Biophysical Chemistry 136 (2008) 67–73

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

pH-dependent structures and properties of casein micelles

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ARTICLE INFO

Article history:
Received 15 February 2008
Received in revised form 30 March 2008
Accepted 30 March 2008
Available online 4 April 2008

Keywords:
Casein micelle
pH
Structure
Electrostatic interaction
Fluorescence

ABSTRACT

The association behavior of casein over a broad pH range has first been investigated by fluorescent technique together with DLS and turbidity measurements. Casein molecules can self-assemble into casein micelles in the pH ranges 2.0 to 3.0, and 5.5 to 12.0. The hydrophobic interaction, hydrogen bond and electrostatic action are the main interactions in the formation of casein micelles. The results show that the structure of casein micelles is more compact at low pH and looser at high pH. The casein micelle has the most compact structure at pH 5.5, when it has almost no electrostatic repulsion between casein molecules.

1. Introduction

Biomaterials based on biologically derived polymers, including peptides, protein, lipids, and oligonucleotides [1–4], have been shown to exhibit properties beneficial to food, cosmetic and medical science and will have significant implications in advances of future generations of materials.

In milk the most abundant protein, casein, is easily available in a high purity at a low cost with several possibilities of technological applications. These applications arise from the multifunctional properties of casein that depend on the supramolecular structure. The casein constituents, α_{s1}-, α_{s2}-, β-, and κ-casein, exist in proportions of approximately 4:1:4:1 by weight. Approximately, caseins can be thought of as amphiphilic block copolymers consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues [5,6]. Caseins exhibit a strong tendency to self-assemble into casein micelles because of its amphiphilic property in aqueous solution.

The mechanism of casein assembly has been the subject of much speculation, and the casein micelle structure is not yet established up to now. Various models of casein micelle structure have been proposed over the last 50 years [7–10]. Among them, two conflicting models for the internal structure of casein micelles have arisen. The submicelle model emphasized the role of hydrophobic interactions in giving rise to submicelles. However, the other model relies solely on the interactions between the caseins and calcium phosphate to hold the micelle together. In the later model, the calcium phosphate is in the form of nanoclusters and the interaction sites on the caseins are the phosphoseryl clusters of the calcium-sensitive caseins (e.g. αs1- and β-casein).

It is well-known that microenvironment such as pH, salt and surfactant can significantly alter the behavior and overall performance of biomolecules [11–16]. In earlier researches, numerous techniques have been employed to study the effect of pH on the properties of casein micelles, including viscosity measurement [17,18], particle size analyzer [19,20], dynamic light scattering [21,22], zeta potential measurement [18] and turbidity measurement [23]. These studies, however, have sometimes produced conflicting results, and the effect of pH on the properties of casein micelle remains unclear. Two explanations can be offered for the inconsistencies among those studies. First, in some of the studied milk system, the presence of other constituents in milk often makes the studied system become complicated, and the effect of pH on the complex of whey protein and casein is actually studied. Second, the concentration of casein, temperature and the ionic strength all affect the properties of casein micelles, which also makes the effect of pH on the casein micelle more complex.

As we all know, fluorescent molecules are useful probes for sensing the microdomain property of polymeric systems [24–27]. Recently, we have studied the effect of surfactant on the structure and property of casein micelles mainly by fluorescence spectra from molecular level [14,15]. In this article, we study the self-assembly process of casein and the effect of pH on the properties of casein micelle by means of

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0301-4622/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.bpc.2008.03.012

fluorescence measurement (including intrinsic fluorescence spectra and fluorescence spectra of extrinsic probes such as pyrene and 1-anilino-8-naphthalenesulfonate (ANS)) together with DLS and turbidity measurements. Therefore, we can obtain detailed and deep-going information of the casein micelle structure and the structure–property relationship of casein micelles at different pHs on molecule level, which will broaden the application range of casein in food, cosmetic and medicine domains.

2. Materials and methods

2.1. Materials

Casein was purchased from Sigma (90%). Pyrene and 1-anilino naphthalene-8-sulfonate (ANS) (99%) were obtained from Aldrich. Casein powders were dispersed in Milli-Q water under stirring at 50 °C, and the dispersions were stored at 4 °C overnight to allow complete hydration. Then, the pH was adjusted to desired values by adding appropriate 1 M NaOH and 1 M HCl. The casein solutions were filtered through a Millipore filter with a 2.0 μm pore size and thermostated at 25 °C for at least 0.5 h before use. All other reagents used were of analytical grade.

2.2. Turbidity measurements

Turbidity measurements were carried out with a Shimadzu 1601 PC UV/vis spectrometer. The turbidity of the casein solutions was monitored by the transmittance at 450 nm. A cuvette with 1 cm pathway was used. All the measurements were conducted at (25±0.1) °C.

2.3. Dynamic light scattering (DLS)

Dynamic light-scattering measurements were made at 25.0±0.1 °C and at a scattering angle of 90° to the incident beam, using an ALV 5022 laser light-scattering instrument equipped with a 22 mW He–Ne laser at 632 nm (JDS model 1145P) in combination with an ALV-5000 digital correlator with a sampling time range of 1.0 μs to 1000 ms. Experiment duration was in the range of 5–10 min, and each experiment was repeated two or more times.

