing the mechanical properties of the adsorbed layers. The results for bare surfactant solutions are not shown here, since in the studied concentration and frequency range their visco-elastic moduli are negligible [50–52].

…

Fig. 3. (A) Surface dilatational rheological parameters: elastic modulus (E’) and loss modulus (E”) of surface layers of bare collagen and collagen/surfactant mixtures after 24 h incubation at 25.C as a function of oscillation frequency (open symbols). Surfactants concentrations in the mixtures were as follows: 5 × 10-6mol L-1…

For the adsorbed layers of bare collagen, E’ increases with increasing frequency of oscillation (f) from 43 mN m-1at 0.005 Hz to 58 mN m-1at 0.1 Hz, while E” decreases from 11 to 7 mN m-1inthe same frequency range, in agreement with our previous report[20]. With the addition of surfactants, both E’(f) and E”(f) curves shift downwards, suggesting a deterioration of mechanical properties of the mixed layers. As far as the storage modulus is concerned, the extent of deterioration follows the opposite trend as observed in reduction of dynamic surface tension: the most pronounced reduction of E’ is observed for TX-100, followed by CTAB and SDS. The loss moduli, E”, in all cases show much lower values and their variation with surfactant concentration is rather minor. On the other hand, the frequency dependence of E” changes with surfactant concentration, passing from decreasing for the bare collagen and low surfactant/collagen ratio mixtures, to increasing, for the high-ratio mixtures (Fig. 3A). The frequency dependence of E’ for all surfactant/collagen mixtures is similar to that of bare collagen, but with increasing the surfactant/collagen ratio the effect of frequency becomes less important. This effect is most pronounced for CTAB and TX-100: the difference between E’ at 0.005 Hz and0.1 Hz drops from about 12 mN m-1at 1:1 collagen/surfactant ratio, to about 4 mN m-1at 1:20. On the other hand, for SDS the difference between E’ at 0.005 Hz and 0.1 Hz is almost the same for collagen/SDS ratio of 1:1 (11.6 mN m-1) and 1:20 (11.2 mN m-1).

With increasing frequency of oscillation, both E’ and E” tend to plateau values, approaching the high-frequency limit, E0[53]. Because of the technical limitations of the oscillating drop setup, the highest experimentally achievable frequency in our experiments is 0.1 Hz, and these values were used to plot the high-frequency concentration dependence of E’ and E” (Fig. 3B). For both collagen and collagen/surfactant mixtures E’ … E”, suggesting that the layers are predominantly elastic. The data in Fig. 3B clearly confirms that the storage modulus of the mixed adsorbed layers worsens in the following sequence: collagen/SDS < collagen/CTAB < collagen/TX-100.

At 1:20 molar ratio, E’ for SDS is still as high as 25 mN m-1, while for the other two surfactants it drops below 10 mN m-1. Similarly, E” for CTAB and TX-100 drops below that of bare collagen, in contrast to SDS, confirming that in the former two mixtures collagen is successfully replaced with uncomplexed surfactant molecules.

The analogous data for the freshly prepared mixtures (without 24 h incubation) are collected in Fig. S3. The less pronounced changes of E’ and E” confirm that the freshly prepared mixtures do not reach an equilibrium immediately.

3.2. UV–vis spectroscopy

Two of the amino acids abundant in collagen: tyrosine (Tyr) and phenylalanine (Phe) possess chromophores absorbing UV light in the range 250–300 nm [54,55]. Thus, in order to shed more light on the mechanism of surfactant-collagen interactions, an UV–vis spectroscopy has been employed. The UV–vis spectra of bare collagen and collagen/surfactant mixtures are collected in Fig. 4, and the absorbance vs. surfactant concentration curves for … = 267 nm are shown as insets.

On the first inspection, the most pronounced changes could be noticed for TX-100 solutions. They originate, however, from the UV light absorption by the aromatic part of the surfactant(max= 275 nm) [56], and not from the collagen/TX-100 interactions. This is clearly seen from the inset showing the absorbance vs. TX-100 concentration in the absence and presence of collagen – the slope of both curves is the same. Similarly, the almost parallel curves for CTAB suggest that the ability to absorb the UV light by the collagen’s Tyr and Phe is not significantly affected by the presence of the surfactant, which in this case is not UV-absorbing (null slope). Only for SDS a clear increase of absorbance in the UV region point to a specific interaction involving the neighborhood of the aromatic amino acids of the collagen. The inflection point is situated around the SDS/collagen molar ratio of 1–2, suggesting that between one and two molecules of SDS are bound per one collagen molecule and that the binding site may be located near Tyr or Phe.

