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ORIGINAL CONTRIBUTION

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Aggregation of sodium dodecyl sulfate in micellar solution of β-casein analyzed by ^{1}H NMR self-diffusion, relaxation and Fourier transform IR spectroscopy

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Abstract Micellization of anionic sodium dodecyl sulfate (SDS) in the presence of β-casein (β-CN) micelles in aqueous media was studied by ^{1}H NMR and Fourier transform IR spectroscopy. At low concentrations SDS molecules incorporate into β-CN micelles and modify the protein secondary structure, increasing the portion of helical domains. It was shown that SDS micelles do not appear until binding sites located in the hydrophobic core of β-CN micelles are saturated.

Keywords Sodium dodecyl sulfate · Micellization · β-Casein · NMR self-diffusion · ^{1}H NMR relaxation

Introduction

Studies of interaction between proteins and surfactants are biologically and biotechnologically significant objectives attracting considerable attention in different areas [1, 2, 3, 4, 5]. Surfactants have a broad use in preparative and analytical biochemistry: they stabilize microemulsions, which is important for micellar enzymology, they influence enzyme functions and they modify texture and gustatory properties of edible proteins.

β-Casein (β-CN) is one of the most abundant proteins of foods. A molecule of this phosphoprotein is formed by a single chain of 209 amino acid residues of known sequence [6] and molecular mass of 24,020 Da (Fig. 1). The N-terminal part of β-CN consists mostly of hydrophilic residues and carries nearly all the protein net charge, whereas the remaining C-terminal portion is mainly hydrophobic and is characterized by almost complete absence of charges [7]. Only short fragments of the protein backbone form small local domains of secondary structures [8, 9] providing noticeable flexibility to the β-CN molecule. Owing to its high emulsifying properties resulting from the combination of flexibility and amphiphilicity, β-CN undergoes self-association and forms spherical micelles in dilute aqueous solutions [10] when the protein content exceeds the critical micelle concentration (cmc), which is about 1.5×10^{-3} g cm^{-3} in deuterium oxide at 4.5 °C [11]. It is known [11, 12] that at concentrations up to 1% (w/w) β-CN micelles have an external radius of around 13.5 ± 1 nm with a dense spherical core with radius 5.5–6.5 nm surrounded by a shell of much lower density containing the majority of the hydrophilic residues.

The emulsifying properties of β-CN determine the functional characteristics of multiple dairy food products and facilitate the formation of many edible colloidal systems. For example, the micellar structure of casein aggregates provides high sorption and increases its delivery of physiologically important ingredients such as

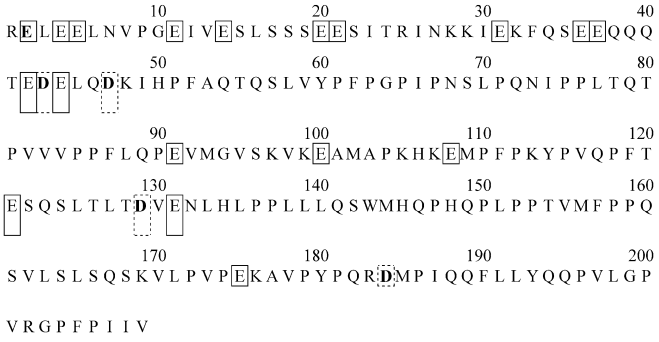

Fig. 1 The sequence of amino acid residues in the chain of the β-casein (β-CN) molecule. Aspartic acid (*D*), glutamic acid (*E*)

vitamins and lipids. The addition of anionic surfactant ligands induces a competitive disruption of hydrophobic protein–protein interactions [13, 14, 15]. In the case of casein, sodium dodecyl sulfate (SDS) can induce changes in the micellar structure, modifying thus the functional properties [16, 17, 18].

The aim of this study was to consider the protein–surfactant interactions from the "other-opposite" side; this means studying the micellization of SDS in β-CN solutions using ^{1}H NMR and Fourier transform (FT) IR spectroscopy. On the one hand, our objective was to use SDS molecules as a probe to check the structure of β-CN micelles. On the other hand, it was to begin studies of polymer–surfactant complexes containing amphiphilic polymer (β-CN). The amphipathy/amphiphilicity of such complexes can be modified easily by changes of temperature, pH, ionic strength, etc.