2.4. Steady-state fluorescence

Steady-state fluorescence experiments were performed with a RF-5301 luminescence spectrometer (Japan Shimadzu Company) equipped with a thermostated water-circulating bath. During the experiments, for casein solution, the excitation and emission slits were fixed at 3.0 and 1.5 nm, respectively, the excitation wavelength was set at 295 nm, and the emission spectra were collected from 300 to 500 nm. In all the measurements, the scan rate was selected at 240 nm/min.

Pyrene was used as the probe to determine the microenvironmental polarity of casein micelles by observing its fluorescence fine structure. The emission spectra were measured in the range of wavelength 350–600 nm with the excitation wavelength being 338 nm. The pyrene concentration was 1.0×10^{-6} M.

The ANS emission spectra were measured in the range of wavelength 400–560 nm with the excitation wavelength being 380 nm. The ANS concentration was 1.0×10^{-5} M.

3. Results and discussion

3.1. The formation of casein micelles probed by pyrene fluorescence

Pyrene has a very low solubility in water (7.0×10^{-7} M), and as a result, it is selectively solubilized in the hydrophobic region or microphases existing in aqueous medium. The vibration fine structure of its mononer fluorescence spectra in solution makes pyrene an excellent probe of local environment polarity changes. The intensity ratio of the first peak to the third (I_1/I_3) of the fluorescence spectrum of pyrene shows the microenvironmental polarity where the probe exists [28]. The abrupt change of I_1/I_3 as a function of surfactant concentration has been commonly used to determine the critical micelle concentration (cmc) of surfactant solutions. Furthermore, an excited monomer can encounter a ground-state pyrene to form an excimer, which produces a broad band at about 450 nm. The ratio of the maximum emission intensity of the excimer (I_e) to the monomer (I_1) for pyrene, I_e/I_1, can be used to judge the efficiency of excimer formation, which can provide further information about the hydrophobic domain in the micelle.

Fig. 1A shows the fluorescence spectra of pyrene in casein solution with different casein concentrations at pH 7.0. Fig. 1B shows I_1/I_3 and I_e/I_1 of pyrene against casein concentration at pH 7.0. As shown in Fig. 1A, the value of I_1/I_3 decreases gradually with increasing concentration of casein over the wide range of concentration from 0.1–1.0 mg/ml. A plateau in the I_1/I_3 vs casein concentration plots appears beyond 1.0 mg/ml. The concentration 1.0 mg/ml can be considered as the critical micellar concentration (cmc). A significant aspect is the gradual decrease in I_1/I_3 between concentrations 0.1 and 1.0 mg/ml, which is unlike the behavior of typical surfactants such as SDS and CTAB. This gradual decline implies that premicellar aggregates are formed and the aggregation number of the premicelle increases with the casein concentration.

Fig. 1B also shows that I_e/I_1 has a maximum at a particular concentration just below 1.0 mg/ml, and diminishes with increasing concentration of casein. It has been reported that a 2×10^{-6} mol/l ethanol solution of pyrene does not exhibit the excimer emission. However, the results in this study show that, compared with ethanol solution, a lower pyrene concentration (1×10^{-6} mol/l) suffices in these systems for efficient excimer formation. Pyrene molecules can be assumed to be dissolved in hydrophobic microdomains, where the local concentration is much higher than the bulk concentration. The appearance of the maximum excimer emission of pyrene in dilute casein solutions below the cmc shows that premicellar aggregates provide a space where the local concentration of pyrene is much higher than the bulk concentration. The pyrene excimer is considered to have formed when pyrene molecules are close to each other in casein aggregates. When the aggregation number of the premicelles is small enough, the pyrene molecules are in intimate contact in a restricted hydrophobic environment. Above cmc, the micelles grow to an infinitely large size to solubilize the pyrene molecules separate. Consequently, I_e/I_1 decreases at this concentration. The pyrene excimer results here also confirm the formation of submicelles of casein.

The pyrene fluorescence spectra were further used to study the self-assemble behavior of casein in the pH ranges of 2.0–3.0, and 5.5–12.0. Fig. 2A and B shows the ratios I_1/I_3 and I_e/I_1 of pyrene against casein concentration at two typical pHs, i.e. pH 2.0 and 12.0. As shown in Fig. 2, a plateau in the I_1/I_3 and the maximum I_e/I_1 vs casein concentration both appear too, which indicates that the casein micelle is formed with the increase of casein concentration at the two pHs. Fig. 2 also shows that the ratios I_1/I_3 and I_e/I_1 of pyrene differ to some extent at different pHs, which will be discussed deeply in the following section. Similar pyrene fluorescence results also suggest the formation of casein micelles at other pHs (data not shown).

The above results clearly indicate that casein micelles are formed at high pH, even at pH 12.0. Contrary to our results, Vala et al. found that casein micelles were disrupted at pH higher than 9.0 [23]. They suggested that the strong electrostatic repulsive might lead to the dissociation of casein micelles. In our case, the hydrophobic interaction between the hydrophobic portions of casein molecules still plays an important role in the self-assembly of casein molecules, and hence the formation of casein micelles at higher pH. This is in agreement with the results of Zhong et al. [29]. In their research, they indicated