3.3. Fluorimetry

The presence of fluorescent aromatic amino acid residues (tyrosine, phenylalanine or tryptophan) in a protein provides a valuable probe for studying its interactions with other species present in the solution [57]. The type I collagen used in this study is rich in Tyr and Phe, but due to a much higher emission yield, Tyr is mostly responsible for the fluorescence spectrum of the collagen solutions [58,59]. Indeed, excitation of the type I calf skin collagen at …exc= 275 nm affords emission in the UV range with a maximum around …em= 303 nm, with a small shoulder at 340 nm, attributable to tyrosine. Addition of the surfactants alters the emission spectra in a way similar to that observed in the spectrophotometric experiments (Fig. 5). The apparent strong response to the presence of TX-100 is linear and is due to the fluorescence properties of the surfactant itself (see Fig. S4 for the corresponding fluorescence spectra of bare TX-100). Also for CTAB, no changes exceeding the scattering due to experimental errors in fluorescence intensity could be noticed. This confirms the spectrophotometric observations about the lack of changes in the vicinity of Tyr in presence of the cationic surfactant. With increasing the SDS/collagen ratio in the mixture, first a decrease and then an increase of the emission intensity is observed, accompanied by a successive disappearance of the shoulder at 340 nm. The shape of the fluorescence intensity vs. SDS concentration suggests formation of a surfactant-protein complex with molar ratio around 1–2, in agreement with the spectrophotometric data. This confirms that at least part of the SDS molecules binding to collagen are probably located in the vicinity of the fluorescently active tyrosine group.

In order to better understand the nature of SDS-collagen interaction, a competition experiment was performed where analogous titration of collagen by SDS was performed in the presence of excessNa2SO4. For this purpose the concentration of the inorganic sulfate salt was fixed at 1 × 10-4mol L-1, which corresponds to the initial collagen/Na2SO4ratio of 1:20. The presence of inorganic sulfate ions shifted the binding curve to the right (Fig. S5) suggesting that they may indeed be bound at the same binding site as the organic sulfate of SDS.

3.4. Differential scanning calorimetry (DSC)

Proteins can be unfolded by many factors, one of the simplest to achieve being an increase of temperature. Also some surfactants, especially SDS, possess the ability to denaturate proteins. In the case of collagen, denaturation involves unfolding the triple helix with formation of random coils. The process can be conveniently followed by using differential scanning calorimetry (DSC) [60–62].

In this study, DSC measurements were carried out to compare the effect of surfactants on thermal stability of collagen after 24 h incubation at 25.C. The denaturation temperature (Td), enthalpy (…Hd) and entropy (…Sd) are shown in Table 1 for bare collagen and its mixtures with SDS, CTAB and TX-100, all at 1:20 collagen/surfactant ratio. The thermodynamic parameters of the helix-to-coil transition reported in Table 1 for collagen are similar to those reported in the literature [61,63]. Interestingly, addition of even large excess of the synthetic surfactants does not alter significantly the type I collagen’s helix-to-coil transition. This confirms that even if the surfactant-protein complexes are formed, the process does not stabilize preferentially neither the helical, nor the unfolded forms of the type I collagen. Thus, the synthetic surfactants used in this study do not affect the triple helix of the protein.

…

Fig. 4. UV–vis spectra for bare collagen (-) and collagen/surfactant mixtures after 24 h incubation at 25.C. Surfactants concentrations in the mixtures were as follows: 5 × 10-6mol L-1…

…

Fig. 5. Fluorescence emission spectra of bare collagen (-) and collagen/surfactant mixtures after 24 h incubation under 25.C. Surfactants concentrations in the mixtures were as follows: 5 × 10-6mol L-1…

…

Fig. 6. Thermodynamic parameters of collagen/SDS and collagen/CTAB complexes determined from ITC at 21.C: Gibbs free energy (…G), enthalpy change (…H), and entropy change (expressed as –T…S).

3.5. Isothermal titration calorimetry (ITC)

Further thermodynamic characterization of collagen/surfactant interactions was performed using isothermal titration calorimetry (ITC). In the ITC experiments, the heat generated or absorbed upon titrating the collagen solutions with the concentrated surfactant solutions was measured in order to obtain information about the stoichiometry (n), enthalpy change (…H) and Gibbs free energy(…G). …H and …G were then used to calculate the entropic contribution (T…S) [64].