Experimental

Materials

Micellization of SDS (BDH Chemicals, Poole, UK) was studied in 1% (w/w) water solution of β-CN purified from cow milk according to Zittle and Custer [19]. Deuterium oxide (CEA-ORIS, Bureau des Isotopes Stables, France) was used as the bulk medium in order to weaken the ^{1}H NMR water signal. The protein was dissolved in deuterium oxide and then SDS was added to this stock solution in amounts from 2 to 400 mM. All measurements were performed at 25 °C.

Methods

NMR measurements were performed with a modified Tesla BS 587A spectrometer with a ^{1}H resonance frequency of 80 MHz. A typical NMR spectrum of the system studied is shown in Fig. 2. The spectrometer was equipped with homebuilt field gradient units able to achieve a gradient magnitude up to 0.5 T m^{-1}. The diffusion studies were carried out using the FT–pulsed-gradient spin-echo technique [20, 21]. The pulse sequence 90°–τ–180°–τ–echo was used to determine the self-diffusion coefficients of SDS using the main resonance peak from methylene protons of the surfactant. The spin–lattice relaxation times, T_1, of methylene and methyl protons of SDS were measured by applying the inversion recovery pulse sequence (180°–τ–90°). The proton NMR spectra for the linewidth studies were recorded in the continuous mode. The spectral width was 1.6 kHz with 4,096-points resolution.

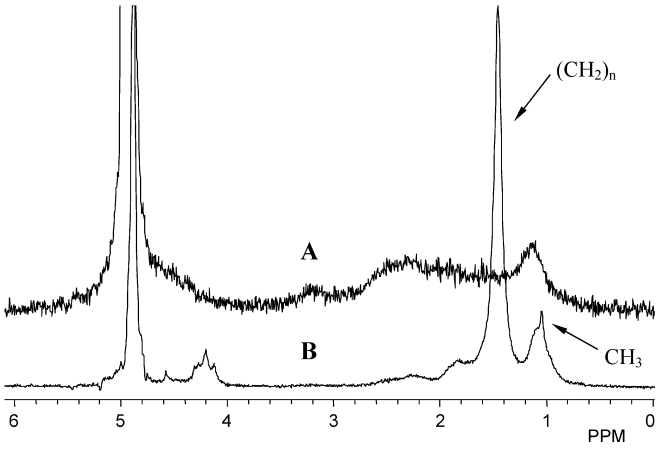

Fig. 2 Proton NMR spectra of **a** 1% β-CN water solution and **b** the sodium dodecyl sulfate (*SDS*)–β-CN system. The signals from SDS methyl and main methylene protons, used for diffusion, relaxation and linewidth measurements, are marked by *arrows*. The difference in the amplification of the spectra is about 5 times

The IR spectra were registered using a Vector-22 FTIR spectrometer (Bruker) in the frequency range 4,000–1,000 cm^{-1} with 4-cm^{-1} resolution and 256 accumulations. The samples were placed in a temperature-controlled demountable CaF_2 cell with 100-μm space gap. The water vapor and solvent absorptions were subtracted from the experimental spectra with subsequent nine-point smoothing by the Savitski–Goley algorithm. All spectra processing, including calculation of the second derivative, was executed using the OPUS program from the spectrometer software. The assignment of the resolved amide I components to the secondary structure features was performed on the basis of the known correlations [22].

