Thermodynamic Analysis of the Impact of the Surfactant−Protein Interactions on the Molecular Parameters and Surface Behavior of Food Proteins

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This paper reports on the thermodynamics of the interactions between surfactants (anionic, CITREM, SSL; nonionic, PGE; zwitterionic, phospholipids) and food proteins (sodium caseinate, legumin) depending on the chemical structure and molecular state (individual molecules, micelles) of the surfactants and the molecular parameters (conformation, molar mass, charge) of the proteins under changes of pH in the range from 7.2 to 5.0 and temperature from 293 to 323 K. The marked effect of the protein−surfactant interactions on the molecular parameters (the weight-average molar mass, the gyration and hydrodynamic radii) and the thermodynamic affinity of the proteins for an aqueous medium were determined by a combination of static and dynamic laser light scattering. Thermodynamically justified schematic sketches of the molecular mechanisms of the complex formation between like-charged proteins and surfactants have been proposed. In response to the complex formation between the proteins and the surfactants, the more stable and fine foams have been detected generally.

Introduction

By now, considerable experimental evidence postulates that the interactions between proteins and small-molecule surfactants play the crucial role in the formation and stabilization of the food colloids when they are used in combination. Actually, it was suggested that these interactions lead to significant changes in the protein self-aggregation[1−6] and absorption at the interfaces[7−12] that could alter such key structural functions of the proteins as gelation and the capacity to form and stabilize emulsions and foams, respectively.[3,5,6,9,13−18] However, though the importance of the interactions between proteins and surfactants for the formation and physical stability of the food colloids is now accepted and well-demonstrated, nonetheless the fundamental understanding of the molecular mechanisms underlying both the protein−surfactant interactions and the impact of them on the formation and properties of the food colloids is still incompletely understood. Besides, many questions concerning the molecular parameters of the protein + surfactant complexes still remain to be answered. Moreover, most literature data are related to the interactions of the limited numbers of the model proteins and surfactants that evidently require the inclusion of more of them, both in the nature and in the molecular structure, as objects for further systematic investigations. In so doing, of special interest is the study of the interactions between industrially important food proteins and anionic surfactants, which are like-charged during the conditions of their expected extensive industrial use, i.e., in the range of pH from 5.0 to 7.0.

By this means, the present paper will review our recent studies on the elucidation of the main factors controlling the specific character of the interactions of the industrially important food proteins and likely signed surfactants primarily. In addition, the potentialities of the molecular design of both the novel complex nanosized particles and the novel structural functionality of the proteins through their interactions with the surfactants will be demonstrated. To accomplish these ends, we have used the thermodynamic approach, for the most part, which was based on the application of a combination of the thermodynamic methods such as mixing calorimetry, differential scanning calorimetry (DSC), and laser light scattering. In so doing, we have attempted to get a greater understanding of the basic relationships in the set: the molecular structure of the proteins and surfactants, the character of the interactions between them, the molecular parameters of the protein + surfactant complexes, and the foaming capacities of the complexes.

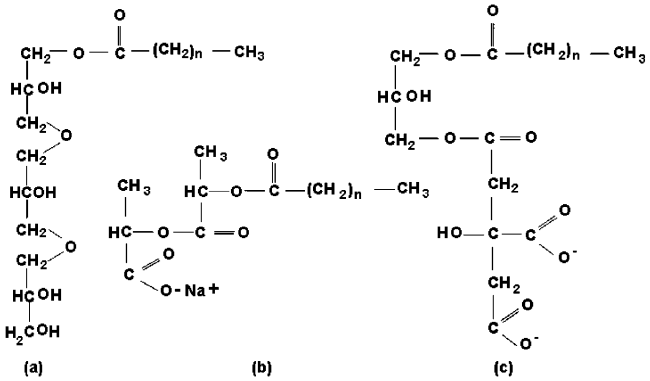

Figure 1. The chemical structures of the small-molecule surfactants, n =14 or 16: (a) PGE, (b) SSL, and (c) CITREM.[19]

As the main objects of our investigation, we have chosen a series of commercially important water-dispersible small-molecule surfactants, which have similarity in the length of the hydrocarbon "tails", but difference in the chemical structure of the polar "heads" (functional groups, charge, size). Namely, anionic surfactants CITREM and SSL, as well as the nonionic surfactant PGE, which generally represent the mixture of stearic and palmitic acid esters with citric acid or lactic acid, or polyglycerol, respectively (Figure 1).[19]

To estimate the role of the molecular state of the surfactants in the interactions, we have chosen the surfactant concentrations both below and above their critical micelle concentrations (cmc).

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In addition, we have also considered zwitterionic biosurfactants such as the soy phospholipids, which generally present as micelles (liposomes) at rather low concentrations in an aqueous medium, because of their very low cmc.[20]

From the protein side, we have studied sodium caseinate, which is the sodium form of the major milk protein, involving the four main fractions of conformationally disordered caseins (38% α_{s1}−, 10% α_{s2}−, 36% β−, 13% κ-casein). These fractions are combined within the discrete particles with size on the nanometer scale, which have rather porous structure determined by the rather open conformation of the constituent caseins.[21] Besides, we have studied the plant oligomeric globular protein legumin (11S globulin), which is the major storage protein of the broad beans (*Vicia faba*), in its native (globule) and heat-denatured (coil) states.[22]

Materials and Methods

Materials. The sample of spray-dried sodium caseinate (>82 wt % dry protein, <6 wt % fat and ash, 5.8 wt % moisture, 0.09 wt % calcium, 1.45 wt % sodium, 2.67 wt % phosphate) was supplied by DMV international (Veghel, Netherlands). Legumin (11S globulin) was isolated from broad beans (var. "Agat") by the method described previously.[23] Homogeneity of the isolated 11S globulin was assessed by a sedimentation velocity analysis in phosphate buffer at pH 8.0, ionic strength of 0.05 M. It was found to be a single peak of 11S globulin with a sedimentation coefficient of about 12S. Small-molecule surfactants which generally represent the mixture of esters of stearic and palmitic acids with either citric acid (CITREM) or lactic acid (SSL), or polyglycerol (PGE), were supplied by Danisco Cultor (Denmark).[19] The soy phospholipid Lipoid S-21 was purchased from Lipoid GmbH Company (Germany). Phosphate buffer (pH 7.2 and pH 6.0) and acetate buffer (pH 5.5 and pH 5.0) with the required ionic strengths were prepared using analytical-grade reagents (99.9% pure): Na_2HPO_4, NaH_2PO_4 and CH_3COOH, NaOH, respectively. Sodium azide (0.01 wt %) was added to the buffers as an antimicrobial agent. All solutions were prepared using double-distilled water.

Preparation of Protein Solutions. Protein solutions (1% w/v) were prepared using the appropriate buffers. Centrifugation (4000 rpm, 30 min, 20 °C) of the protein solutions was carried out to remove a small fraction of insoluble material. The concentration of the protein in solution after centrifugation was checked using a refractometer (Shimadzu, Japan), with reference to the known value of the protein refractive index increment, ν.[5,18]

Preparation of Thermodenatured Legumin. The protein solutions with required concentrations were made in a 0.05 mol L^{-1} phosphate buffer (pH 7.2) as described above. Aliquots (25 mL) of the protein solutions were placed in glass vials, and the vials were heat-sealed. The vials were heat-treated at 90 °C for 30 min in a water bath and after cooling were allowed to equilibrate for 20 h at room temperature (22 ± 2 °C). The level of protein denaturation as a result of the heat treatment was checked by DSC and was indicative of a complete loss of the native conformation of the protein; i.e., no change of the heat capacity of heat-treated protein solutions with increasing temperature was found on the thermogram.

Preparation of Surfactant and Phospholipid Solutions, and Mixtures with the Protein Solutions. Stock solutions of the small-molecule surfactants (100−1000 mg L^{-1}) and phospholipids (100 mg L^{-1}) were prepared by ultrasound treatment over 1 h at a frequency of 4.5 kHz while the solutions were shaken at 65 °C (CPLAN water bath shaker, type 357, Poland). As a result, the fine homogeneous dispersions of the small-molecule surfactants and phospholipids in an aqueous medium were formed. Thereafter, the stock solutions, cooled to room temperature, were used for the preparation of both the concentration series of the surfactant and phospholipid solutions and the mixed solutions with the protein of the required concentrations, whereupon the mixed solutions were shaken at 40 °C for 1 h (CPLAN water bath shaker, type 357, Poland) and then cooled to room temperature.

Mixing Calorimetry. Calorimetric measurements were made using a LKB 2277 flow calorimeter set in the temperature range 20−50 °C. A peristaltic pump introduced the reactants into the instrument. The pump was calibrated by measuring the time required to pump a known volume of solution into the calorimeter. The flow rate was 4×10^{-6} L s^{-1}. The ratio of the flow rates in the two channels was close to 1, i.e., the solutions of proteins or small-molecule surfactants or phospholipids in all cases were diluted by a factor of 2 under mixing. The mixing solutions were thermally equilibrated before entering the reaction vessel. A calibration of the calorimeter itself was done electrically at the measurement temperature. The sensitivity of the calorimetric measurement is no less than 3×10^{-6} J s^{-1}.

Thermal effects were observed during dilution of the following: (i) the protein solution by the pure buffer, $Q_{\text{protein-buffer}}$; (ii) the solution of small-molecule surfactant or phospholipids (phl) by the pure buffer, $Q_{\text{small-molecule surfactant/phl-buffer}}$; and (iii) the protein solution by the solution of small-molecule surfactant or phospholipids, Q_Σ. These thermal effects Q were measured in the thermal power units (J s^{-1}).