Addition of TX-100 does not produce any measurable heat effects, suggesting that it is not strongly bound to collagen under the employed conditions. On the other hand, in the case of CTAB and SDS the results are surprisingly similar, but repeatable (Fig. 6). In both cases the Gibbs free energy is negative suggesting that the SDS-collagen and CTAB-collagen complexes are formed spontaneously and have comparable thermodynamic stability. Interestingly, the process is driven mostly by an entropic contribution, the enthalpy change being small and positive. This suggests a release of the bound water molecules upon interaction of collagen to the surfactants and/or rearrangement of the whole collagen molecule. The latter seems, however, less probable given the little effect of the surfactants observed in DSC results above.

Thus, the most probable origin of the high entropic contribution seem to be the hydrophobic interactions between the collagen and the alkyl chains of SDS and CTAB. The stoichiometry of both collagen/SDS and collagen/CTAB complexes determined from fitting of the ITC data, were ncoll-SDS= 1.6 ± 0.1 and ncoll-CTAB= 1.5 ± 0.1.4.

Discussion

Interactions between the surfactants and the globular (e.g. …-lactoglobulin, lysozyme, albumin) or random coil (e.g. …-casein) proteins has been extensively studied in the past because of the synergistic effects often observed in such mixtures. On the other hand, fibrous proteins attracted much less attention, and their interaction with surfactants has been mostly studied from the point of view of bulk properties [41,65–67]. The general conclusion that can be derived from studies on the globular protein-surfactant mixtures is that their interaction is largely determined by the ionic character of both types of molecules. This turns out to be true also for the collagen/surfactants mixtures analyzed in this paper, and is evident from both surface (surface tension, surface dilatational rheology) and bulk (DSC, ITC, UV–vis absorbance and fluorescence) techniques. Our study additionally points to a hydrophobic interaction as a possible important contribution to binding of both SDS and CTAB. The SDS and CTAB molecules share some similarities. Both contain acyclic aliphatic chain and charged group, although SDS has anionic, and CTAB – cationic one. The hydrophobic parts of these molecules are much bigger than the hydrophilic ones. In contrast, TX-100 has no charged group and the hydrophobic part is smaller than hydrophilic one.

TX-100, being a nonionic surfactant, does not seem to interact measurably with the type I collagen, even though both the surface tension and optical properties of the mixtures change significantly in its presence. As far as the solution/air interface is concerned, TX-100 simply replaces the protein because of its intrinsic surface activity [49]. The phenyl and ether groups of TX-100 are not specifically bound to any fragment of the collagen molecule, which was confirmed by ITC. Thus, the solution/air interface is quickly covered with much smaller and fast-diffusing TX-100 (Fig. 1), and the subsequent adsorption of large and slow-diffusing collagen is hindered. The degradation of mechanical properties of the mixed collagen/TX-100adsorbed layers confirms that already in the equimolar mixtures only a minor part of the interface is covered by the protein.

The complex formation between CTAB and the type I collagen at pH 1.8 is somehow surprising given the protein’s high isoelectric point (pI = 8.26 [68]) and a cationic character of the surfactant. The complex formation in the bulk is confirmed with ITC and some indication of its presence at the interface can be deduced from the surface tension and rheology data. The lack of changes in the spectroscopic experiments suggests that the interaction does not affect the chromophoric and fluorophoric groups of collagen (Tyr and Phe). The dominant entropic contribution to the Gibbs energy of CTAB-collagen binding suggests a hydrophobic character of interaction. The stoichiometry of the CTAB-collagen complex obtained from ITC suggests that on average between one and two CTAB molecules are bound to each collagen’s triple helix, but the lack of changes in optical absorption and emission spectra does not allow us to speculate on the possible location of these molecules along the triple helix.

The hydrophobic attraction between CTAB and collagen is also operational at the water/air interface, where a clear reduction of surface tension is observed in the low CTAB-to-collagen ratio mixtures (Fig. 2). On the other hand, with increasing CTAB concentration the mechanical properties of the mixed adsorbed layers clearly deteriorate (Fig. 3). Similarly to other low-molecular weight synthetic surfactants, CTAB itself forms adsorbed layers with poor mechanical properties [16,20]. Thus, worsening of the mechanical properties of the mixed layers suggests that the protein and surfactant molecules are co-adsorbing at the interface, but the collagen’s ability to form elastic networks is reduced by binding with CTAB. In other words, CTAB probably reduces the collagen’s ability to self-associate at the interface.