Results and discussion

The SDS and water self-diffusion coefficients for different SDS concentrations in the absence and in the presence of 1% β-CN are shown in Fig. 3. In the case of pure SDS solutions one can observe the standard micellization curve (Fig. 3b). At the minimum SDS content the self-diffusion coefficient reflects the translational motion of its monomeric molecules. When the surfactant concentration reaches the cmc at 8–10 mM [23, 24], SDS forms micelles, which are characterized by much lower self-diffusion coefficients in comparison with the monomeric molecules. As a result of fast (on the NMR experiment time scale) exchange of surfactant molecules between micellar and molecular states, the experimentally observed SDS self-diffusion coefficient can be expressed as

$$D = P_{\mathrm{mon}} D_{\mathrm{mon}} + P_{\mathrm{mic}} D_{\mathrm{mic}}, \quad (1)$$

where D_{mon} and D_{mic} are self-diffusion coefficients of SDS monomers and micelles, respectively; P_{mon} and

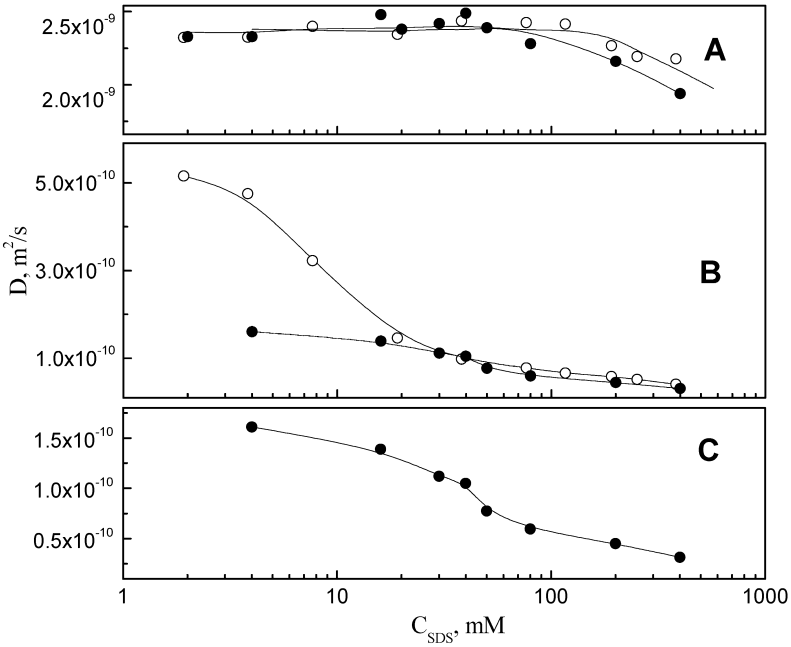

Fig. 3 Self-diffusion coefficients of **a** water and **b**,**c** SDS in the absence (*open symbols*) and in the presence (*closed symbols*) of β-CN. Clearer details of the SDS micellar transition with β-CN present are shown in **c**

P_{mic} are corresponding fractions. When the SDS concentration exceeds the cmc all additional surfactant molecules take part in the formation of micelles, resulting in a steep increase in P_{mic} since $P_{mon} + P_{mic} = 1$.

When β-CN is present one can also observe stepwise behavior of the SDS self-diffusion concentration dependence (Fig. 3c); however, some basic differences should be highlighted. In the low-concentration domain the surfactant self-diffusion coefficient is much lower than in the β-CN free system and its decrease in the transition zone, being shifted towards higher SDS concentrations, is not so radical (Fig. 3b). What could be the most probable reasons for these distinctions? The decrease in the SDS self-diffusion coefficient at low concentrations cannot be a consequence of the increase in the medium viscosity when protein is present. Indeed, small changes in water diffusion for two systems can be observed (Fig. 3a). The obstruction effect produced by β-CN micelles (about 1% of the volume) cannot induce a threefold decrease of the SDS self-diffusion coefficient either. Most probably the distinctions are due to the binding of a considerable amount of SDS with the micelles of β-CN. Because of the large width and low amplitude of the peak assigned to the mixture of all β-CN protons at a resonance frequency of 80 MHz (spectrum a in Fig. 2) it is impossible to determine the self-diffusion coefficient of protein micelles in our experiment. The small-angle neutron scattering data [12] show that the self-diffusion coefficient of β-CN micelles is approximately equal to 2×10^{-11} m^2 s^{-1} for an average micelle radius of about 10 nm. Under the assumption that at low SDS concentrations surfactant is distributed between β-CN micelles ($D = 2\times10^{-11}$ m^2 s^{-1}) and the monomeric state ($D = 4.9\times10^{-10}$ m^2 s^{-1} in 4 mM SDS solution), on the basis of an equation similar to Eq. (1) it is possible to estimate that for an SDS concentration of 4 mM about 70% of its molecules are bound to casein micelles, which corresponds to seven SDS molecules per molecule of β-CN. Apparently, it is energetically advantageous for SDS molecules to aggregate with β-CN, forming mixed micelles, rather than to exist in the monomeric state in aqueous media.