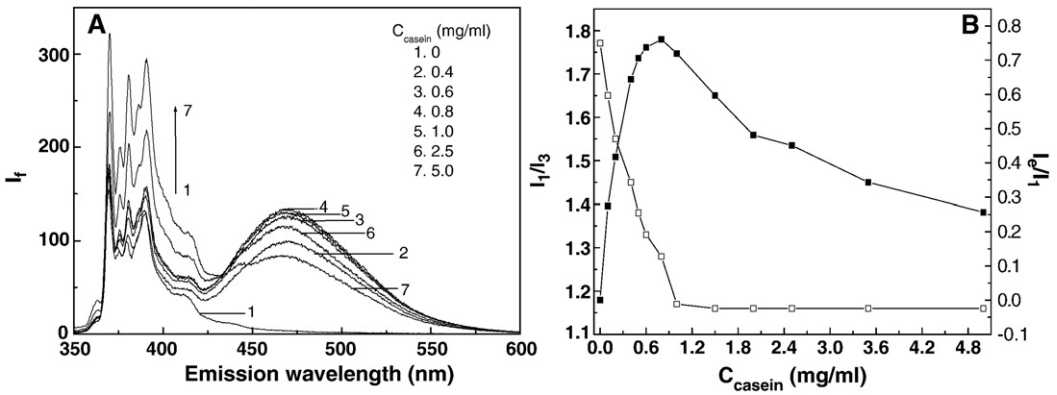

Fig. 1. (A) Fluorescence emission spectra of pyrene (1×10^{-6} M) in casein solution with different concentration. (B) Plots of I_1/I_3 (□) and I_e/I_1 (■) of pyrene against casein concentration.

that casein molecules could self-assemble by the hydrophobic interaction even at pH 12.0.

It is also notable that the calcium phosphate nanoclusters are considered to be an important factor in the formation of casein micelles in some earlier researches. However, the calcium phosphate nanoclusters are mainly dissolved at pH lower than 3.0. Thus, the casein micelles are formed through the hydrophobic interaction between the hydrophobic portion of casein molecules, the hydrogen bond and cation–π interaction at acid condition (as discussed further in the next section). This indicates that the calcium phosphate nanocluster is not a crucial factor in the formation of casein micelles.

3.2. The effect of pH on the casein micelle

3.2.1. Trp fluorescence

It is known to all that the fluorescence of protein usually comes from the tyrosine, tryptophan, and phenylalanine residues. The fluorescence spectrum of protein is sensitive to the microenvironment because of these chromophores and it allows non-intrusive measurements of protein at low concentration under physiological conditions. K_{s1}-casein contains two tryptophan residues at positions 164 and 199, and β-casein has a Trp residue at position 143, respectively. The three Trp residues are mainly located in a primarily hydrophobic portion of casein molecules, and can provide important information about the self-assembly of the casein molecules [30,31].

The fluorescence emission spectra of casein with different pHs excited at 295 nm are shown in Fig. 3A. Fig. 3B shows the influence of pH on the emission intensity and the maximum wavelength of Trp residues. Fig. 3B reveals that the emission intensity increases in the pH range of 2.0–3.0, but then decreases in pH 5.5–12.0 with increasing pH. Fig. 3B also shows that the emission maximum wavelength (λ_{max}) shifts blue a little in the pH range of 2.0–3.0, and then increases in pH 5.5–12.0 with increasing pH. The maximum I_f and the minimum λ_{max} are reached at pH 5.5. In the pH range of 3.0–5.5, where the pH is near to the theoretical p*I* values of casein (the average p*I* of casein is 4.8), and the system is not stable because of the low net charge of casein molecules. The precipitate is formed, which could not provide valuable information. Therefore, our fluorescence experiment is not conducted in this pH range.

The maximum emission wavelength (λ_{max}) is very useful in estimating the hydrophobicity of the microenvironment around tryptophan residues. λ_{max} at 335–350 indicates that tryptophan residues are located in the less polar region, that is, they are buried in a hydrophobic domain in protein; λ_{max} at 350–353 nm suggests that tryptophan residues are exposed to water.

λ_{max} centering at about 338 nm indicates that Trp residues are located in a more hydrophobic domain below pH 3.0. Below pH 3.0, where the pH is below the theoretical p*I* values of casein (the average p*I* of casein is 4.8), many amino acid residues in casein are effectively protonated. Since the ability of H^+ in $-NH_3^+$ to form hydrogen bond is much stronger than that of the neutral H atom in $-NH_2$, there exists strong hydrogen bond between casein molecules. Meanwhile, it is noteworthy that the so-called cation–π interaction is a strong and specific interaction between a cation and the π face of an aromatic ring [32]. In proteins, cation–π interactions can occur between the cationic side chains of lysine (Lys), arginine (Arg), or histidine (His), on the one hand, and the aromatic side chains of phenylalanine (Phe), tyrosine (Tyr), or tryptophan (Trp), on the other [33,34]. Thus, both the hydrogen bond action and the cation–π interaction play key roles in the self-assembly of casein, in addition to the hydrophobic interaction, which