The most pronounced changes in bulk and interfacial properties were observed for SDS. They are consistent with those reported in the literature for other fibrous protein such as keratin [69] and fibroin [70]. Because of the anionic characters of SDS, both electrostatic and hydrophobic interactions are expected to play an important role here. Even though SDS is capable of unfolding many globular proteins, no changes were observed in the thermodynamic parameters of the collagen thermal denaturation. Thus, the changes induced by binding of SDS are restricted to local modifications of the triple helix, which itself remains intact. The thermodynamic parameters for SDS-collagen complexation are similar to those for CTAB-collagen, which may suggest a similar mechanism of interaction. However, while electrostatic interactions are clearly more important for SDS, the hydrophobic ones would probably be more pronounced for CTAB, which has a longer alkyl chain (16 carbon atoms, instead of 12 for SDS). The similarities between SDS and CTAB are thus probably incidental, especially that in the case of SDS the changes in optical properties of collagen solutions are different than for CTAB. Overall, the electrostatic and hydrophobic interactions seem equally probable in the case of binding of SDS to collagen.

The spectroscopic results suggest that SDS may be bound in the vicinity of tyrosine [57], although not necessarily by this particular amino acid. Mertz et al. reported that at physiological pH (6.8) the phosphate and sulfate ions are specifically bound to collagen fibrils [71]. The authors reported the existence of 1–2 binding sites for sulfate per collagen molecule inside the fibrils. The binding site was postulated to locate near the positively charged amino acid residues (such as arginine and lysine) within regions of high net positive charge. In fact, the special affinity of sulfate (together with phosphate) ions to arginine is known from the literature [72–74].

Although the interaction is of electrostatic nature, its strength is modulated by the Hofmeister effect [75,76]. The competition experiment using an inorganic sulfate salt seems to confirm that the sulfate group plays an important role in recognition of the SDS molecule. By combining the expected high affinity of SDS to arginine and the observed effect of SDS binding on tyrosine, the possible binding site for at least the first SDS molecule should be located in the regions where the two amino acids are spatially close. The type I collagen contains about 50 arginine residues per one -chain (Uniprot ID P02453), and 12 ± 1 tyrosine residues per collagen molecule [57] located mainly in the non-helical part of the collagen chains (Uniprot ID P02453). By analyzing the protein’s amino acid sequence, we postulate that the most probable binding site for SDS is located at the end of the non-helical part of 1-collagen chain 1213Arg-Tyr-Tyr1215(Uniprot ID P02453), remaining after cleavage of the C-terminal propeptide 1216–1463. The fact that even in the presence of excess inorganic sulfate, the SDS molecules can still be bound to collagen suggests that also the SDS’ n-dodecyl chain participates in the surfactant binding.

5. Conclusions

Despite being a fibrous protein, the type I collagen displays some surface activity and its adsorbed layers at water/air interface are highly visco-elastic in dilatation, with E’ … E”. In the presence of the low-molecular weight synthetic surfactants, both surface tension and surface dilatational rheology of collagen are modified to the extent depending on the nature of the surfactant and the protein-to-surfactant ratio. The most pronounced enhancement of surface activity was observed for SDS, where surface tension could be reduced down to = 42 mN m-1(for comparison, in bare collagen solution at the same concentration, = 57 mN m-1, and for SDS does not drop below 64 mN m-1). With increasing SDS concentration the mechanical properties of the adsorbed layers deteriorate, but below the collagen/SDS ratio of 1:5 are still satisfactory (E’ > 30 mN m-1). Thus, from the practical point of view, SDS-collagen mixtures with slight excess of the surfactant offer significant reduction of surface tension while keeping the adsorbed layers mechanically resistant, which makes them potentially attractive components of foams and possibly also emulsions.

The mechanistic studies described in this contribution point to a specific binding of the SDS’s sulfate group to the collagen’s arginine groups located in the vicinity of tyrosine, probably at the end of the non-helical part of 1-collagen chain1213Arg-Tyr-Tyr1215. Even though the interaction is electrostatic in nature, it is probably specific to sulfate ions (Hofmeister type). Additionally, the SDS’ n-dodecyl chain may be hydrophobically attracted to the collagen’s triple helix. It is possible that the proposed mechanism holds also for other types of collagens and fibrillar proteins (e.g. keratin, elastin), because of the common occurrence of arginine in their amino acid sequence. In the present paper we profited from the spatial proximity of arginine and tyrosine, where the latter served as a reporter moiety thanks to its optical properties. Thus, binding of the sulfate group to arginine in other collagens does not have to lead to such distinct changes in UV–vis and fluorescence spectra. For instance, in type III collagen, which is a homotrimer ([1(III)]3), the1-chains do not contain the specific arginine-tyrosine sequence (Uniprot ID P04258). On the other hand, another homotrimeric collagen, type II ([1(II)]3), contains the sequence, but located in N- and C- terminal propeptides (Uniprot ID P02459), which are removed during the later stages of biosynthesis [22]. To the best of our knowledge, only the type V collagen (a heterotrimer existing as either [1(V)]22(V)] or [1(V)2(V)3(V)] [22]), contains the Arg-Tyr sequence in each -chain (Uniprot ID G3MZI7, F1N2Y2, F1MJQ6). Consequently, one may expect changes of optical properties upon binding of SDS to type V collagen, although surface properties should be affected equally for other types as well.