The concentration dependence of the SDS self-diffusion coefficient in β-CN micellar solution reflects the complicated character of surfactant redistribution between the three possible states: monomers, SDS micelles and mixed micelles formed by surfactant and β-CN giving the measured self-diffusion coefficient:

$$D = P_{mon}D_{mon} + P_{mic}D_{mic} + P_{cs}D_{cs}, \quad (2)$$

where D_{cs} is the self-diffusion coefficient of mixed micelles formed by β-CN and SDS molecules and P_{cs} is the portion of SDS bound to these micelles. When the saturation of β-CN micelles by SDS is reached, all additional SDS molecules remain monomeric. A further increase of SDS concentration in the system raises the concentration of free surfactant to cmc_1 and one can observe a micellar transition at an SDS concentration of 40–50 mM.

In analyzing the results of nuclear magnetic relaxation, it is necessary to remember that as in the case of self-diffusion, the relaxation parameters are the sum of weight-average contributions from protons of SDS molecules located in different aggregation states: monomers, SDS micelles and mixed micelles of SDS and β-CN.

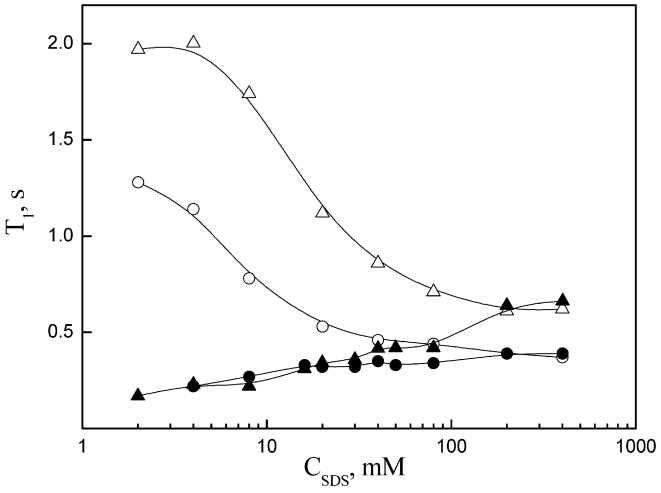

Fig. 4 ^{1}H NMR T_1 values for methyl (*triangles*) and main methylene (*circles*) protons of SDS in the absence (*open symbols*) and in the presence (*closed symbols*) of β-CN

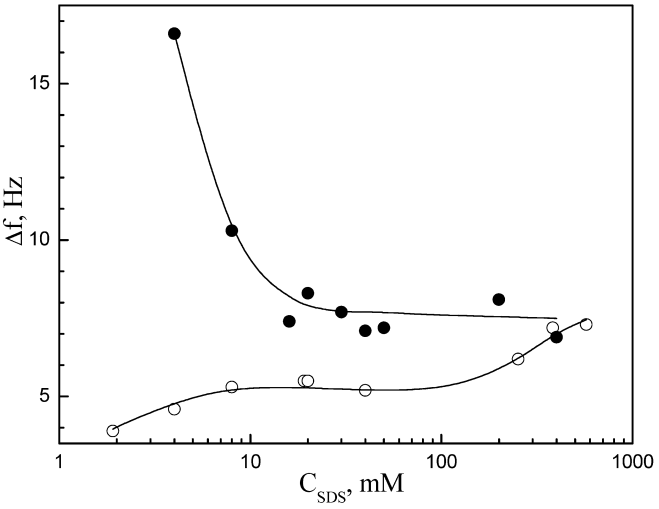

Fig. 5 Half-width of the signal of the main methylene protons of SDS in the absence (*open circles*) and in the presence (*closed circles*) of β-CN