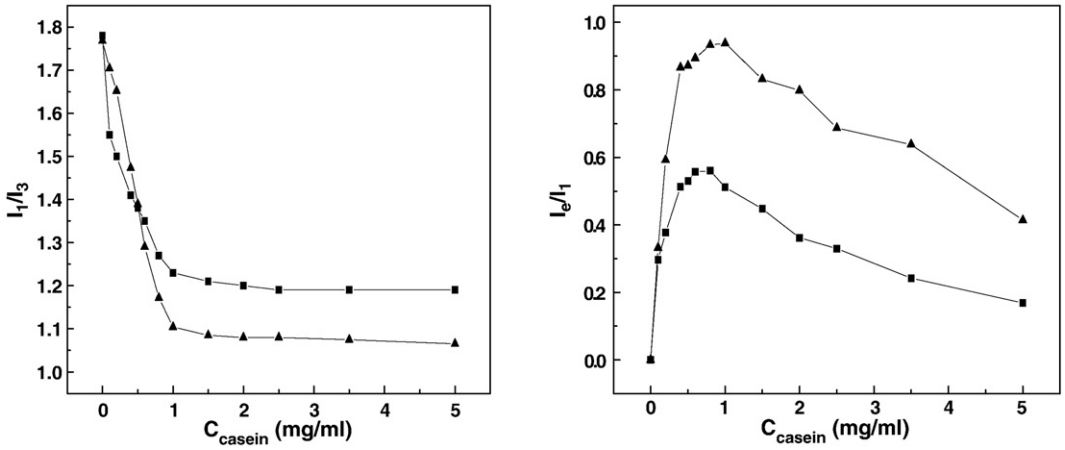

Fig. 2. Plots of I_1/I_3 (A) and I_e/I_1 (B) of pyrene against casein concentration (■, pH 2.0 and ▲, pH 12.0).

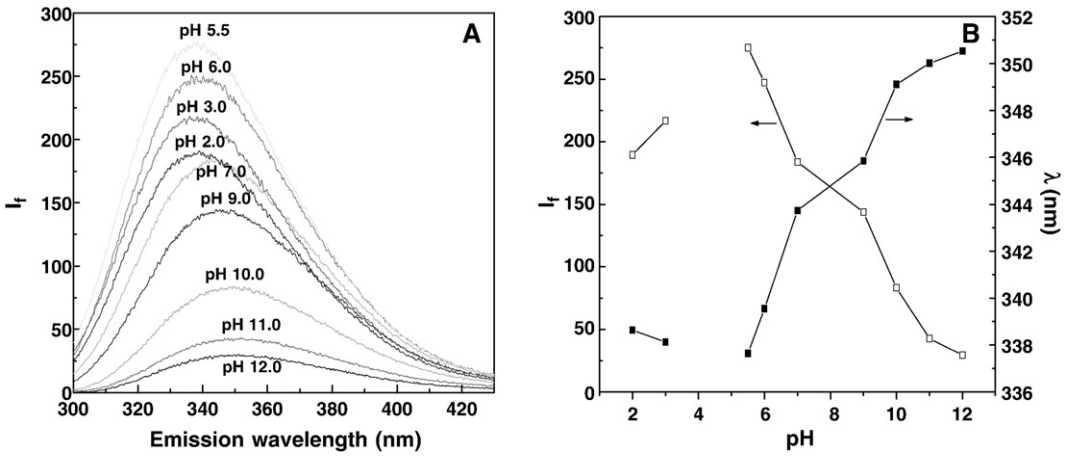

Fig. 3. (A) Fluorescence emission spectra of casein (2.0 mg/ml) with different pHs (λ_{ex}=295 nm). (B) Plots of fluorescence intensity (□) and maximum wavelength (■) against pH.

leads to the more compact structure of casein micelle below pH 3.0, and hence the smaller λ_{max}. At pH 3.0, where the pH is near to the p*I* of casein, the structure of the casein micelle is more compact because of the low negative charge of casein micelle, so λ_{max} is smaller and I_f is higher than that at pH 2.0.

In the pH range of 5.5–12.0, the deprotonation of the carboxylic groups of aspartic acid (Asp) and glutamic acid (Glu) residues gives rise to many changes in casein micelle formation, including electrostatic repulsions, the destruction of salt bridges and the formation of buried isolated charges, eventually leading to the loose structure of the casein micelle with increasing pH. Therefore, the Trp residues are located at less hydrophobic microdomain in casein micelles with the increase of pH, which leads to the increase of λ_{max} and the decrease of I_f with increasing pH.

Obviously, at pH 5.5, where the pH is near to the theoretical p*I* values of casein, the net charge of casein molecules is almost zero. Thus, the structure of the casein micelle is the most compact, and the Trp residues are located at the most hydrophobic domain, leading to the smallest λ_{max} and highest I_f.

3.2.2. Pyrene fluorescence

The influence of pH on the properties of casein micelle is also detected by the pyrene probe measurement. Fig. 4 shows that I_1/I_3 increases and I_e/I_1 decreases in the pH range of 2.0–3.0, but then I_1/I_3 decreases and I_e/I_1 increases in pH 5.5–12.0 with increasing pH. The maximum I_1/I_3 and the minimum I_e/I_1 are reached at pH 5.5.

The structure of the hydrophobic microdomains are tightly packed at pH lower than 3.0, as indicated by the above intrinsic fluorescence results, so the pyrene molecules cannot penetrate into the deeper hydrophobic domain of casein micelles, which leads to a higher I_1/I_3; meanwhile, fewer pyrene molecules are located in the same hydrophobic region of casein micelles, which hampers the formation of excimer complexes, and hence a lower I_e/I_1. At pH 3.0, where the pH is near to the p*I* of casein, the structure of the casein micelle is more compact, so I_e/I_1 is smaller and I_1/I_3 is higher than that at pH 2.0.

In the pH range of 5.5–12.0, more carboxylic groups are ionized, and the resultant repulsion among the carboxylic groups leads to some loosening of the hydrophobic nanodomains with increasing pH. Thus, the pyrene molecules can penetrate into the deeper hydrophobic region, which leads to a lower I_1/I_3; at the same time, more pyrene molecules can be located in the same hydrophobic region of casein micelles, which leads to the formation of more excimer complexes, and hence a higher I_e/I_1. The structure of the casein micelle is the most compact at pH 5.5, which causes the pyrene molecules to be located at the shallowest hydrophobic microdomain, and hence the highest I_1/I_3 and the lowest I_e/I_1.