The specific enthalpy of the interactions between proteins and small-molecule surfactants or phospholipids was obtained from the relationship

$$\Delta H_{\text{protein-surfactant/phl}} = -(Q_\Sigma - Q_{\text{protein-buffer}} - Q_{\text{surfactant/phl-buffer}})/\Delta n \quad (1)$$

where Δn is the number of grams of proteins mixed with small-molecule surfactants or phospholipids per second (g s^{-1}).

The reproducibility of the measurements was no less than ±10%.

Estimation of the Protein and Complex (Protein + Surfactant) Molecular and Interaction Parameters in a Bulk Aqueous Medium. The weight-average molar mass, M_w, the radius of gyration, R_G, and the second virial coefficient, A_{pr-pr}, of the proteins alone and in the complex with small-molecule surfactants or phospholipids were determined by laser multiangle static light scattering in a dilute aqueous solution (1×10^{-4} to 1×10^{-2} g mL^{-1}, with 5−8 protein concentration points at most). Both the protein alone and the complex (protein 0.5% w/v + surfactant at the specified concentration, prepared as described above) were diluted by the pure buffer. The Rayleigh ratio R_θ was measured using the vertically polarized light (633 nm) at angles in the range $40° \le \theta \le 140°$ (13 angles) using a VA Instruments Co., Ltd. LS-01 apparatus (Saint Petersburg, Russia) calibrated with dust-free benzene ($R_{90} = 11.84 \times 10^{-6}$ cm^{-1}). Solutions were filtered directly into the light-scattering cell through a Millipore membrane with a pore size of 0.8 μm. The raw data were used to plot the angular and concentration dependences of the ratio $HC/\Delta R_\theta$ according to the Zimm method.[24] Here, C is the protein concentration (g mL^{-1}), ΔR_θ is the excess light scattering over that of the solvent at angle θ, and H is an instrumental optical constant equal to $4\pi^2 n^2 \nu^2 / N_A \lambda^4$, where N_A is Avogadro's number, λ is the wavelength of incident light in vacuo, n is the refractive index of the solvent, and ν is the refractive index increment of the protein. Values of the weight-average molar mass, M_w, were estimated as averages from the intercepts of both the concentration dependence of $HC/\Delta R_\theta$ as $\theta \to 0$ (the extrapolation was performed on 13 angles) and the angular dependence of $HC/\Delta R_\theta$ as $C \to 0$ (the extrapolation was performed on 5−8 concentrations). Values of the radius of gyration, R_G, were estimated from the slope of the angular dependence of $HC/\Delta R_\theta$ as $C \to 0$. Values of the second virial coefficient, A_{pr-pr}, were estimated from the slope of the concentration dependence of $HC/\Delta R_\theta$ as $\theta \to 0$. The second virial coefficient characterizes primarily the thermodynamic affinity of the protein or complex (protein + surfactant) particles for the solvent (an aqueous medium in our case) (it is poor if $A_{pr-pr} < 0$, or by contrast, it is good if $A_{pr-pr} > 0$, and it is ideal if $A_{pr-pr} = 0$),[25] i.e., so providing the circumstantial evidence for the protein surface hydrophilicity/hydrophobicity.

The values of M_w, A_{pr-pr}, and R_G presented in this work are averaged data for at least two repetitions of each of the experiment. The experimental error in the determinations of M_w and A_{pr-pr} was estimated as ±10%. The error in the R_G determination was ±5%.

Values of the refractive index increments for the proteins alone and in the presence of surfactants/phospholipids were determined at 635 nm and 20 °C using the Shimadzu differential refractometer. The experimental error is ±10%. For legumin in its native and heat-denatured states, these values were unchanged within experimental error in the presence of the surfactants studied: $\nu = 0.2 \times 10^{-3}$ and 0.18×10^{-3} m^3 kg^{-1}, respectively. In the case of sodium caseinate, the values of the refractive index increments were equal to the following: without surfactants, 0.190 (pH 7.2, ionic strength (I) 0.05 and 0.01 M), 0.203 (pH 6.0, I 0.01 M), 0.201 (pH 5.5, I 0.05 and 0.01 M), 0.199 (pH 5.1, I 0.01 M); with surfactants, 0.194 (pH 7.2, I 0.05 M, CITREM 5 mg L^{-1}), 0.160 (pH 5.5, I 0.05 M, CITREM 500 mg L^{-1}), 0.202 (pH 7.2, I 0.05 M, SSL 1 mg L^{-1}), 0.170 (pH 5.5, I 0.05 M, SSL 100 mg L^{-1}), 0.214 (pH 7.2, I 0.05 M, PGE 0.5 mg L^{-1}), 0.194 (pH 7.2, I 0.01 M, phospholipids 10^{-5}mol L^{-1}), 0.212 (pH 6.0, I 0.01 M, phospholipids 10^{-5} mol L^{-1}), 0.196 (pH 5.1, I 0.01 M, phospholipids 10^{-5}mol L^{-1}).

Values of the hydrodynamic radius R_h of both proteins (5×10^{-3} g/mL) alone and in the presence of the small-molecule surfactants/phospholipids were estimated in their aqueous solutions by dynamic light scattering.[26,27] The time correlation function of the scattering intensity was measured at 90° with the vertically polarized light (633 nm) using a VA Instruments LS-01 apparatus (St. Petersburg, Russia). The values of the hydrodynamic radius R_h presented in this work are averaged data for 10 repetitions of each of the measurements generally. The error in the R_h determination was estimated as ±10%. To determine the hydrodynamic radius from the time correlation function, a special program was used (DYNALS release 1.5, all rights reserved by A. Golding and N. Sidorenko, VA Instruments Co., Ltd. (Saint Petersburg, Russia)).

The temperature during the light-scattering experiments was always 20 °C.

Differential Scanning Calorimetry. Calorimetric measurements were made using a DASM-4M differential adiabatic scanning microcalorimeter (Special Design Office of Biological Instrument Making, Russian Academy of Sciences) in the temperature range 20−110 °C, at a scanning rate of 2 °C min^{-1} and an excess pressure of 2.5 atm. The concentration of the protein samples was 0.005 g mL^{-1}. The accuracy of the measurements is about 10% of the values of the heat capacities. The sensitivity of the calorimetric measurement is no less than 5×10^{-6} J s^{-1}. The thermodynamic parameters of protein denaturation were calculated as proposed before.[28] The values of the thermodynamic parameters of protein denaturation presented in this work are averaged data for at least two repetitions of each of the experiments. The reproducibility of the measurements was no less than ±10%.

Estimation of the Protein Foam Ability. Foam (25 mL) had been generated through a glass membrane (1 μm) using the bubbling method. The velocity of the supply of purified air through the glass membrane was 1.8 mL/s. In these experiments, both the volume of the foaming solutions and the protein concentration were maintained at constant 5 mL and 1% w/v, respectively. The change of the foam stability with time was characterized using a digital photo camera.

Results and Discussion

Thermodynamic Analysis of the Relationships between Some of the Structural Features of Both Proteins and Surfactants and the Predominant Character of their Interactions in an Aqueous Medium (below the cmc). The overall interactions between proteins and surfactants are added up from an average over the great many different physicochemical processes involving the various functional groups and segments (polar, charged, hydrophobic) of both these amphiphilic molecules.[4]

Depending on the aqueous environmental conditions (pH, ionic strength, temperature) and the features of both the chemical structures (the availability of the specific functional groups and segments) and the physical molecular parameters (molar mass, size, conformation) of the interacting molecules, the overall character of the protein−surfactant interactions may differ strongly between exothermic or endothermic.

An accessible thermodynamic function that directly reflects the predominant character of the protein−surfactant interactions in an aqueous medium is the enthalpy of the interactions $\Delta H_{pr-surf}$ measured from mixing calorimetry.[29] To illustrate, the negative values of $\Delta H_{pr-surf}$, measured around the room temperature (293−298 K), are indicative of the exothermic in the character interactions: predominantly electrostatic between opposite charges and generally hydrogen bonding. In contrast, the positive values of $\Delta H_{pr-surf}$ in the same temperature range are ordinarily the reflection of the predominantly endothermic character of the interactions, determined by the hydrophobic protein−surfactant interactions between nonpolar parts of their molecules.[30,31] Besides, the measured value of $\Delta H_{pr-surf}$ could be dictated by the heat effects from the following processes: (i) the transfer of the surfactant molecules from aqueous medium into the protein interior, and (ii) the micellization/demicellization of the surfactant molecules as a result of the interactions with proteins in the specific concentration ranges, which are close to the critical micelle concentration (cmc) of the surfactants.[5,32,33]

Recently, it was shown that the character of the interactions between proteins and the individual molecules of surfactants might change dramatically, depending on their net charges, sizes, and the protein conformation (globule, random coils). As this takes place, evidently the like net charges of proteins and surfactants determine the strength of the electrostatic repulsion forces acting between them, and thereby, the probability of the formation of contacts between their different functional groups (oppositely charged and uncharged polar groups or nonpolar ones). In turn, both the size of the polar heads of the surfactants and the protein conformations control the capacity of the surfactant molecules with similar lengths of hydrocarbon chains to penetrate into the protein interior and to be involved in the interactions of both polar and nonpolar parts of their molecules.[5,18]

By way of illustration, it has been possible to follow the influence of both the charge and size of the polar head of the surfactants, having hydrocarbon chains of similar lengths, on the character of their interactions with the like-charged proteins, on the basis of the calorimetric measurements. This influence can be demonstrated most clearly with an example of the interactions sodium caseinate and anionic surfactants, both of like sign at neutral pH (7.2) but differing in the chemical structure of their polar heads, namely, CITREM (two carboxylic groups) and SSL (one carboxylic group) (Figure 1).

