Physicochemical study of k- and b-casein dispersions and the effect of cross-linking by transglutaminase

Physicochemical Study of κ- and β-Casein Dispersions and the Effect of Cross-Linking by Transglutaminase

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The milk proteins β- and κ-casein are in a monomeric state below their critical micelle concentration (cmc) and spontaneously self-associate above the cmc, at or above room temperature. Small-angle neutron scattering (SANS) measurements at 5 g L^{-1} confirmed the high specific volume of these micelles, and ^{1}H-NMR and calorimetric measurements showed that the polypeptide chains remain open and their side chains largely flexible in the associated state. The experimental values of parameters used to describe both types of micelle were radius of gyration of 8 nm, scattering radius (in ^2H_2O) of 11 nm, and interaction radius close to 15 nm. The scattering radius is obtained by assuming a homogeneous sphere, and the interaction radius by assuming hard-sphere-like behavior. The micelle size, structure, and interaction radius were independent of concentration. The interparticle structure factor of the micelles was determined using a polydisperse hard-sphere model, which showed that the wave vector position of the peak of the interparticle structure factor was independent of concentration over the range 5−20 g L^{-1}, consistent with effective hard-sphere behavior. The SANS and ^{1}H-NMR experiments indicated that the β-casein micelles were less compact and more dynamic than the κ-casein micelles. The micelle dispersions were treated with the cross-linking enzyme transglutaminase. Scattering results showed that the cross-linking hardly influenced the structure or interaction of the micelles, indicating that there was little or no interparticle cross-linking. Addition of 6 M urea did not change the structure of the cross-linked micelles, whereas addition of the urea to the non-cross-linked micelles caused extensive dissociation.

1. Introduction

In bovine milk, the caseins form large protein structures (10^8 Da, ∼200 nm diameter), held together, for the most part, by a small (about 7%) weight fraction of calcium phosphate. The physiological functions of these casein micelles probably include the safe transport of calcium phosphate through the mammary gland to the neonate.[1] There are four major fractions, α_{s1}-, α_{s2}-, β-, and κ-casein, which differ in many respects including charge (α_{s1}- > α_{s2}- > β- > κ-casein) and sensitivity to precipitation by calcium ions (α_{s2}- > α_{s1}- > β- ≫ κ-casein).[2] The primary structures of the β- and κ-caseins have either discrete polar anionic or nearly apolar and neutral regions at the termini, giving them a distinctly amphipathic nature. At neutral pH, the hydrophilic N-terminus of β-casein (residues 1−21) is highly charged (net charge, −11.5). In contrast, it is the C-terminus of κ-casein that is very hydrophilic, and in the native casein micelle, it behaves as a brush, providing steric stabilization. If this C-terminal part of 65 residues is removed, as in cheese making, by the proteinase chymosin, the casein micelles lose their colloidal stability and flocculate or gel.

The word micelle is used to describe both the native colloidal casein particles present in milk and the self-associated state of pure preparations of both β- and κ-caseins. An analogy with soap micelles is implied in relation to the properties of the two individual caseins but is less appropriate for the native particle, not least because of the cementing action of the micellar calcium phosphate. The amphipathic character of the primary structures of β- and κ-caseins also suggests that hydrophobic bonding is important in forming their micellar state. Traditionally, β- and κ-casein micelles are assumed to exhibit monomer−micelle association equilibria of the "all or nothing" or closed-association type,[3−6]

$$n \text{ monomers} \leftrightarrow \text{micelle}$$

This description of micelle formation is also termed the two-state model. The micellization number n is supposedly fixed and of the order of 20−50. Here we refer to the pioneering work of Vreeman and Payens.[4,5,26,28,32] They made extensive studies of the micellization of β- and κ-casein and showed that the apparent molecular mass of the micelles is a clear function of temperature and concentration. Andrews et al.[7] used small-angle X-ray scattering (SAXS) to study the micellization of β-casein and found n to be 14 in their experiments. Recently, however, Mikheeva et al.[8] showed that the β-casein