The low affinity of pyrene for the hydrophobic domain of casein micelles with compact structure may be due to its planar configuration. Such a rigid configuration could hinder the suitable fit of the pyrene to the more hydrophobic domain of casein micelles.

3.2.3. The size of casein micelles

DLS measurement was used to further investigate the effect of pH on the size of casein micelles. Fig. 5 shows the effect of pH on the radius of

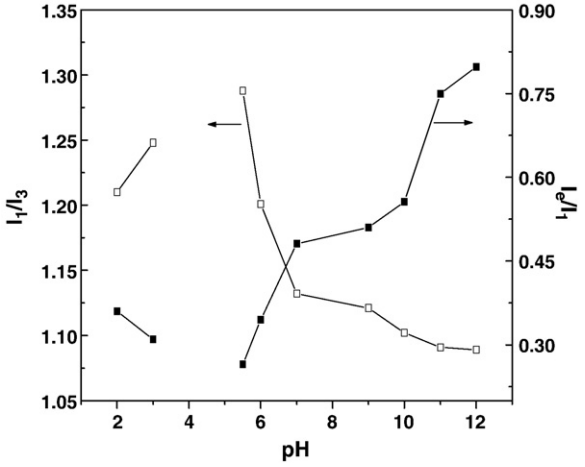

Fig. 4. Plots of I_1/I_3 (□) and I_e/I_1 (■) of pyrene in casein solution (2.0 mg/ml) against pH.

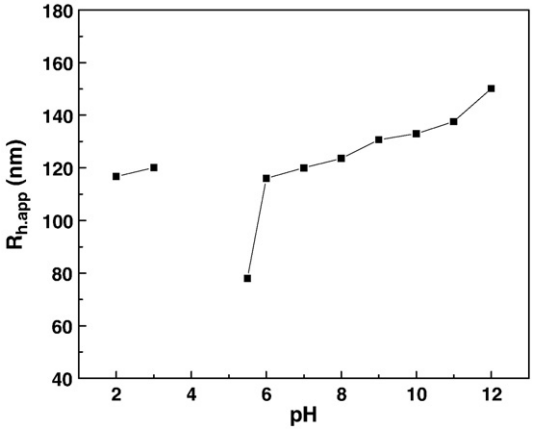

Fig. 5. The relationship of the apparent hydrodynamic radii of casein micelles with pH.

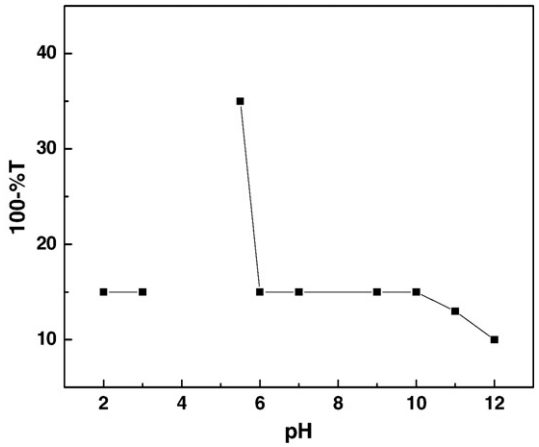

Fig. 6. Turbidity 100-%T of casein (2.0 mg/ml) as a function of pH.

casein micelles. As shown in this figure, the radius of casein micelles increases smoothly with pH in the pH range of 6.0 to 12.0, and a remarkable low radius value is reached at pH 5.5. Below pH 3.0, the radius is very similar to that at pH 7.0, and remains almost unchanged with pH. For light-scattering measurements between pH 3.0 and 5.5, flocculated aggregates exceed the upper limit of the Malvern Zetasizer (2000 nm) and absolute values are meaningless.

In the pH range from 6.0 to 12.0, individual casein molecules become more negatively charged and are separated further from each other due to a stronger electrostatic repulsion with the increase of pH. This leads to the looser structure of casein micelles and increase the size of the casein micelles with pH.

Upon pH decreasing to 5.5, the decrease in the electrostatic charge repulsion between casein molecules enhances the hydrophobic interaction between casein molecules at a pH closer to p*I*. This leads to the casein molecules closer to each other in casein micelles, and to the more compact structure of casein micelles, and hence the decrease of the radius of casein micelles.

xAt pH lower than 3.0, the net positive charge of casein molecules can stabilize casein micelles. Although the calcium phosphate nanoclusters are mainly dissolved at pH lower than 3.0, the mean size of casein micelles remains almost unchanged compared with that at pH 7.0. This indicates that the hydrogen bond together with the hydrophobic interaction matters a great deal in the formation of casein micelles at acidic pH.

3.2.4. Turbidity

Fig. 6 shows the effect of pH on the turbidity of casein solution. As shown in Fig. 6, the turbidity remains constant at pH lower than 3.0 and in the pH range of 6.0 to 10.0, and increases sharply at pH lower than 6.0, and decreases smoothly at pH higher than 10.0 with increasing pH.

It is well-known that turbidity depends much on the size and the scattering factor of particles. The big size and high scattering factor of particles usually lead to the high turbidity of the system. Obviously, the sharp increase in turbidity at pH lower than 6.0, and the smooth decrease in turbidity at pH higher than 10.0 are inconsistent with the small radius at pH lower than 6.0 and the bigger radius at pH higher than 10.0. Therefore, the particle scattering factor plays a crucial role here. The most compact structure of casein micelles leads to the highest scattering factor of particle, and hence a sharp increase in the turbidity of casein solution at pH lower than 6.0. The looser structure of casein micelles leads to the lower scattering factor of particle, and hence a decrease in the turbidity of casein solution at pH higher than 10.0.