Thus, the predominantly endothermic character of the interactions was observed at room temperature (293 K) for CITREM, and in contrast, the predominantly exothermic character was observed for SSL, which transform into exothermic and endothermic with increasing temperature, respectively (Figure 2a,b).

The pronounced, predominantly endothermic character of the protein−CITREM interactions (Figure 2a) could be attributed primarily to the hydrophobic interactions between the hydrocarbon chains of the CITREM molecules and the nonpolar groups of the protein. Besides, it could be caused by the transfer of the hydrocarbon chains of the surfactant molecules from an aqueous medium into the hydrophobic interior of the protein that may be endothermic at this temperature.[32−34] For both of

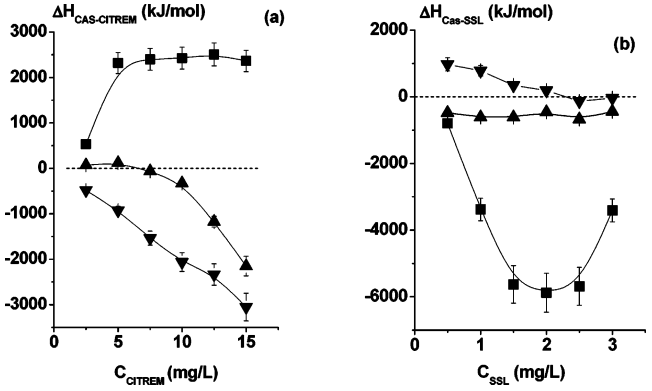

Figure 2. The effect of temperature on the concentration dependencies of the enthalpy of the interactions between anionic surfactants and sodium caseinate (0.5% w/v) in an aqueous medium (pH 7.2, ionic strength 0.05 M): (■) 293 K, (▲) 313 K, and (▼) 323 K for (a) CITREM (cmc 12.5−17.5 mg/L)[5] and (b) SSL (cmc 2.5−3.5 mg/L).[5]

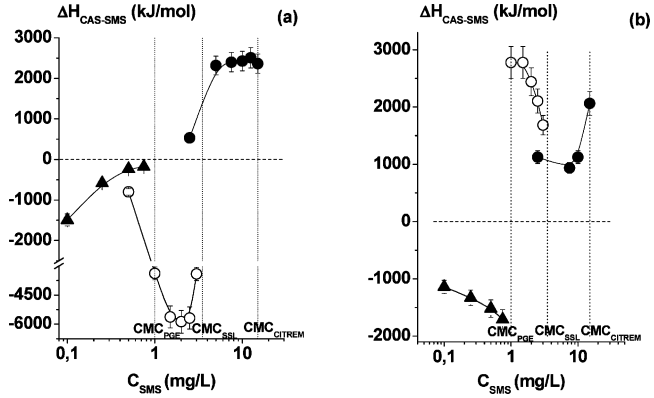

Figure 3. The effect of pH on the character of the interactions between sodium caseinate (0.5% w/v) and surfactants in an aqueous medium (ionic strength 0.05 M) at $T = 293$ K: (●) CITREM, (○) SSL, and (▲) PGE for (a) pH 7.2 and (b) pH 5.5.

these endothermic processes (the positive contribution from the enthalpy into the change of the free energy of the system), the main driving factor may be considered to be the increase in the entropy of the system due to the release of a great number of water molecules, caused by the dehydration of both surfactant and protein molecules under their interactions.

In addition, the revealed apparent independence of the endothermic heat effect of the interactions on the surfactant concentration at 293 K could be attributable to the compensative contributions to the total heat effect from the exothermic interactions between polar functional groups of CITREM and sodium caseinate.

The increase in temperature leads to the change of the interactions from endothermic to exothermic, much as in the case of the change in the character of the transfer of the hydrocarbon chains of the surfactant molecules from water into the hydrophobic interior of the surfactant micelles under their formation in the same temperature range.[32−34] As this takes place, the pronounced increase in the exothermic heat effects of the interactions with increasing concentration of the individual CITREM molecules (Figure 2a) can be attributable to the increase in the number of the surfactant molecules involved in the interactions with the protein.

It seems likely that the negative charge (similar with that of the protein[35]) of the anionic CITREM (causing the electrostatic repulsions between them), in combination with the relatively big size of its polar head, hinders the penetration of the polar head of CITREM into the protein interior and essentially decreases the probability of interactions between them such as electrostatic (between opposite charges) and hydrogen bonding, primarily. Under these circumstances, the hydrophobic interactions between the hydrocarbon chains of CITREM molecules and the hydrophobic patches of the protein seem to be more preferable. The similar character of the interactions was revealed previously under the interactions of the like-charged sodium dodecyl sulfate (SDS) and soy protein at pH values of 7.0 and 8.2.[36]

In contrast to this, the exothermic character of the interactions found for SSL at 293 K (Figure 2b) could be attributable to facilitation of the hydrogen bonding and electrostatic attraction of the oppositely charged functional groups of the surfactant and protein. Both the less-marked protein−surfactant electrostatic repulsions (lower charge) and the smaller size (small polar head) of SSL are favorable for the penetration of SSL molecules as a whole into the interior of the protein nanoparticles. This easier penetration can increase the probability of different kinds of protein−surfactant interactions. As this takes place, the overall energies of the interactions between polar functional groups of the protein and the surfactant (electrostatic (160 kJ/mol)[30] and hydrogen bonding (10−40 kJ/mol)[30]) are superior generally to those between nonpolar parts of their molecules (hydrophobic (5−10 kJ/mol)[30]) at room temperature (293 K), and we can see the superiority of the former group in the total heat effects measured. In addition, the revealed exothermic character of the interactions evidently provides the gain in free energy of the system despite the evident entropy loss under the formation of the new bonds.

Moreover, the concentration dependence of the exothermic heat effect of the interactions has progressed to the maximum value and then diminished with increasing concentration of SSL toward the cmc. This result could reflect the opposing contribution from the endothermic transfer of the hydrocarbon chains of the surfactant molecules from an aqueous medium into the hydrophobic interior of the protein that becomes more marked near the cmc.

However, with increasing temperature, when the exothermic interactions between the polar functional groups become weaker, we can see strengthening in the endothermic interactions, probably between nonpolar parts of the surfactant and protein (Figure 2b). Such interactions may be considered entropically driven primarily because of dehydration of both surfactant and protein molecules under the interactions between them. In this case, the slight decrease in the endothermic heat effect of the interactions found at high temperature (323 K) with increasing surfactant concentration can be attributable to the rise of the opposing contribution from the exothermic (at this temperature) transfer of the hydrocarbon chains of the surfactants from an aqueous medium into the hydrophobic interior of the protein.

The net electrical charge on both the protein and the ionic surfactant that is a determining factor for their interactions in an aqueous medium, as noted above, may be governed by the solution pH. This is of great importance for the case of proteins, because their charge is dramatically dependent on how far the pH values differ from the protein isoelectric points. To illustrate, Figure 3 shows the effect of reducing the pH on the character of the interactions between sodium caseinate ($pI_{pr} \approx 4.8$) and surfactant.

In the case of CITREM, the character of the interactions with the protein becomes less endothermic with reduction of the pH. Most likely, the reduced protein charge, and consequently the reduced electrostatic protein−surfactant repulsions, are favorable for an increase in the probability of interactions between the

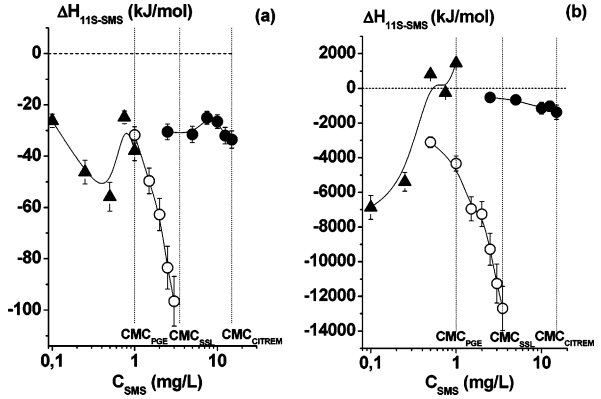

Figure 4. The enthalpy of the interactions of legumin (0.5% w/v) with the surfactants in an aqueous medium (pH 7.2, ionic strength 0.05 M) at $T = 293$ K: (●) CITREM, (○) SSL, and (▲) PGE for (a) native legumin and (b) heat-denatured legumin.

polar functional groups (oppositely charged ionic and nonionic) of the anionic surfactant and the protein, which are generally exothermic. Such interactions could contribute to the diminishing total endothermic heat effect of the interactions (compare Figure 3a and b). It is significant to note here that, by relying on the invariant cmc values with reduced pH for the surfactants studied,[5] it may be assumed that the net charge of the anionic surfactants is kept constant in the range of pH from 7.2 to 5.5.

For the evidently less charged SSL, the dramatic change in the character of the interactions with the protein, i.e., the transfer from predominantly exothermic to endothermic, occurs with reduction of the pH. This result could be attributed to the increase in the contribution to the total heat effect of the formation of the predominantly hydrophobic contacts between nonpolar parts of the surfactant and the less-charged protein at lower pH (compare Figure 3a and b).