Under the applied conditions, the type I collagen is not denatured in the presence of SDS or the other two surfactants used: CTAB and TX-100. While for the latter, no evidence of binding to collagen could be found (both in the bulk and at the surface), the former seems to be complexed by the protein. In this case, however, the spectroscopic evidence did not allow for any speculation on the possible location of the binding site for CTAB. Because of the net positive charge of both CTAB and collagen under the employed conditions, electrostatic interaction should be less favored than hydrophobic binding. Overall, the type I collagen in mixtures with the anionic, cationic and neutral synthetic surfactant behaves qualitatively in a similar way as the globular proteins. The extent of interaction and consequently – enhancement of the surface properties decreases in the order SDS > CTAB TX-100. With increasing the surfactant-to-protein ratio the complex is successively replaced by the uncomplexed surfactant molecules.

Acknowledgements

This work was financially supported by the Warsaw University of Technology. The calorimetry equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT), and cosponsored by European Regional Development Fund and Innovative Economy, The National Cohesion Strategy of Poland.

Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.colsurfb.2016.08.058>.

References

[1] R. Marchant, I.M. Banat, Biosurfactants: a sustainable replacement for chemical surfactants? Biotechnol. Lett. 34 (9) (2012) 1597–1605.

[2] I.M. Banat, S.K. Satpute, S.S. Cameotra, R. Patil, N.V. Nyayanit, Cost effective technologies and renewable substrates for biosurfactants’ production, Front. Microbiol. 5 (2014).

[3] G. Bognolo, Biosurfactants as emulsifying agents for hydrocarbons, Coll. Surf. A 152 (1–2) (1999) 41–52.

[4] L.V. de Araujo, C.R. Guimaraes, R.L.D.S. Marquita, et al., Rhamnolipid and surfactin: anti-adhesion/antibiofilm and antimicrobial effects, Food Control 63 (2016) 171–178.

[5] M. Nitschke, G.M. Pastore, Biosurfactants: properties and applications, Quim. Nova 25 (5) (2002) 772–776.

[6] M. Nitschke, S.G.V.A.O. Costa, Biosurfactants in food industry, Trends Food Sci. Technol. 18 (5) (2007) 252–259.

[7] R. Marchant, I.M. Banat, Microbial biosurfactants: challenges and opportunities for future exploitation, Trends Biotechnol. 30 (11) (2012) 558–565.

[8] S.K. Satpute, A.G. Banpurkar, P.K. Dhakephalkar, I.M. Banat, B.A. Chopade, Methods for investigating biosurfactants and bioemulsifiers: a review, Crit. Rev. Biotechnol. 30 (2) (2010) 127–144.

[9] V.S. Alahverdjieva, D.O. Grigoriev, J.K. Ferri, et al., Adsorption behaviour of hen egg-white lysozyme at the air/water interface, Coll. Surf. A 323 (1–3) (2008) 167–174.

[10] J.M. Ruso, A. González-Pérez, G. Prieto, F. Sarmiento, Study of the interactions between lysozyme and a fully-fluorinated surfactant in aqueous solution at different surfactant-protein ratios, Int. J. Biol. Macromol. 33 (1–3) (2003) 67–73.

[11] A. Kezwon, K. Wojciechowski, Interaction of Quillaja bark saponins with food-relevant proteins, Adv. Colloid Interface Sci. 209 (2014) 185–195.

[12] R.S.H. Lam, M.T. Nickerson, Food proteins: a review on their emulsifying properties using a structure-function approach, Food Chem. 141 (2) (2013) 975–984.

[13] M. Hu, D.J. McClements, E.A. Decker, Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate, J. Agric. Food Chem. 51 (6) (2003) 1696–1700.

[14] K. Wojciechowski, A. Kezwon, J. Lewandowska, K. Marcinkowski, Effect of ß-casein on surface activity of Quillaja bark saponin at fluid/fluid interfaces, Food Hydrocolloids 34 (2014) 208–216.