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micellization can be described quite adequately using the so-called shell model of Kegeles,[9,10] from which a distribution of micelle sizes follows that is centered around $N/2$ where N is the maximum (as determined by steric constraints) micellization number. Thus, the shell model describes the association behavior as

$$\begin{aligned}
&\beta\text{-cas}_1 + \beta\text{-cas}_1 \leftrightarrow \beta\text{-cas}_2\\
&\beta\text{-cas}_1 + \beta\text{-cas}_2 \leftrightarrow \beta\text{-cas}\\
&\cdots\cdots\cdots\cdots\cdots\cdots\\
&\beta\text{-cas}_1 + \beta\text{-cas}_i \leftrightarrow \beta\text{-cas}_{i+1}\\
&\cdots\cdots\cdots\cdots\cdots\cdots\\
&\beta\text{-cas}_1 + \beta\text{-cas}_{N-1} \leftrightarrow \beta\text{-cas}_N
\end{aligned}$$

The essence of the shell model is that the association constant of the first step is very much smaller than the association constants of all subsequent steps.

Since there is a large similarity in various properties of β- and κ-caseins, we assume that the κ-casein micelle can also be described using the shell model. The purpose of this paper is to determine the structure and interaction of β- and κ-casein micelles using small-angle neutron scattering (SANS). We employed a special method to study the structure of the micelles formed, by cross-linking them internally by using the enzyme transglutaminase (TGase). It catalyzes the formation of γ-(carboxyamide-ϵ-amino) cross-links between protein molecules.[11,12] Although both β- and κ-casein are good substrates for TGase, no large aggregates were found while incubating the protein solutions with TGase at 40 °C. This led to the supposition that intermolecular cross-links are formed within, but not between, the micelles. To verify this hypothesis, the chaotropic agent urea was added to the non-cross-linked casein solutions to a final concentration of 6 M, causing the micelles to dissociate into monomers. When the micelles were cross-linked, the addition of the urea did not result in the micelles falling apart.

2. Theory of Scattering

Neutrons can be considered as plane waves,[13−15] and therefore we use Rayleigh−Gans−Debye theory[16−18] to describe our neutron scattering experiments. In a SANS experiment, the normalized scattered intensity $I(Q)$ depends on the wave vector Q, which is defined as $4\pi \sin(\theta/2)/\lambda$, where θ is the angle at which the scattered intensity is detected and λ is the neutron wavelength. For monodisperse sols, $I(Q)$ is related to the interparticle structure factor $S(Q)$ and the particle scattering factor $P(Q)$ by[13−18]

$$I(Q) \propto \phi P(Q) S(Q) \tag{1}$$

where ϕ is the volume fraction of colloidal particles. The proportionality constant depends on the difference between the scattering length densities of the particle and the solvent. For spherical colloids of radius a, the particle scattering factor is

$$P(Q) = \left\{ 3\left(\frac{\sin(Qa) - Qa\cos(Qa)}{(Qa)^3} \right) \right\}^2 \tag{2}$$

which is approximated closely by $P(Q) = \exp(-Q^2a^2/5)$ for $Qa < 2$. Hence, a so-called Guinier plot of $\ln\{P(Q)\}$ versus Q^2 gives the radius of gyration from the slope.

It is still possible to use eq 1 for moderately polydisperse particles. In that situation, one may define an operational interparticle structure factor:

$$S(Q)_2 = \frac{c_1 I(Q)_2}{c_2 I(Q)_1} \tag{3}$$

where the subscript 2 refers to a concentration where there is appreciable interaction between the micelles and the subscript 1 refers to a low concentration for which $S(Q) = 1$ for all Q-values. The interparticle structure factor of a dispersion of polydisperse hard spheres can be calculated for a p-component particle system by using Fournet's expression[18] for the scattered intensity $I(Q)$. The coherent scattering part is then

$$I(Q) = \sum_{i,k=1}^{p} f_i f_k B_i(Q) B_k(Q) (\rho_i \rho_k)^{1/2} [\delta_{ik} + H_{ik}(Q)] \tag{4}$$

where ρ_i is the number of particles i per volume, f_i is the scattering amplitude of particle i at zero scattering angle, $B_i(Q)$ is the intraparticle interference factor of particle i, δ_{ik} is the Kronecker delta function, and the function H_{ik} is defined as

$$H_{ik}(Q) = (\rho_i \rho_k)^{1/2} \int_0^\infty 4\pi r^2 (g_{ik}(r) - 1) \frac{\sin(Qr)}{Qr} \mathrm{d}r \tag{5}$$