For reference, a principal change of the character of the interactions between sodium caseinate and the nonionic surfactant PGE with reduction in the pH has not been found, as expected. Owing to the assumed predominant hydrogen bonding between the functional groups of PGE and the protein, the similar exothermic character of their interactions was found at both pH values studied (pH 7.2 and pH 5.5) (compare Figure 3a and b). Only for the concentration dependence of the exothermic heat effect revealed it can be safely suggested that its increase up to the cmc (pH 5.5) could be attributable to the increase in the concentration of the interacting surfactant molecules, whereas its lowering (pH 7.2) could be caused by the compensative contribution from the opposing endothermic processes (interactions between nonpolar parts of the surfactant and protein or transfer of the surfactant from water into the protein interior at 293 K).

The role of the protein conformation on the character of the interactions of proteins with surfactants could be most clearly defined from the comparison of the characteristics of the interactions of a native and thermodenatured protein by the example of the globular plant protein legumin. Figure 4 shows that the values of the enthalpy of the interactions of the heat-denatured protein with surfactants (Figure 4b) are 2 orders of magnitude greater than those measured for the native protein globule (Figure 4a) for both the anionic (CITREM, SSL) and nonionic (PGE) surfactants.

On one hand, this result could be attributable to the greater accessibility for the surfactants of the reacting groups or parts of the protein molecules due to their transformation from globules to random coils, as a result of the heat denaturation (90 °C, 30 min). On the other hand, an increase in the number of binding sites in the protein for the surfactants may be suggested, owing to the formation of soluble aggregates of the heat-denatured legumin ($M_w = 6300$ kDa).[18]

Table 1. Relationships between the Values of the Enthalpy of the Protein (0.5 wt %/v)−Surfactant Interactions and the Protein Architecture, as well as the Protein Relative Hydrophilicity, Reflecting the Net Protein Charge (pH 7.2, $I = 0.05$ M, $T = 293$ K)[a]

	sodium caseinate	native legumin	heat-denatured legumin
$A_{pr-pr}\ 10^5$ (m^3 mol kg^{-2})	2.9	0	−21
	$\Delta H_{protein-surfactant}$ (kJ mol^{-1})		
CITREM (7.5 mg/L)	2202	−30	−762
SSL (1 mg/L)	−3366	−34	−4333

[a] The experimental error in the determinations of A_{pr-pr} and $\Delta H_{protein-surfactant}$ was estimated as ±10%.

For legumin, as for sodium caseinate, the concentration dependences of the heat effects of the interactions between proteins and surfactants can help to reveal their complicated nature, especially when we can find a sharp change in the character of the interactions with increasing surfactant concentration, as in the case of nonionic PGE. Thus, the reduced exothermic character of the protein−PGE interactions with increasing PGE concentration could be attributable to the compensative contribution from the opposing endothermic processes (interactions between nonpolar parts of the surfactant and protein or transfer of the surfactants from water into the protein interior at 293 K).

The commonness of the role of protein conformation shows itself most clearly in the comparison between the values of the enthalpy of the protein−surfactant interactions measured from mixing calorimetry for the different proteins (Table 1). In such a manner, the enthalpy of the interactions is a minimum for the native globular legumin, having the compact conformation of the globule ($M_w = 330$ kDa, $R = 3-5$ nm[22,37,38]), the interior of which does not seem to be practically accessible for the rather long surfactant molecules. In contrast, both the sodium caseinate nanoparticles ($M_w = 4000$ kDa, $R_h = 117$ nm)[5] and the thermoaggregates of the heat-denatured legumin ($M_w = 6300$ kDa, $R_h = 58$ nm)[18] show the pronounced heat effects under the interactions with the surfactants. This result could be attributable to the similar, more open architecture of both the sodium caseinate nanoparticles and the heat-denatured legumin aggregates, which most likely provides the great access to the protein interior for the surfactant molecules and, therefore, the abundant binding sites for them there. Such protein architecture is dictated by the rather open coil conformation of the constituent caseins[21,39,40] and the heat-unfolded protein chains.[37,41]

As this takes place, it is interesting to note that the heat effects, which are similar in character (exothermic) and order of magnitude, were revealed in the case of the interactions of both sodium caseinate and heat-denatured legumin with anionic surfactant SSL, having both the lower charge and smaller size of the polar head and hence more easily penetrating into the protein interior, in comparison with another anionic surfactant studied, CITREM.

In contrast, despite the similar architecture, the dramatic change in the character of the protein−surfactant interactions was revealed for CITREM, having the greater charge and size of the polar head, as compared with SSL, in passing from the nanoparticles of the sodium caseinate to the thermoaggregates

Table 2. Molecular and Thermodynamic Parameters of the Protein + Surfactant Complexes Formed as a Result of the Interactions of the Sodium Caseinate and Legumin with the Individual Molecules of Surfactants, that is below Their cmc[5] in an Aqueous Medium at 293 K (pH 7.2, ionic strength 0.05 M)[a]

system	$C_{surfactant}$ (mg L^{-1})	M_w 10^{-6} (Da)	k_1[b]	A_{pr-pr} 10^5 (m^3 mol kg^{-2})	A_{pr-pr} (m^3 mol^{-1})	R_G[c] (nm)	k_2[b]	$\rho = R_G/R_h$
				Sodium Caseinate				
protein without surfactant	0	4		2.9	0.93	117		1.1
protein + CITREM	5	29	7.3	11.2	188	211	1.8	2.0
protein + SSL	1	25	6.3	6.3	78	207	1.8	2.2
protein + PGE	0.5	15.6	3.9	5.9	29	191	1.6	2.0
				Native Legumin				
protein without surfactant	0	0.33		0	0			
protein + CITREM	6	0.38	1.2	−24.5	−0.07			
protein + SSL	1	0.69	2.0	−0.7	−0.007			
protein + PGE	0.5	2.6	7.9	22	2.97			
				Heat-Denatured Legumin				
protein without surfactant	0	6.3		−21	−17	40		0.7
protein + CITREM	6	19.2	3.0	1.4	10	108	2.7	1.7
protein + SSL	1	55	8.7	32.8	1984	273	6.8	3.1
protein + PGE	0.5	8.8	1.4	−8.5	−13	119	3.0	1.7

[a]The experimental error in the determinations of M_w, A_{pr-pr}, and R_h was estimated as ±10%. The error in the R_G determination was ±5%. [b] k_1 is the extent of the protein aggregation $k_1 = M_w^{protein+surfactant}/M_w^{protein}$; k_2 is the extent of the increase in the size of the protein nanoparticles $k_2 = R_G^{protein+surfactant}/R_G^{protein}$. [c] It was not possible to estimate the R_G from the laser light scattering measurements for the case of the native legumin because of the lack of angular asymmetry of the light scattering that is caused by the evident small size of both the native[22,37,38] and modified by the interactions with surfactants globules of legumin.

of the heat-denatured legumin. It is safe to assume that this result could be attributable to the different net charges of the proteins, which can be manifested indirectly by the different thermodynamic affinities of the proteins for an aqueous medium (their different relative hydrophilicities) that, in turn, can be estimated through the values of the second virial coefficients.[25] Actually, the higher hydrophilicity and consequently the larger net charge of the sodium caseinate nanoparticles in comparison with those of the heat-denatured legumin are mirrored in the different signs of the second virial coefficients,[24,25] A_{pr-pr}, obtained for these proteins from static light scattering (Table 1), i.e., the positive value for sodium caseinate, as opposed to the negative value for the heat-denatured legumin. By this means, the relatively high hydrophilicity found for sodium caseinate[5] proves additionally that the expected rather strong electrostatic repulsions between the like-charged protein and polar heads of the CITREM molecules prevent the development of both ionic interactions (between the opposite charges) and hydrogen bonding between them, whereas the formation of the hydrophobic contacts between the nonpolar parts of their molecules turn out to be more preferable, as was discussed above. By contrast, the lower hydrophilicity and hence the expected lower net charge of the heat-denatured legumin, composed of the most hydrophobic basic constituent chains,[37,41] seems to facilitate the development of ionic interactions (between the opposite charges) and hydrogen bonding between the protein and the polar head groups of CITREM that prevail over the hydrophobic interactions between the nonpolar parts of their molecules in the total heat effect of their interactions (Table 1).

Modification of the Molecular Parameters of the Proteins by the Interactions with Individual Surfactant Molecules (below the cmc). As a result of the interactions of proteins with the individual molecules of surfactants, a marked protein aggregation was revealed generally by the increase in the values of both the weight average molar mass and the radius of gyration of the proteins, measured by laser light scattering [5,18] and presented in Table 2 for the purposes of illustration, as well as obtained from turbidity measurements,[4] as if the individual surfactant molecules fulfill the role of the powerful cross-linking agents.

It is worthy to note here that the surfactant concentrations chosen for the investigation in this case were below their cmc and were specified by the following cmc values:[5] CITREM, 12.5−17.5 mg/L; SSL, 2.5−3.5 mg/L; PGE, 0.75−1.0 mg/L. In so doing, it is notable that the extent of the protein aggregation found as a result of the interactions of the proteins with SSL is close to or even higher than that obtained in the presence of CITREM despite the fivefold smaller weight concentration (approximately fourfold smaller molar concentration) of SSL in the system as compared with CITREM. This result could be attributable to the lower charge and smaller size of the polar heads of the SSL molecules that facilitate the formation of the contacts with the like-charged protein nanoparticles, as discussed above. However, the lowest extent of the protein aggregation revealed for both the nanoparticles of sodiuin caseinate and heat-denatured legumin in the presence of PGE may be attributed to some extent to the lowest PGE concentration in the system studied.