3.3. The binding of ANS to casein micelles

Casein micelles are able to encapsulate hydrophobic compounds, and the micelles are potential drug carriers that are nontoxic and biocompatible. The effect of pH on the binding of different charged probe on casein micelles can provide more information about the interaction between casein micelles and drug molecules with different property. Since pyrene is a neutral hydrophobic probe, here, another anionic probe ANS has been chosen to study the surface hydrophobicity of casein micelles at different pHs.

ANS has been widely used to monitor exposed hydrophobic patches on the surface of proteins during folding and to monitor conformational changes. Thus, ANS can also be used to detect the accessibility of hydrophobic microenvironment with affinity for ANS molecules on casein micelles at different pHs. ANS fluorescence is strongly dependent on the polarity of the local environment around the chromophore. In water the emission is weak, with a maximum around 520 nm. Upon binding to hydrophobic patches of proteins, the fluorescence spectrum is blue-shifted with a maximum around 470 nm and the emission intensity is increased.

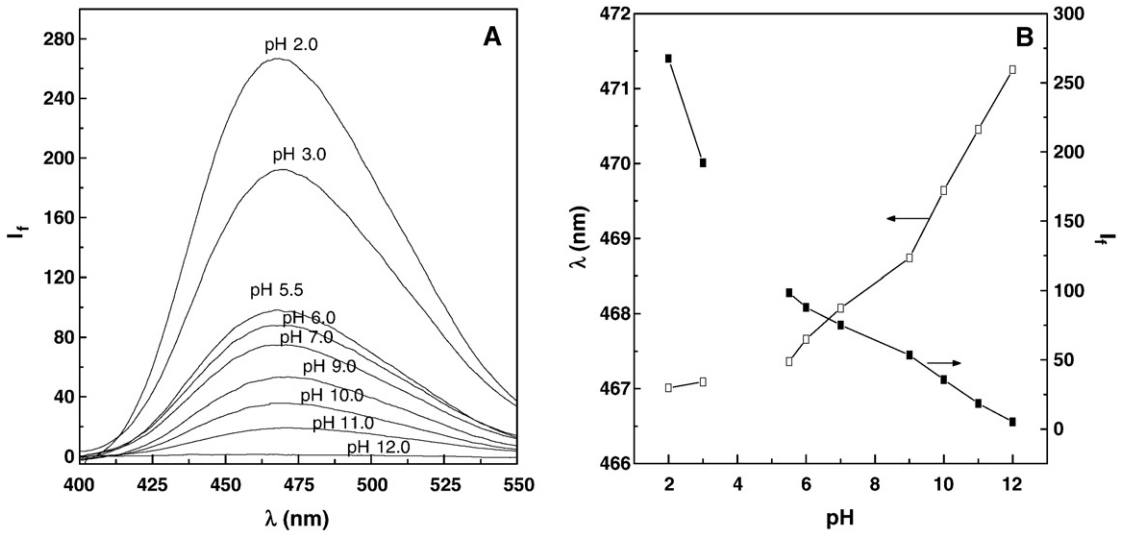

Fig. 7. (A) Fluorescence emission spectra of ANS in casein solution (2.0 mg/ml) with different pHs. (B) Plots of fluorescence intensity (■) and maximum wavelength (□) of ANS against pH.

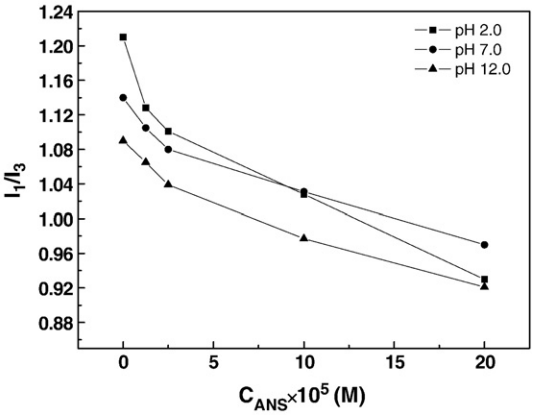

Fig. 8. The effect of ANS concentration on the ratio I_1/I_3 of pyrene at three typical pHs.

The fluorescence emission spectra of ANS in casein solution with different pHs are shown in Fig. 7A. Fig. 7B shows the pH dependence of the fluorescence maximum intensity and wavelength of ANS in the presence of 2.0 mg/ml casein. In the pH range of 2.0–3.0 and 5.5–12.0, I_f decreases sharply, and λ_{max} increases, with the increase of pH.

Since the fluorescence of ANS alone is not dependent on pH in the range studied, the pH-induced ANS fluorescence property variation in the presence of casein micelles may be the result of the variation of affinity of ANS molecules for casein micelles at different pHs.

It is clear that pH has different effects on the microenvironment around pyrene and ANS molecules in casein solution (Fig. 4), which indicates that casein micelles provide different hydrophobic domains to accept pyrene and ANS molecules, respectively. This can be verified by the casein micelle micropolarity determined by the ratio I_1/I_3 of pyrene. Fig. 8 shows the effect of ANS concentration on the ratio I_1/I_3 of pyrene at three typical pHs. It can be seen from this figure that the addition of ANS makes the ratio I_1/I_3 of pyrene decrease at all pHs. Obviously, the added ANS makes pyrene transfer to the more hydrophobic domain of casein micelles.