The T_1 concentration dependence, observed for the SDS methyl and main methylene protons in water solution (Fig. 4), depicts the typical micellization process. The inflexion points coincide exactly with the self-diffusion data, particularly for methylene protons of SDS, and reflect the changes in the size of the molecular aggregates. The T_1 values for these protons in the β-CN–SDS system indicate that at low surfactant concentrations almost all SDS molecules interact with large β-CN micelles. As the surfactant concentration increases, redistribution of the portions of surfactant in different states takes place and only at SDS concentrations exceeding 200 mM the contribution from SDS bound to β-CN micelles becomes negligible.

The resonance line of the main methylene protons consists of several signals with a small chemical shift difference, and the alteration in its width cannot easily be separated from changes in the chemical shift. Although the half-width of this signal does not give an exact description of spin–spin relaxation it gives a qualitative understanding of changes in the size of the micellar aggregates [25]. The initial broadening of the resonance signal of the main methylene protons in aqueous SDS solutions (Fig. 5) can be mainly explained by the formation of SDS micelles and to some extent by the changes in the packing of surfactant alkyl chains during the micellization process. Beyond the cmc one can see a region with the unchanged structure of SDS micelles. At higher concentrations one can observe changes in the form and/or in the size of SDS micelles. When β-CN is present at low surfactant concentrations the half-width indicates (Fig. 5) that most of the surfactant molecules tumble with large-scale aggregates, the β-CN micelles being the large ones. During the increase in the surfactant concentration, the contribution from

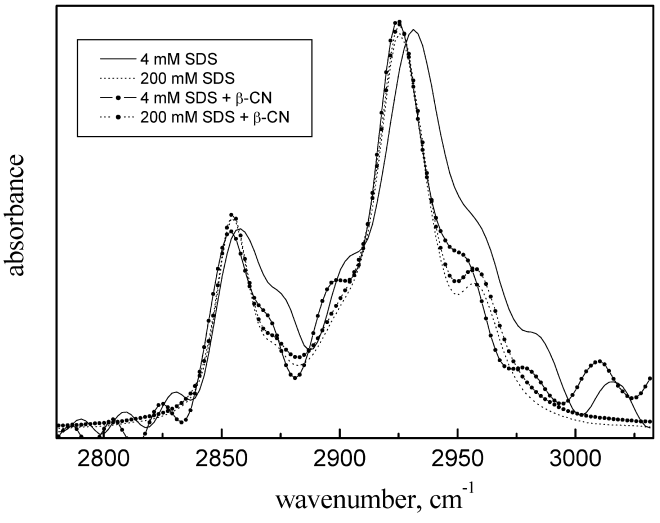

Fig. 6 Absorbance spectra of SDS alkyl chains normalized to the optical density at the maximum of the band at 2,925 cm^{-1} (ν_{as} CH_2)

SDS molecules bound to casein micelles is lowered at the expense of the contribution to the growth of the SDS micelles.

IR spectroscopy was used to study surfactant solutions at two concentrations: one of them (4 mM) being below the cmc and the other one (200 mM) being essentially higher. The environments of the SDS molecules at these concentrations are different as judged from the spectral band positions of its alkyl groups (Fig. 6). SDS (4 mM) shows a high-frequency position (2932 cm^{-1}) of the most intensive CH_2 stretching band, which is characteristic of a polar environment [26]. The observed low-frequency shift in the case of 200 mM SDS suggests a transition of its CH_2 groups to a more