Besides, the calculation of the molar masses of the hypothetical protein plus surfactant complexes, which rested on the assumption that all the amounts of the added surfactants were bound equally by the original protein nanoparticles in solution, provides the evidence for the protein aggregation caused by the interactions with surfactant molecules, by virtue of the fact that the calculated values of the M_w are several times or even orders of magnitude smaller than those found by light scattering.

The protein aggregation is followed generally by the pronounced increase in the relative protein surface hyrophilicity[5,18] that is reflected by either the marked increase in the positive values of the second virial coefficient, as in the cases of the interactions of the anionic surfactants (CITREM and SSL) with both sodium caseinate and heat-denatured legumin (Table 2), or by the decrease in their absolute negative values, as for the interactions of PGE with the heat-denatured legumin. Table 2 shows that this result makes itself evident in the values of the

second virial coefficients expressed both in weight (m^3 mol kg^{-2}) and mole (m^3 mol^{-1}) units. What this means is that the increase in surface hydrophilicity of the modified protein is an inherent characteristic of both the protein weight units and the protein mole units, respectively. As this takes place, this increase is more pronounced through the values of the second virial coefficients expressed in mole units, i.e., when the molar weights of the protein particles are taken into account and the surfaces of the protein particles are considered as a whole.

In line with this result, the marked increase in the solubility of soy protein was reached in response to the interaction with sodium dodecyl sulfate (SDS).[42]

On the other hand, the protein aggregration may be followed by the clearly defined increase in the relative hydrophobicity of the protein surface ($A_{pr-pr} < 0$), as is revealed for the cases of the interactions of the anionic surfactants (CITREM and SSL) with the native legumin (Table 2). This result checks well with the possibility of insoluble protein−surfactant complex formation due to net protein charge neutralization as a result of the interaction between oppositely charged protein and surfactant.[4]

The changes found in the hydrophilic/lipophilic properties of the protein surface in the presence of the surfactants (Table 2) are most likely dictated by the ultimate spatial arrangement of the hydrophobic and hydrophilic parts of the protein and the surfactant molecules in response to the predominant nature of their interactions. What this means is that either most of the hydrophobic parts of both interacting molecules are exposed into aqueous medium ($A_{pr-pr} < 0$) or, on the contrary, they are hidden in the interior of the protein aggregates, whereas the hydrophilic parts are directed into aqueous medium ($A_{pr-pr} > 0$). As this takes place, either greater polarity of the surfactant molecules or greater extent of the ultimate protein aggregation, k_1, leads to the formation of more hydrophilic surface of the protein aggregates, and more positive values of the A_{pr-pr} were found.

Moreover, one would expect that the peculiar features of the changes of the molecular properties of the native globular legumin, in particular, the decrease in the protein surface hydrophilicity ($A_{pr-pr} < 0$), revealed as a result of the interactions with surfactants could be attributable to the associated changes of the conformational stability of the protein globule. The changes in the protein conformational stability, reflecting the extent of folding/unfolding of the protein globule, could be characterized by the alteration of the thermodynamic parameters, describing the protein heat denaturation, and measured in a DSC experiment in an aqueous medium.[1−4,43] These are the specific enthalpy of the protein denaturation, ΔH_d;[28,44] the distinctions between the specific heat capacities of the native and heat-denatured protein, $\Delta_d C_p$, which reflects the differences between the degree of the exposure, or the solvating, of hydrophobic groups in the native and denatured states;[44,45−47] the parameter cooperativity, $\Delta T_{1/2}$, that is, the width of the calorimetric transition at a half heat peak height (K) on the thermogramme; and the temperature of denaturation, T_d. A decrease in the values of ΔH_d, $\Delta_d C_p$, and T_d generally indicates a decrease in the protein conformational stability, and the opposite is the truth for the increase in protein conformational stability.

Actually, Table 3 shows that the greatest decrease in protein conformational stability was found in the case of the interactions of native legumin with PGE. This result suggests the disruption of the important bonds in the interior of the protein, which are responsible for the conformational stability of the original protein globule, as a result of the interactions with the surfactant. This disruption causes the partial protein unfolding before heating. That is most clearly apparent in the lower value of $\Delta_d C_p$, which shows that the degrees of exposure of the hydrophobic groups at the protein surface before and after heating approach each other. It is interesting to note that the most marked protein aggregation in an aqueous medium, obviously hydrophobic in nature, was revealed in this case, as if the maximum increase in the original protein hydrophobicity was reached by summing over both the greatest unfolding of the protein globule and the attachment to the protein of the hydrocarbon tails from the nonionic surfactant.

Table 3. Impact of the Interactions of the Individual Molecules of Surfactants (below the cmc) on the Thermodynamic Parameters of the Heat Denaturation of Legumin (C_{pr} = 0.5% w/v) at pH 7.2 and Ionic Strength of 0.05 M[a]

system	$C_{surfactant}$ (mg/L)	ΔH_d (J/g)	$\Delta_d C_p$ (J/(gK))	$\Delta T_{1/2} \pm 0.3$ (K)	$T_d \pm 0.3$ (K)
protein without surfactant	0	25.1	0.35	11.8	83.7
protein + CITREM	5	22.1	0.34	9.2	81.8
protein + SSL	1	33.1	0.42	11.0	81.1
protein + PGE	0.5	19.5	0.23	9.5	81.4

[a] The experimental error in the determinations of ΔH_d, $\Delta_d C_p$ was estimated as ±10%.

In addition, the DSC measurements testify that the formation of the additional surfactant−protein bonds due to the interactions between them can lead to the apparent increase in the conformational stability of the modified protein, as reflected by the increase in the values of ΔH_d and $\Delta_d C_p$ for the case of SSL (Table 3). At the same time, the values found in this case decrease in T_d despite the increase in the specific enthalpy of the protein denaturation, ΔH_d, can infer the greater change in the specific entropy of the protein denaturation, ΔS_d, according to the determination of the T_d by the following relation: $T_d = \Delta H_d/\Delta S_d$.[30] This result could be attributable to the great release of the surfactant molecules from their complex with protein with increasing temperature and the protein denaturation. The rather high predominant exothermic character of the interactions between native legumin and SSL at 293 K (Figure 4), which suggests the decrease in the strength of the attractive forces between their polar groups with increasing temperature, agrees well with this assumption.

The apparent increase in the conformational stability of the protein, modified by the interactions with SSL (Table 3), is followed by the lesser extent of the protein aggregation as compared with the effect of PGE (Table 2). It seems likely that in this case the protein aggregation is caused only by the increase in the original protein surface hydrophobicity at the expense of the attachment of the hydrocarbon tails of the surfactant to the original protein surface primarily.

It is interesting to note that both the lowest change in the original protein conformational stability (Table 3) and the lowest extent of the protein aggregation (Table 2) were found to be in response to the interactions between the protein and the CITREM molecules with the most like charges, even despite their higher concentration. This result agrees well with the lowest heat effect of the interactions measured in this case (Figure 4).

In addition, for all these cases, the parameter of cooperativity, $\Delta T_{1/2}$, decreases (Table 3), which indicates that the protein denaturation occurs within a more narrow temperature range, and thus, the transition is the more cooperative process[48] that could be attributable to the formation of the rather prolonged zones of the new contacts between protein and surfactants, which could be destroyed simultaneously by heating.

Table 4. Theoretical Estimation of the Type of Protein Aggregation Caused by the Interactions of the Protein (0.5% w/v) with the Surfactants in an Aqueous Medium at 293 K (pH 7.2, ionic strength 0.05 M)

			$k_2{}^{d*}$			
system	$C_{surfactant}$ (mg L^{-1})	k_1[a]	collinear aggregation along a rigid rod $d^* = 1$	random aggregation $d^* = 2.5$	shell-like aggregation $d^* = 3.0$	shrinking of the unimer during aggregation $d^* = 3.5$
			Sodium Caseinate			
protein + CITREM	5	7.3	1.8	4.3	5.8	7.8
protein + SSL	1	6.3	1.8	4.3	5.8	7.8
protein +PGE	0.5	3.9	1.6	4.3	4.1	5.2
			Heat-Denatured Legumin			
protein + CITREM	6	3.0	2.7	11.9	19.7	32.3
protein + SSL	1	8.7	6.8	120.5	314.4	819.9
protein + PGE	0.5	1.4	3.0	15.6	27	46.8

[a] k_1 is the extent of the protein aggregation $k_1 = M_w^{protein+surfactant}/M_w^{protein}$; k_2 is the extent of the increase in the size of the protein nanoparticles $k_2 = R_G^{protein+surfactant}/R_G^{protein}$.

At the same time, a consideration of the extent of the increase (k_2) in the radius of gyration (R_G) of the protein aggregates shows that this value is generally less than that (k_1) for the weight average molar mass (M_w) (Table 2). For polymer aggregates, the relationship between M_w and R_G could be described by the power-law equation[26]

$$M_w = KR_G^{d^*}, \quad (2)$$

where the exponent d* has the meaning of a dimensionality, which helps to describe the aggregate structures formed by well-defined geometrical models.[26] Hence, it is safe to assume that, as the first approximation, $k_1 = k_2{}^{d^*}$ under the protein aggregation caused by the protein interactions with the surfactants. Table 4 shows the results of the comparison of the values k_1 and $k_2{}^{d^*}$. The value of $k_2{}^{d^*}$ was calculated using the exponent d*, fitting to the different type of polymer aggregation.