[15] J.L. Li, Y.Q. Cheng, P. Wang, W.T. Zhao, L.J. Yin, M. Saito, A novel improvement in whey protein isolate emulsion stability: generation of an enzymatically cross-linked beet pectin layer using horseradish peroxidase, Food Hydrocolloids 26 (2) (2012) 448–455.

[16] A. Kezwo´n, I. Chromi´nska, T. Fraczyk, K. Wojciechowski, Effect of enzymatic hydrolysis on surface activity and surface rheology of type I collagen, Colloids Surf. B: Biointerfaces 137 (2016) 60–69.

[17] J.Y. Exposito, U. Valcourt, C. Cluzel, C. Lethias, The fibrillar collagen family, Int. J. Mol. Sci. 11 (2) (2010) 407–426.

[18] S. Ricard-Blum, The collagen family, Cold Spring Harbor Perspect. Biol. 3 (1) (2011).

[19] K.E. Kadler, C. Baldock, J. Bella, R.P. Boot-Handford, Collagens at a glance, J. Cell Sci. 120 (12) (2007) 1955–1958.

[20] A. Kezwon, K. Wojciechowski, Effect of temperature on surface tension and surface dilational rheology of type I collagen, Colloids Surf. A 460 (2014) 168–175.

[21] M. Van der Rest, R. Garrone, Collagen family of proteins, FASEB J. 5 (13) (1991) 2814–2823.

[22] D.J.S. Hulmes, T. Scheibel, Vertebrates collagens – structures, functions and biomedical applications Fibrous Proteins, Vol. 1, CRC Press, 2008.

[23] J.A. Fallas, V. Gauba, J.D. Hartgerink, Solution structure of an ABC collagen heterotrimer reveals a single-register helix stabilized by electrostatic interactions, J. Biol. Chem. 284 (39) (2009) 26851–26859.

[24] A.V. Persikov, J.A.M. Ramshaw, A. Kirkpatrick, B. Brodsky, Electrostatic interactions involving lysine make major contributions to collagen triple-helix stability, Biochemistry 44 (5) (2005) 1414–1422.

[25] T.I. Nikolaeva, E.I. Tiktopulo, R.V. Polozov, Y.A. Rochev, Thermodynamic and structural characteristics of collagen fibrils formed in vitro at different temperatures and concentrations, Biophysics 52 (2) (2007) 191–195.

[26] V.K. Yadavalli, D.V. Svintradze, R.M. Pidaparti, Nanoscale measurements of the assembly of collagen to fibrils, Int. J. Biol. Macromol. 46 (4) (2010) 458–464.

[27] M. Fang, E.L. Goldstein, E.K. Matich, B.G. Orr, M.M. Banaszak Holl, Type I collagen self-assembly: the roles of substrate and concentration, Langmuir 29(7) (2013) 2330–2338.

[28] C.H. Lee, A. Singla, Y. Lee, Biomedical applications of collagen, Int. J. Pharm. 221 (1–2) (2001) 1–22.

[29] D.P. Chang, F. Guilak, G.D. Jay, S. Zauscher, Interaction of lubricin with type II collagen surfaces: adsorption, friction, and normal forces, J. Biomech. 47 (3) (2014) 659–666.

[30] Y.W. Du, L.N. Zhang, X. Ye, et al., In vitro and in vivo evaluation of bone morphogenetic protein-2 (BMP-2) immobilized collagen-coated polyetherether ketone (PEEK), Front. Mater. Sci. 9 (1) (2015) 38–50.

[31] H. Sun, Q. Yu, B. Yang, G. Xu, Surface hydrophilic modification of poly(etherether ketone) and immobilization of collagen, Gaodeng Xuexiao HuaxueXuebao/Chem. J. Chin. Univ. 37 (6) (2016) 1154–1160.

[32] Y.L. Chi, Q.X. Zhang, X.P. Liao, J. Zhou, B. Shi, Physicochemical properties and surface activities of collagen hydrolysate-based surfactants with varied oleoyl group grafting degree, Ind. Eng. Chem. Res. 53 (20) (2014) 8501–8508.

[33] C. Li, W. Liu, L. Duan, Z. Tian, G. Li, Surface activity of pepsin-solubilized collagen acylated by lauroyl chloride along with succinic anhydride, J. Appl. Polym. Sci. 131 (14) (2014).

[34] C. Li, H. Tian, L. Duan, Z. Tian, G. Li, Characterization of acylated pepsin-solubilized collagen with better surface activity, Int. J. Biol. Macromol. 57 (2013) 92–98.

[35] R. Miller, V.B. Fainerman, A.V. Makievski, et al., Dynamics of protein and mixed protein/surfactant adsorption layers at the water/fluid interface, Adv. Colloid Interface Sci. 86 (1) (2000) 39–82.