The results here confirm that ANS and pyrene molecules are located at different microenvironment in casein micelles. ANS resides in the hydrophobic microdomain on casein micelle surface and pyrene resides in the deeper hydrophobic microdomain on casein micelle. Therefore, the planar configuration of molecules will not limit the affinity of ANS molecules for casein micelles. It has been established that ANS binding to a polypeptide chain is primarily determined by the formation of ion pairs between ANS-anion (the sulfonate SO_3^- group) and the cationic groups (lysine, arginine, and histidine side chains) of a protein [35]. The electrostatic ionic interactions among ANS and casein increase with a decrease in pH, resulting in an increase in the number of ANS molecules bound to casein micelle at low pH. Thus, the fluorescence intensity of ANS increases with the decrease of pH.

In the higher pH range from 5.5–12.0, the negative charge of the casein micelle increases with pH, so the electrostatic repulsive action suppresses the affinity of ANS for casein micelle. Therefore, the fluorescence intensity of ANS decreases, and the maximum wavelength of ANS increases, with the increase of pH.

Therefore, the electrostatic interaction plays a crucial role in the binding of the ANS to casein micelles. Matulis and Lovrien [36] reported that electrostatic interactions are the predominant interactions between ANS and bovine serum albumin, and hydrophobic affinity is a minor interaction between them, which is in agreement with our results.

In the work of Chakraborty and Basak the binding of ANS to casein molecules at different pHs is also studied [27]. In their work, the same effect of pH on the ANS intensity was observed at pH higher than 5.5. However, the decrease of the ANS intensity was found at very acidic condition. The authors suggested that the hydrophobic interaction between ANS and the hydrophobic domain on casein molecules played an important role in the affinity of ANS for casein molecules, and they neglected the electrostatic interaction between ANS and casein molecules. This is different from our results. The difference can be resolved by nothing but the fact that the casein samples in their studies were pure casein (e.g. α, β-casein) with low casein concentration, and molecules mainly exists as casein micelle. However, in our case, ANS accessible hydrophobic clusters or patches on casein micelle surface can be created by assembly of the individual hydrophobic portions of casein molecules at lower pH.

4. Conclusions

For the first time, systematical studies of casein micelle properties over a broad pH range have been conducted by fluorescent technique together with DLS and turbidity measurements. The main results of this study can be summarized as follows: (1) Casein molecules can self-assemble into casein micelle in the pH range of 2.0 to 3.0, and 5.5 to 12.0. (2) The hydrophobic interaction, hydrogen bond and electrostatic action are the main interactions in the formation of casein micelles. (3) The structure of the casein micelle is more compact at low pH and looser at high pH. (4) ANS has high affinity for casein micelle only at acid condition and the affinity decreases sharply with increasing pH. It is believed that the above findings will provide new insights into the form–function relationship of casein micelles modulated by pH, which will broaden the application range of casein micelles in food, cosmetic and medicine domains.

Acknowledgment

This work was supported by the National Nature Science Foundation of China (20633010).

References

[1] T.L. Raguse, J.R. Lai, P.R. LePlae, S.H. Gellman, Toward β-peptide tertiary structure: self-association of an amphiphilic 14-helix in aqueous solution, Org. Lett. 3 (2001) 3963–3966.
[2] Y.S. Gu, A.E. Decker, D.J. McClements, Production and characterization of oil-in-water emulsions containing droplets stabilized by multilayer membranes consisting of β-lactoglobulin, -carrageenan and gelatin, Langmuir 21 (2005) 5752–5760.
[3] S. Li, P. He, J. Dong, Z. Guo, L. Dai, DNA-directed self-assembling of carbon nanotubes, J. Am. Chem. Soc. 127 (2005) 14–15.
[4] J. Barauskas, M. Johnsson, F. Tiberg, Self-assembled lipid superstructures: Beyond vesicles and liposomes, Nano Lett. 5 (2005) 1615–1619.
[5] C.F. Narambuena, F.S. Ausar, I.D. Bianco, D.M. Beltramo, E.P.M. Leiva, Aggregation of casein micelles by interactions with chitosans: a study by Monte Carlo simulations, J. Agric. Food Chem. 53 (2005) 459–463.
[6] C.G. de Kruif, Casein micelle interactions, Int. Dairy J. 9 (1999) 183–188.
[7] D.S. Horne, Casein micelle structure: models and muddles, Curr. Opin. Colloid Interface Sci. 11 (2006) 148–153.
[8] H.M. Farrell Jr., E.L. Malin, E.M. Brown, P.X. Qi, Casein micelle structure: what can be learned from milk synthesis and structural biology? Curr. Opin. Colloid Interface Sci. 11 (2006) 135–147.
[9] S.R. Euston, D.S. Horne, Simulating the self-association of caseins, Food Hydrocoll. 19 (2005) 379–386.
[10] P. Walstra, Casein sub-micelles: do they exist? Int. Dairy J. 9 (1999) 189–192.
[11] A. Pourjavadi, M. Kurdtabar, Eur. Polym. J. 43 (2007) 877.
[12] S.E. Burke, C.J. Barrett, pH-responsive properties of multilayered poly(L-lysine)/hyaluronic acid surfaces, Biomacromolecules 4 (2003) 1773–1783.
[13] Z.J. Sui, J.A. Jaber, J.B. Schlenoff, Polyelectrolyte complexes with pH-tunable solubility, Macromolecules 39 (2006) 8145–8152.
[14] Y. Liu, R. Guo, Interaction between casein and sodium dodecyl sulfate, J. Colloid Interface Sci. 315 (2007) 685–692.
[15] Y. Liu, R. Guo, Interaction between casein and the oppositely charged surfactant, Biomacromolecules 8 (2007) 2902–2908.
[16] D.K. Debnath, K. Mukhopadhyay, S. Basak, Acid-induced denaturation and refolding of prothrombin, Biophys. Chemist. 116 (2005) 159–165.
[17] S.G. Anema, E.W. Lowe, Y.M. Li, Effect of pH on the viscosity of heated reconstituted skim milk, Int. Dairy J. 14 (2004) 541–548.
[18] C. Guillaume, L. Jiménez, J.L. Cuq, S. Marchesseau, An original pH-reversible treatment of milk to improve rennet gelation, Int. Dairy J. 14 (2004) 305–311.