The comparisons presented in Table 4 are indicative of some collapse of the sodium caseinate nanoparticles, combined into the protein aggregates, most likely due to pronounced surfactant cross-linking in their interior as a result of the interactions with the anionic surfactants, which is most marked in the case of CITREM. It seems that this result is attributable to the rather porous and flexible structure of the original sodium caseinate nanoparticles. In line with this result, the surfactant-induced contraction of the gelatin chain up to the cmc by almost 30% was revealed recently as a result of its interaction with anionic surfactant α-olefin sulfonate.[49]

In turn, the shell-like aggregation is a characteristic of the sodium caseinate aggregation caused by the interactions with the nonionic surfactant, PGE.

In the case of the heat-denatured legumin, the data suggest the collinear aggregation of the original protein particles along a rigid rod. This result is in accord with the change of the highly structure sensitive parameter, $\rho = R_G/R_h$,[26] in this case (Table 2). This is indicative of the transformation of the architecture of the protein nanoparticels from the spherelike, $0.6 \leq \rho \leq 1$, to the more rigid and open architecture, as encountered with rigid polydisperse rods when $\rho \gg 2$.[26]

It is interesting to note that an increase in ρ from 1 to 2 (Table 2) suggests that the architecture of the sodium caseinate aggregates modified by surfactants becomes generally more open despite the assumed shrinking of their constituent protein nanoparticles.

On the strength of the combination of data from mixing calorimetry and laser light scattering, a schematic sketch of the molecular mechanism of the protein−surfactant interaction can be proposed. As an example of the most common case, Figure 5 shows this mechanism for the case of sodium caseinate with CITREM.

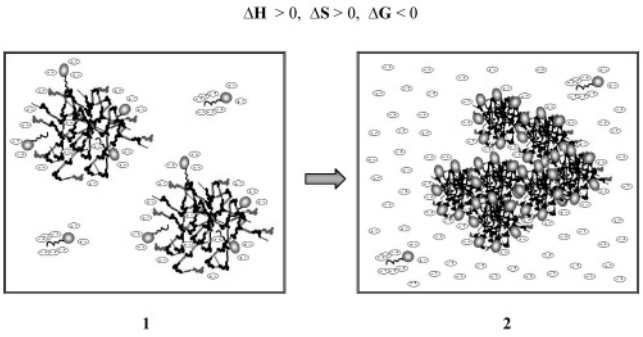

Figure 5. A schematic sketch of the molecular mechanism of the protein−surfactant interaction for the case of sodium caseinate (C = 0.5% w/v) with CITREM (C = 5 mg L^{-1}) in an aqueous medium (pH 7.2, ionic strength 0.05 M) at 293 K. The first picture shows only the water molecules bound with polar groups of both protein and surfactant as well as the water molecules structured as a result of the hydrophobic hydrotation that occurs around the hydrocarbon chain of the surfactant. The second picture demonstrates the release of the bound and structured water molecules as a result of the predominantly hydrophobic interactions between the protein and the surfactant. The free water molecules are not shown.

Thus, at the beginning (Figure 5, part 1), we have the separate sodium caseinate nanoparticles with the specific size (R_G = 117 nm) and the thermodynamic affinity for the aqueous medium (A_{pr-pr} = 0.93 m^3 mol^{-1}) possessing the spherical architecture as indicated by the value close to unity of the structure-sensitive parameter, ρ. Ultimately (Figure 5, part 2), the protein aggregates with high hydrophilicity (A_{pr-pr} = 188 m^3 mol^{-1}) were formed because of hydrophobic interactions predominantly revealed between nonpolar parts of the surfactant and the protein (Figures 2 and 3), followed, most likely, by the location of the polar heads of the surfactants primarily at the surface of the protein aggregates. As this takes place, the contracted protein nanoparticles modified by the surfactant are combined into the new complex protein plus surfactant nanoparticle with both greater size and architecture more open than hard sphere ($\rho = 2$). The main driving factor of the interactions may be the increase in the entropy of the system ($\Delta S > 0$) due to the release of a great number of water molecules, caused by the dehydration of both surfactant and protein molecules under their interactions.

We would like to note here that the information on the effect of surfactants on protein self-aggregation in aqueous medium

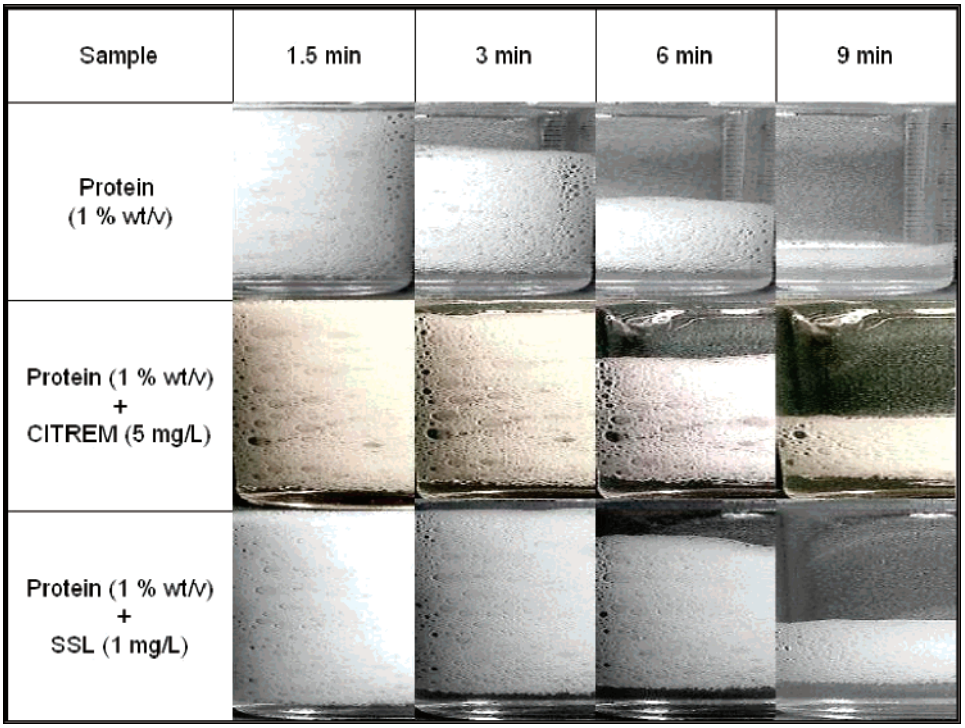

Figure 6. The effect of the anionic surfactants on the time stability of the foams stabilized by sodium caseinate (C = 1% w/v) at 293 K (pH 7.2, ionic strength 0.05 M).

is very scarce in the literature,[1−6,18,43] which in our opinion hinders a deeper insight into the molecular mechanisms of the effects of surfactants on the well-studied structural−functional properties of food proteins. Most likely, more systematic studies on this subject are required in order to get this information.

Modification of the Foaming Abilities of the Proteins by the Interactions with Individual Surfactant Molecules. The impact of the protein aggregation and the increase in the thermodynamic affinity of the surfactant-modified protein nanoparticles for an aqueous medium on the protein functionality can be most clearly illustrated with the example of sodium caseinate in the presence of anionic surfactants. Thus, such a modification of the protein nanoparticles (Table 2) leads generally to the longer lifetime of the protein foams (Figure 6) due to, most likely, both strengthening of the steric characteristics of the protein adsorbed layers and the rise in their hydrophilicity that is favorable to the confinement of water between air bubbles. Because it is well-known that the overall foam destabilization (the half-life time of the foams) and the specific destabilization processes (drainage, coarsening, and coalescence) are related primarily to the interfacial characteristics [protein concentration at the interface (thickness); protein−protein and protein−surfactant attractions (network formation), interfacial shear and dilatational characteristics (viscoelastic behavior), charge (determining the electrostatic repulsions between bubbles), solvation by the aqueous medium] of the protein film adsorbed around the bubbles.[50−56]

However, it is clear that in order to confirm properly our speculations on the foaming ability of the protein plus surfactant complexes further investigations are required.

It is necessary to note here that the surfactants at the studied concentrations are not able to form stable foams at all.

Features of Both the Protein−Surfactant Interactions and Protein Molecular Modification above the Surfactant cmc. When we deal with surfactants, one of the main questions is, what is the impact of the micellar state of the surfactants on the character of their interactions with protein and, further, on the protein molecular properties and functionality?[57] Previous study shows that proteins, depending mainly on their charge and size, can be solubilized in a nonpolar media, fractionated, and purified by incorporation into the interior of reverse micelles.[57] Measurements of protein structure show that solubilization usually causes little or no denaturation of protein molecules if the interior of a reverse micelle is able to accommodate a protein molecule without having to alter its optimum size significantly.[57]

It is believed also that the following intrinsic features of the surfactant micelles can be suggested as important for their interactions with proteins: (i) their thermodynamic stability, which evidently could be expressed in terms of the ΔG_{mic} and could be favorable or not to the participation of the hydrophobic "core" of the micelles in the interaction with protein, (ii) the net charge and associated relative hydrophilic/lipophilic properties of the micellar surface,[5] (iii) the size of micelles. The two latter factors can contribute to the impact of the excluded volume effects on the protein−surfactant repulsive interactions.