[36] T.A. Khan, H.C. Mahler, R.S.K. Kishore, Key interactions of surfactants in therapeutic protein formulations: a review, Eur. J. Pharm. Biopharm. 97(2015) 60–67.

[37] R. Miller, V.B. Fainerman, M.E. Leser, M. Michel, Kinetics of adsorption of proteins and surfactants, Curr. Opin. Colloid Interface Sci. 9 (5) (2004) 350–356.

[38] A. Dan, G. Gochev, J. Krägel, E.V. Aksenenko, V.B. Fainerman, R. Miller, Interfacial rheology of mixed layers of food proteins and surfactants, Curr. Opin. Colloid Interface Sci. 18 (4) (2013) 302–310.

[39] A.R. Mackie, Structure of adsorbed layers of mixtures of proteins and surfactants, Curr. Opin. Colloid Interface Sci. 9 (5) (2004) 357–361.

[40] M.A. Bos, T. Van Vliet, Interfacial rheological properties of adsorbed protein layers and surfactants: a review, Adv. Colloid Interface Sci. 91 (3) (2001)

437–471.

[41] N.N. Fathima, A. Dhathathreyan, Effect of surfactants on the thermal, conformational and rheological properties of collagen, Int. J. Biol. Macromol. 45 (3) (2009) 274–278.

[42] A.V. Makievski, V.B. Fainerman, R. Miller, M. Bree, L. Liggieri, F. Ravera, Determination of equilibrium surface tension values by extrapolation via longtime approximations, Colloids Surf. A 122 (1–3) (1997) 269–273.

[43] G. Loglio, P. Pandolfini, R. Miller, et al., Drop and bubble shape analysis as a tool for dilational rheological studies of interfacial layers, Stud. Interface Sci. 11 (2001).

[44] K. Lunkenheimer, F. Theil, K.-H. Lerche, Investigations on the hydrolysis of sodium n-alkyl sulfates in aluminum oxide suspensions, Langmuir 8 (2) (1992) 403–408.

[45] V.B. Fainerman, S.V. Lylyk, E.V. Aksenenko, J.T. Petkov, J. Yorke, R. Miller, Surface tension isotherms, adsorption dynamics and dilational visco-elasticity of sodium dodecyl sulphate solutions, Colloids Surf. A 354 (1–3) (2010) 8–15.

[46] V. Pradines, V.B. Fainerman, E.V. Aksenenko, J. Krägel, N. Mucic, R. Miller, Adsorption of alkyl trimethylammonium bromides at the water/air and water/hexane interfaces, Colloids Surf. A 371 (1–3) (2010) 22–28.

[47] R. Wüstneck, J. Krägel, R. Miller, P.J. Wilde, D.C. Clark, The adsorption of surface-active complexes between -casein, -lactoglobulin and ionic surfactants and their shear rheological behaviour, Colloids. Surf. A 114 (1996) 255–265.

[48] E.V. Aksenenko, V.B. Fainerman, J.T. Petkov, R. Miller, Dynamic surface tension of mixed oxyethylated surfactant solutions, Colloids Surf. A 365 (1-3) (2010) 210–214.

[49] V.B. Fainerman, S.V. Lylyk, E.V. Aksenenko, et al., Adsorption layer characteristics of Triton surfactants. 1. Surface tension and adsorption isotherms, Colloids Surf. A 334 (1–3) (2009) 1–7.

[50] P.A. Yazhgur, B.A. Noskov, L. Liggieri, et al., Dynamic properties of mixed nanoparticle/surfactant adsorption layers, Soft Matter 9 (12) (2013) 3305–3314.

[51] A. Rao, J. Kim, R.R. Thomas, Interfacial rheological studies of gelatin-sodium dodecyl sulfate complexes adsorbed at the air-water interface, Langmuir 21(2) (2005) 617–621.

[52] H. Fruhner, K.D. Wantke, K. Lunkenheimer, Relationship between surface dilational properties and foam stability, Colloids Surf. A 162 (1–3) (2000) 193–202.

[53] E.H. Lucassen-Reynders, A. Cagna, J. Lucassen, Gibbs elasticity, surface dilational modulus and diffusional relaxation in nonionic surfactant monolayers, Colloids Surf. A 186 (1–2) (2001) 63–72.

[54] N.O. Metreveli, K.K. Jariashvili, L.O. Namicheishvili, et al., UV–vis and FT-IR spectra of ultraviolet irradiated collagen in the presence of antioxidant ascorbic acid, Ecotoxicol. Environ. Saf. 73 (3) (2010) 448–455.