[19] A. Jaubert, C. Durier, A. Kobilinsky, P. Martin, Structural organization of the goat casein micelle: effect of the physico-chemical environment (pH, temperature, ionic strength) on its mineral and protein composition, Int. Dairy J. 9 (1999) 369–370.
[20] S.L. McSweeney, D.M. Mulvihill, D.M. O'Callaghan, The influence of pH on the heat-induced aggregation of model milk protein ingredient systems and model infant formula emulsions stabilized by milk protein ingredients, Food Hydrocoll. 18 (2004) 109–125.
[21] R. Tuinier, C. Rolin, C.G. de Kruif, Electrosorption of pectin onto casein micelles, Biomacromolecules 3 (2002) 632–638.
[22] P. Muller-Buschbaum, R. Gebhardt, E. Maurer, E. Bauer, R. Gehrke, W. Doster, Thin casein films as prepared by spin-coating: influence of film thickness and of pH, Biomacromolecules 6 (2006) 1773–1780.
[23] B. Vala, M.A. Smiddy, A.L. Kelly, T. Huppertz, Solvent-mediated disruption of bovine casein micelles at alkaline pH, J. Agric. Food Chem. 54 (2006) 8288–8293.
[24] C.D. Grant, M.R. DeRitter, K.E. Steege, T.A. Fadeeva, E.W. Castner, Fluorescence probing of interior, interfacial, and exterior regions in solution aggregates of poly (ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers, Langmuir 21 (2005) 1745–1752.
[25] M.M. Shokri, K. Khajeh, J. Alikhajeh, A. Asoodeh, B. Ranjbar, S. Hosseinkhani, M. Sadeghi, Comparison of the molten globule states of thermophilic and mesophilic α-amylases, Biophys. Chemist. 122 (2006) 58–65.
[26] T.A. Dar, L.R. Singh, A. Islam, F. Anjum, A.A. Moosavi-Movahedi, F. Ahmad, Guanidinium chloride and urea denaturations of β-lactoglobulin A at pH 2.0 and 25 °C: the equilibrium intermediate contains non-native structures (helix, tryptophan and hydrophobic patches), Biophys. Chemist. 127 (2007) 140–148.
[27] A. Chakraborty, S. Basak, pH-induced structural transitions of caseins, J. Photochem. Photobiol., B 87 (2007) 191.
[28] K. Christine, B. Jean, D. Shan, Y.F. Jeremy, B.P. Chen, Self-assembling peptide as a potential carrier of hydrophobic compounds, J. Am. Chem. Soc. 126 (2004) 7522–7532.
[29] Q.X. Zhong, C. Daubert, O.D. Velev, Physicochemical variables affecting the rheology and microstructure of rennet casein gels, J. Agric. Food Chem. 55 (2007) 2688–2697.
[30] H.A. Michael, M.F.J. Harold, W.G. Markus, Conformational analysis of the hydrophobic peptide α_{s1}-casein(136–196), Biochim. Biophys. Acta 1431 (1999) 410–420.
[31] M.F.J. Harold, E.D. Wickham, J.J. Unruh, P.X. Qi, P.D. Hoagland, Secondary structural studies of bovine caseins: temperature dependence of β-casein structure as analyzed by circular dichroism and FTIR spectroscopy and correlation with micellization, Food Hydrocoll. 15 (2001) 341–354.
[32] J.C. Ma, D.A. Dougherty, The cation–p interaction, Chem. Rev. 97 (1997) 1303–1324.
[33] J.P. Gallivan, D.A. Dougherty, A computational study of cation–p interactions vs salt bridges in aqueous media: implications for protein engineering, J. Am. Chem. Soc. 122 (2000) 870–874.
[34] H. Minoux, C. Chipot, Cation–π interactions in proteins: can simple models provide an accurate description? J. Am. Chem. Soc. 121 (1999) 10366–10372.
[35] V.N. Uversky, J.R. Gillespie, I.S. Millett, A.V. Khodyakova, A.M. Vasiliev TV Chernovskaya, R.N. Vasilenko, G.D. Kozlovskaya, Natively unfolded human prothymosin α adopts partially folded collapsed conformation at acidic pH, Biochemistry 38 (1999) 15009–15016.
[36] G.A. Manderson, M.J. Hardman, L.K. Creamer, Effect of heat treatment on bovine β-lactoglobulin A, B, and C explored using thiol availability and fluorescence, J. Agric. Food Chem. 47 (1999) 3617–3627.