A recent mixing calorimetric study postulates that, when both the surfactant micelles and the protein carry a rather high like net charge, then the micelles cannot take part in the interactions with the proteins on account of the strong electrostatic repulsions between them. Hence, in this case, the interactions occur only between the protein and the individual surfactant molecules, which are in equilibrium with their micelles. This is reflected in the lack of any changes of the character of the protein−surfactant interactions in going through the cmc, as was found for the interactions of both sodium caseinate and native legumin with the anionic surfactants, namely CITREM and SSL, at pH 7.2.

In contrast, under the experimental conditions, which are favorable to the decrease in the like net charge of the interacting protein and surfactant micelles, the dramatic changes in the character of the interactions of the proteins with the surfactants, occurring above the cmc, postulate the participation of the surfactant micelles in the interactions with proteins. Let us

Table 5. Molecular and Thermodynamic Parameters of the Protein + Surfactant Complexes Formed as a Result of the Interactions of Sodium Caseinate with Micelles of the Anionic Surfactants, in an Aqueous Medium (pH 5.5, ionic strength 0.05 M) at 293 K[a]

system	$C_{surfactant}$ (mg L^{-1})	M_w 10^{-6} (Da)	k_1[b]	A_{pr-pr} 10^5 (m^3 mol kg^{-2})	A_{pr-pr} (m^3 mol^{-1})	R_G (nm)	k_2[b]	$\rho = R_G/R_h$
				Sodium Caseinate				
protein without surfactant	0	15		2.0	9	200		2.2
protein + CITREM	500	123	8.2	1.7	517	186	0.93	0.6
protein + SSL	100	18	1.2	5.9	38	187	0.94	1.4

[a] The experimental error in the determinations of M_w, A_{pr-pr}, and R_h was estimated as ±10%. The error in the R_G determination was ±5%. [b] k_1 is the extent of the protein aggregation $k_1 = M_w^{protein+surfactant}/M_w^{protein}$; k_2 is the extent of the increase in the size of the protein aggregates $k_2 = R_G^{protein+surfactant}/R_G^{protein}$.

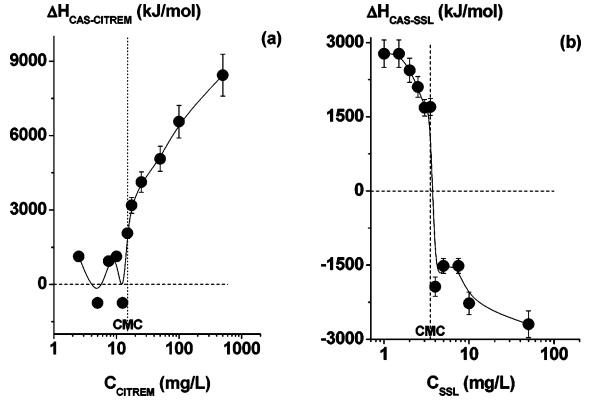

Figure 7. The effect of the molecular state of the surfactants on the character of their interactions with sodium caseinate (0.5% w/v) in an aqueous medium (pH 5.5, ionic strength 0.05 M) at 293 K for (a) CITREM and (b) SSL.

consider some features of such participation in more detail by the example of the interactions of the anionic surfactant micelles with sodium caseinate at pH 5.5 (Figure 7).

In the case of CITREM, we have found a sharp increase in the endothermic heat effect of the interactions between CITREM and protein (Figure 7a). This result could be most likely attributable to the disruption of the surfactant micelles under the interactions with the protein followed by a simultaneous release of a great number of surfactant molecules that increases the number of hydrophobic protein−surfactant contacts significantly. As a consequence of this, the intensive inter- and intramolecular protein cross-linking occurs that leads to the formation of large protein aggregates with much higher hydrophilicity and more compact structure and spherical architecture as compared with the protein alone (Table 5).

In the case of SSL, the dramatic change in the character of the protein−surfactant interactions from endothermic to exothermic also suggests the involvement of the micelles in the interactions and, in addition, that the micelles are probably involved as a single whole (Figure 7b). As this takes place, the predominant interactions between nonpolar parts of the protein and the surfactants transfer to interactions between their polar groups primarily.

These results are in good accord with the greater negative value of the Gibbs free energy of formation of the micelles in the case of SSL ($\Delta G_{mic} = -6$ kT) in comparison with the micelles of CITREM ($\Delta G_{mic} = -4$ kT) that points to the higher thermodynamic stability of the former.[5]

When micelles are involved as a single whole in the interactions with the protein, as in the case of SSL, the molecular parameters of the original protein particles do not change significantly (Table 5). There is no pronounced protein cross-linking, while there is an apparent addition of the hydrophilicity of the micellar surface to the original protein surface (Table 5). Most likely, the additional charge added to the protein from the attached micelles prevents the further aggregation of the protein nanoparticles because of the increasing electrostatic repulsions between them.

A schematic sketch of the molecular mechanism of the interactions between protein and surfactant micelles (Figure 8) can be proposed, as an example, on the basis of the combination of the data from mixing calorimetry (Figure 7) and laser light scattering (Table 5).

In addition, to gain a more penetrating insight into the mechanism of the interactions of the ionic surfactant micelles as a single whole with the protein, let us consider in more detail the interactions of sodium caseinate with the very stable thermodynamically micelles of the soy phospholipids ($\Delta G_{mic} < -23$ kT) under changes of pH. These micelles are defined as zwitterionic liposomes in the range of the pH studied, i.e., from 7.2 to 5.0.[20]

First and foremost, let us note that the pronounced aggregation of the sodium caseinate nanoparticles was induced by the pH lowering from neutrality (pH 7.2) toward the protein isoelectric point (p*I* 4.8), and as a result, the protein nanoparticles, differing structurally, were formed (Table 6).[6,58] With this taken into account, under the construction of the binding isotherms for the binding of phospholipids to sodium caseinate, the binding extent, ν, was calculated as the number of moles of phospholipids (a ligand) bound per mole of protein nanoparticles originally existing at pH 7.2 and being considered as the building blocks of the protein aggregates formed with the pH lowering from neutrality to acidic values (Figure 9). The shift of binding isotherms to higher concentrations of a free ligand postulates an essential decrease in the binding affinity of the protein building blocks relative to the liposomes of phospholipids with the lowering pH from 7.2 to 5.0.[6] This result infers a significant contribution of the electrostatic interactions between the opposite charges of the protein and the phospholipids into their binding affinity for each other in an aqueous medium at room temperature.

This supposition is in good accord with the predominantly exothermic character of the interactions between them revealed by mixing calorimetry at the same temperature (293 K) and neutral pH (6.0 and 7.0), in particular.[6]

In contrast, when the binding extent, ν, was calculated as the number of moles of phospholipids bound per mole of protein existing at each pH, i.e., with regard to their weight average molar mass, M_w, (Table 6) the significant rise in the binding extent, ν, was found with the pH lowering. In other words, the larger the protein aggregates and the number of binding sites in the interior of the protein nanoparticles are, the higher the binding extent of the liposomes of phospholipids relative to the sodium caseinate nanoparticles.[6]

On the strength of the light scattering data, obtained at pH 7.2 and 293 K, i.e., under the conditions of the highest binding

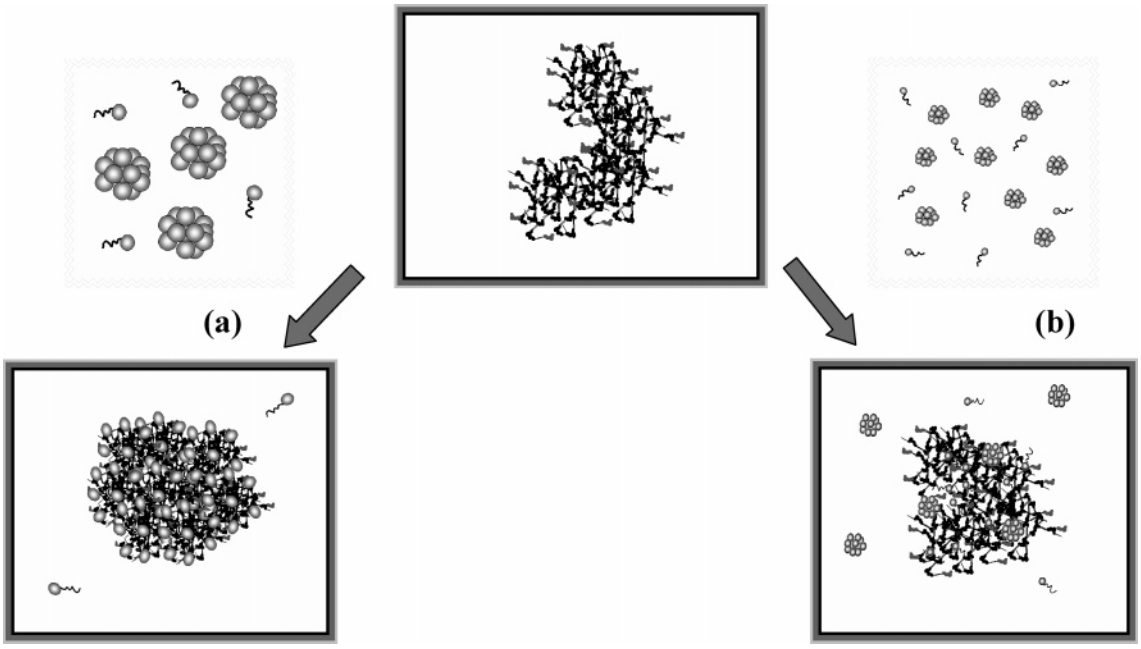

Figure 8. A schematic sketch of the molecular mechanism of the interactions of protein with surfactant micelles for the case of sodium caseinate (C = 0.5% w/v) in an aqueous medium (pH 5.5, ionic strength 0.05 M) at 293 K for (a) CITREM (C = 500 mg L^{-1}) and (b) SSL (C = 100 mg L^{-1}).