[55] Y.K. Lin, D.C. Liu, Comparison of physical-chemical properties of type I collagen from different species, Food Chem. 99 (2) (2006) 244–251.

[56] A.S. Wexler, Determination of phenolic substances by ultraviolet difference spectrometry, Anal. Chem. 35 (12) (1963) 1936–1943.

[57] K. Wu, W. Liu, G. Li, The aggregation behavior of native collagen in dilute solution studied by intrinsic fluorescence and external probing, Spectrochim. Acta – Part A 102 (2013) 186–193.

[58] J.M. Menter, Temperature dependence of collagen fluorescence, Photochem. Photobiol. Sci. 5 (4) (2006) 403–410.

[59] A. Sionkowska, Photochemical transformations in collagen in the presence of melanin, J. Photochem. Photobiol. A: Chem. 124 (1–2) (1999) 91–94.

[60] O. Kaewdang, S. Benjakul, T. Kaewmanee, H. Kishimura, Characteristics of collagens from the swim bladders of yellowfin tuna (Thunnus albacares), Food Chem. 155 (2014) 264–270.

[61] U. Freudenberg, S.H. Behrens, P.B. Welzel, et al., Electrostatic interactions modulate the conformation of collagen I, Biophys. J. 92 (6) (2007) 2108–2119.

[62] Y. Chen, R. Ye, Y. Wang, Acid-soluble and pepsin-soluble collagens from grasscarp (Ctenopharyngodon idella) skin: a comparative study on physicochemical properties, Int. J. Food Sci. Technol. 50 (1) (2015) 186–193.

[63] L. Wang, Q. Liang, T. Chen, Z. Wang, J. Xu, H. Ma, Characterization of collagen from the skin of Amur sturgeon (Acipenser schrenckii), Food Hydrocolloids 38(2014) 104–109.

[64] S. Choudhary, N. Kishore, Drug-protein interactions in micellar media: thermodynamic aspects, J. Colloid Interface Sci. 413 (2014) 118–126.

[65] P.L. Kronick, P. Cooke, Destabilization of collagen in hide and leather by anionic surfactants II. Calorimetry of the reaction of collagen with sulfates, J. Polym. Sci. Part B: Polym. Phys. 36 (5) (1998) 805–813.

[66] J. Krej¡cí, Interaction of mixture of anionic surfactants with collagen, Int. J. Cosmet. Sci. 29 (2) (2007) 121–129.

[67] F. Maldonado, M. Almela, A. Otero, J. Costa-López, The binding of anionic and nonionic surfactants to collagen through the hydrophobic effect, J. Protein Chem. 10 (2) (1991) 189–192.

[68] Z. Zhang, G. Li, B. Shi, Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes, J. Soc. Leather Technol. Chem. 90 (1) (2006) 23–28.

[69] G. Özdemir, O.E. Sezgin, Keratin-rhamnolipids and keratin-sodium dodecyl sulfate interactions at the air/water interface, Colloids Surf. B: Biointerfaces52 (1) (2006) 1–7.

[70] X. Wu, J. Hou, M. Li, J. Wang, D.L. Kaplan, S. Lu, Sodium dodecyl sulfate-induced rapid gelation of silk fibroin, Acta Biomater. 8 (6) (2012) 2185–2192.

[71] E.L. Mertz, S. Leikin, Interactions of inorganic phosphate and sulfate anions with collagen, Biochemistry 43 (47) (2004) 14901–14912.

[72] C.P. Schneider, D. Shukla, B.L. Trout, Arginine and the hofmeister series: the role of ion-ion interactions in protein aggregation suppression, J. Phys. Chem. B 115 (22) (2011) 7447–7458.

[73] A.L. Patrick, N.C. Polfer, H2SO4 and SO Transfer Reactions in a sulfopeptide-basic peptide complex, Anal. Chem. 87 (19) (2015) 9551–9554.

[74] A.S. Woods, S.C. Moyer, S.N. Jackson, Amazing stability of phosphate-quaternary amine interactions, J. Proteome Res. 7 (8) (2008) 3423–3427.

[75] A.S. Woods, S. Ferré, Amazing stability of the arginine-phosphate electrostatic interaction, J. Proteome Res. 4 (4) (2005) 1397–1402.

[76] A.S. Woods, H.Y.J. Wang, S.N. Jackson, Sulfation, the up-and-coming post-translational modification: its role and mechanism in protein-protein interaction, J. Proteome Res. 6 (3) (2007) 1176–1182.