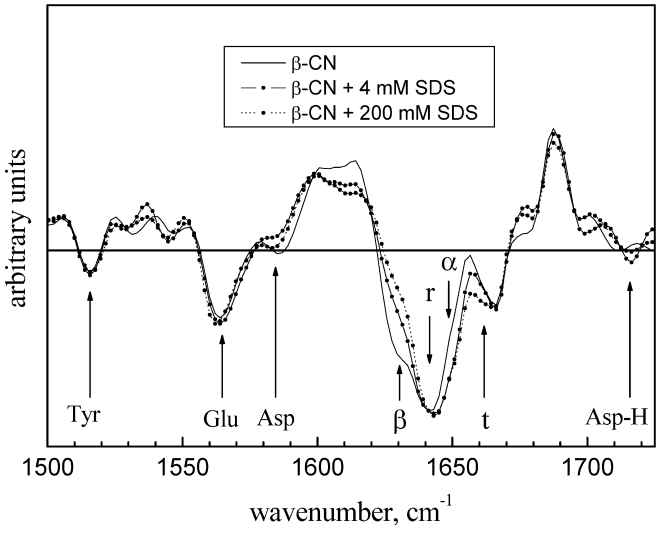

Fig. 7 The second derivative of the β-CN spectrum in the range of amide I absorbance. The *symbols* point to the wavelengths typical of the following protein secondary structures: β β-extended, α α-helix, *r* random, and *t* β-turn

hydrophobic and tightly packed environment, i.e. the surfactant micellization. Addition of protein results in an approximately identical hydrophobic environment for alkyl chains for both SDS concentrations with little difference from the hydrophobic core of the SDS micelles. These data, as well as NMR results, indicate that even at minimal concentrations SDS is bound to β-CN micelles, presumably in their hydrophobic core.

β-CN in deuterium oxide is predominantly in a random conformation with the amide 1 band centered on 1,641 cm^{-1} [22] (Fig. 7). The considerable portion of β-turns at 1,662 cm^{-1} can also be attributed to the disordered structure and high content of prolines. Besides, a small but distinct amount of extended structure is seen at 1,630 cm^{-1}, which can be attributed either to the presence of extended loops in the molecule of β-CN or to regular β-structures. The addition of 4 and 200 mM SDS shifts progressively the visible maximum of the amide 1 band to higher frequencies. The second-derivative analysis (Fig. 7) reveals a decrease in the content of the extended structure and an increase in the absorbance at 1,652 cm^{-1}, typical of a helical structure, which is in agreement with the known tendency of proteins to become more helical in less polar media [27].

SDS induces the decrease of the absorbance of ionized carboxylic groups (COO^-) of the β-CN aspartic acid residues (1,585 cm^{-1}). It is noteworthy that, at the same time, the glutamic acid absorbance (1565 cm^{-1}) remains unchanged. Taking into account that aspartate residues are mainly located in the hydrophobic region of the β-CN molecule whereas glutamates are mostly in the hydrophilic N-terminal region (Fig. 1), one may deduce that SDS is bound preferably in the hydrophobic core of β-CN micelles, where C-terminal parts of this protein should be particularly abundant.

On the one hand, β-CN–SDS behaves as an ordinary uncharged polymer–detergent system, where polymer–surfactant complexes are formed well below the cmc [24, 28, 29, 30]. On the other hand, there is no evidence for self-aggregation of SDS below the cmc when β-CN is present or for the formation of SDS micelles bound to the biopolymer chain. The main specificity of the system studied lies in the self-association of amphipathic β-CN molecules. The hydrophilic residues in the outer shell of the β-CN micelle, bearing nearly all the protein negative charges [7], do not allow anionic SDS to bind in this domain because of electrostatic repulsion. The results of the present study demonstrate that incorporation of SDS into the hydrophobic core of the β-CN micelles is taking place. The binding of SDS by β-CN involves hydrophobic interactions of the surfactant alkyl chains with nonpolar amino acid residues of the protein and Coulombic interactions of SDS with small amounts of positive charges placed in the C-terminal part of the protein molecule. As can be seen in Fig. 1 the positive charges in the N-terminal part of β-CN are neutralized by the neighboring negative charges harbored by aspartate, glutamate and phosphorylated serines.

Conclusions

The addition of β-CN to SDS water solutions results in the shift of the surfactant micellization curve towards higher concentrations in comparison with pure SDS aqueous solutions. SDS is bound mostly in the hydrophobic core of β-CN micelles and modifies the protein secondary structure, increasing the proportion of helical domains. Only after the saturation of binding centers in β-CN micelles by surfactant (SDS) SDS molecules can form micellar structures by themselves.

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