Table 6. Effect of the Phospholipids (10^{-5} M) on the Molecular and Thermodynamic Properties of the Sodium Caseinate Nanoparticles at Different pH Values (ionic strength 0.01 M, T = 293 K)[a]

	sodium caseinate nanoparticles					sodium caseinate nanoparticles with phospholipids (10^{-5} M)				
pH	M_w 10^{-6} (Da)	A_{pr-pr} 10^5 (m^3 mol kg^{-2})	A_{pr-pr} (m^3 mol^{-1})	R_G (nm)	$\rho = R_G/R_h$	M_w 10^{-6} (Da)	A_{pr-pr} 10^5 (m^3 mol kg^{-2})	A_{pr-pr} (m^3 mol^{-1})	R_G (nm)	$\rho = R_G/R_h$
7.2	4	2.9	0.93	117	1.1	14	596	2335	177	1.7
6.0	16	4.7	24	211	1.9	6	58	42	147	1.3
5.1	1023	8.9	18.6×10^4	357	4.6	11	119	287	70	0.7

[a] The experimental error in the determinations of M_w, A_{pr-pr}, and R_h was estimated as ±10%. The error in the R_G determination was ±5%.

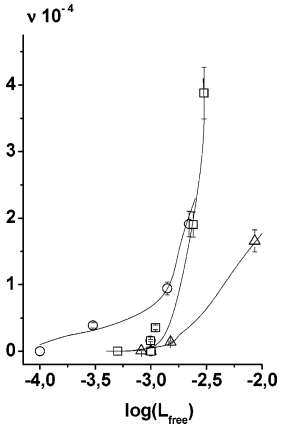

Figure 9. The effect of pH on the binding isotherms of the phospholipids to the sodium caseinate nanoparticles (C = 0.5% w/v) (ionic strength 0.01 M, T = 293 K); the binding extent, ν, is plotted as a function of the logarithm of a free ligand concentration in the system, log(L_{free}): (○) pH 7.0, (□) pH 6.0, and (△) pH 5.0.

affinity of the liposomes of phospholipids for the protein, it may be safely suggested that the liposomes can play an important part as the efficient cross-linking compound for the protein. As a result of this, the protein aggregation was revealed (Table 6). This aggregation was followed by the pronounced increase in the thermodynamic affinity of the protein plus phospholipids complexes for the aqueous medium (Table 6) that could be attributable to the addition of hydrophilicity from the attached liposomes of phospholipids to the protein.

In contrast, at lower pH (6.0 and 5.1), when the original protein nanoparticles are more associated (Table 6), the attachment of the liposomes of phospholipids to the protein leads to the dissociation of the protein nanoparticles most likely owing to electrostatic repulsions between the like-charged phospholipid liposomes closely added to the protein (Table 6). As this takes place, the protein disaggregation is the most dramatic at pH 5.1, i.e., in the close vicinity of the protein isoelectric point. In this case, a great number of less hydrophilically modified protein nanoparticles is formed (see the values of A_{pr-pr} in mole units in Table 6).

In addition, recently the calorimetric data postulate the micelle-like cluster formation in the interior and/or at the surface of the sodium caseinate nanoparticles at the concentrations of the surfactants in close vicinity of their cmc.[4−6] Earlier, it was established that surfactants can actually bind to the protein cooperatively, forming the micelle-like clusters rather than noncooperatively as monomers.[59,60] As this takes place, the micelle-like clusters formed in the protein interior tend to be smaller than the free surfactant micelles[60] and postulate the importance of the effect of the specific features of the protein interior for the character of the surfactant micellization.[5] Evidently, to gain greater insight into the impact of this kind of surfactant micellization on the molecular properties of proteins and their functionality, further systematic investigations are required.

Efficiency of the Modifications of the Protein Foaming Ability by the Interactions with Surfactant Micelles. As a

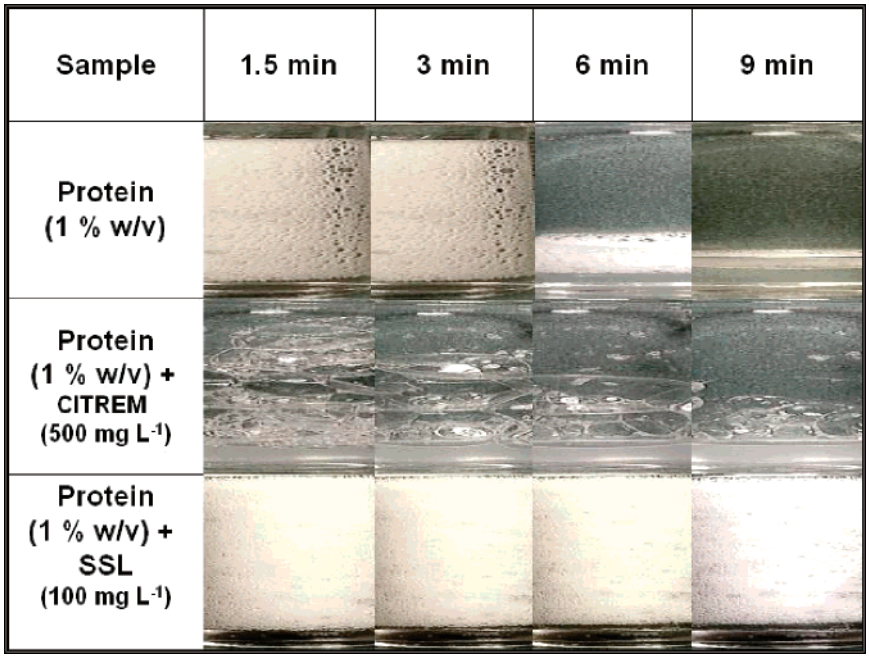

Figure 10. The effect of the anionic surfactant micelles on the time stability of the foams stabilized by sodium caseinate (C = 1% w/v) (pH 5.5, ionic strength 0.05 M) at 293 K.

result of the related modification (Tables 5 and 6), the newly formed protein nanoparticles perform the following structural functionality, as illustrated by the example of sodium caseinate:

(i) The pronounced protein aggregation in the case of CITREM can lead evidently to a significant decrease in the number density of the complex protein nanoparticles, which could form the protective adsorbed layers at the air bubbles. As a consequence, stable foams cannot be formed[50] (Figure 10). On the other hand, the negative contribution from the competitive adsorption of CITREM to the foam time stability can be also expected,[61] because it is necessary to point out that the surfactants alone are not able to stabilize the foams at all at the studied concentrations.

(ii) In contrast, in the case of SSL when the number density of the modified nanoparticles does not practically change while their hydrophilicity and consequently the confinement of the water by the protein adsorbed layers increases,[50,53] the time stability of the foams also increases dramatically (Figure 10).

(iii) The foams stabilized by the sodium caseinate nanoparticles modified by phospholipid liposomes show a dramatic increase in their time stability as compared to the pure protein foams throughout the pH range studied (Figure 11).

As to the pure phospholipids, we should note that they do not give the fine and stable foams at all at the concentrations presented under the experimental conditions. The origin of the synergistic effect found for the mixtures of the phospholipids with sodium caseinate dramatically depends on pH. At pH 7.2, it is mainly attributable to the strengthening of both the steric characteristics of the protein adsorbed layers at the air bubbles and their hydrophilicity, most likely because of the protein aggregation and the pronounced increase in the thermodynamic affinity of the complexes for the aqueous medium, respectively.[50−56]

In contrast, at the pH lowering, the origin lies in the pronounced protein disaggregation that leads to the formation of a great number of the more (pH 6.0) or less (pH 5.1) hydrophilic protein-based particles, and that could also lead to the higher protein adsorption at the air−water interface and hence to better steric parameters of the adsorbed protein layers at the air bubbles.[52,62]

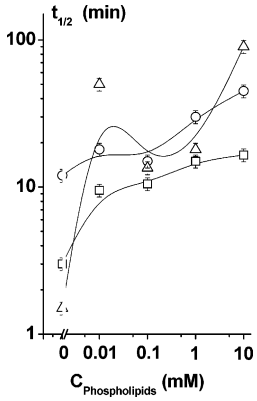

Figure 11. The effect of pH on the time of the half-life, $t_{1/2}$, of the foams stabilized by the sodium caseinate (1% w/v) in the presence of the different concentrations of phospholipids: (○) pH 7.0, (□) pH 6.0, and (△) pH 5.0.

Conclusions

On the strength of the experimental data presented, we could conclude that the molecular parameters and structural functionality of the protein nanoparticles could be dramatically modified by the interactions with surfactants in the aqueous medium that might provide a good basis on which both novel functionality of active food ingredients and novel delivery systems could be designed. In addition, an advantage in using the thermodynamic approach is a more penetrating insight into the molecular mechanism of such a modification that can offer the opportunity for its prediction, regulation, and accomplishment of the desirable molecular design under storage and different processing conditions. Further, more systematic research seems necessary, however, to understand the impact of the changes in the surfactant molecular state on the molecular and functional properties of food proteins as a result of their interactions.

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