where $g_{ik}(r)$ is the radial distribution function as a function of the distance between the particle centers r. In the limit of $\rho \to 0$, with ρ being the total concentration $\sum \rho_i$ over all i's, the scattering intensity depends only on single particle scattering:

$$I_0(Q) = \sum_{i=1}^{p} f_i^2 B_i^2(Q) \rho_i \tag{6}$$

From this, it follows that a polydisperse particle scattering factor $P_{\mathrm{poly}}(Q)$ can be defined as

$$P_{\mathrm{poly}}(Q) = \frac{\sum_{i=1}^{p} f_i^2 B_i^2(Q) \rho_i}{\sum_{i=1}^{p} f_i^2 \rho_i} \tag{7}$$

(in the monodisperse case $P(Q) = B^2(Q)$). It is then

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physically appealing to redefine the total scattering intensity as[20]

$$I(Q) = \rho f_{\text{poly}}{}^2 P_{\text{poly}}(Q) S_{\text{poly}}(Q) \quad (8)$$

where f_{poly} is defined as

$$f_{\text{poly}}{}^2 = \frac{\sum_{i=1}^{p} f_i{}^2 \rho_i}{\sum_{i=1}^{p} \rho_i} \quad (9)$$

Using the Percus−Yevick approximation for $g(r)$ for hard spheres,[19−21] we can thus calculate the interparticle structure factor for polydisperse hard spheres.

3. Experimental Section

3.1. Materials. High-purity β-casein, M = 24 kDa, for the SANS and NMR experiments was obtained from Eurial (Rennes, France). Stock solutions were prepared in 25 mM Na phosphate, pH 6.5 (Merck, D64271, Darmstadt, Germany). The β-casein used in the calorimetry work was prepared by urea fractionation from whole casein using the procedure of Aschaffenburg.[31]

High-purity κ-casein, M = 19 kDa, was obtained from Dr. David Horne from the Hannah Research Institute, Ayr, Scotland. It was prepared according to the method of Leaver and Law.[22]

β-Casein and κ-casein solutions in sodium phosphate buffer were incubated with the protein glutamine γ-glutamyl-transferase (Tgase, E.C. 2.3.2.13, Webb[23]). The TGase was the gift of Dr. J. Sakamoto from AJINOMOTO, Tokyo, Japan. It was added at a level of 15 u/g protein and incubated for 2 h at 40 °C. The enzyme was inactivated by heating the solution to 80 °C for 10 min.

For the SANS measurements, 99.9% ^2H_2O (Sigma) was used to dissolve the proteins which were lyophilized before. The "pD", as measured with a pH meter, of the ^2H_2O solutions was 6.75.[24]

3.2. Methods. *Small-Angle Neutron Scattering.* The SANS experiments were performed using cold neutrons emitted from the cold source of the high-flux nuclear reactor at the Institut Max Von Laue−Paul Langevin in Grenoble (France) using the D22 spectrometer. Hellma QS quartz cells were used with a sample path length of 2 mm. All samples were mixed thoroughly before measuring. The mean wavelength of the emitted neutrons was 1.0 nm with a width at half-height of 9%. Measurements were taken at 25 °C.

Nuclear Magnetic Resonance Spectroscopy. The ^{1}H-NMR spectra were recorded on a Bruker AM400 spectrometer interfaced to an Aspect 3000 computer. The spectra of the caseins in the absence and in the presence of 6 M deuterated urea were taken at 25 °C in 50 mM phosphate buffer, pH 7.1. The resonance of the solvent was suppressed by presaturation.

κ-Casein was dissolved in ^2H_2O, and β-casein spectra were recorded in 90% H_2O/10% ^2H_2O. The protein concentration of the samples was 32 g L^{-1} for κ-casein and 20 g L^{-1} for β-casein; after adding 6 M urea, the concentrations were 22 and 14 g L^{-1}, respectively.

Differential Scanning Microcalorimetry. Freeze-dried protein was dissolved in a buffer of 60 mM NaCl and 10 mM Na phosphate, adjusted to pH 6.7. A Microcal MCS calorimeter was used to scan thermograms of β- and κ-casein dispersions at a concentration of either 5 or 50 g L^{-1} over the temperature range 5−110 °C at a scan rate of 1 °C min^{-1}.

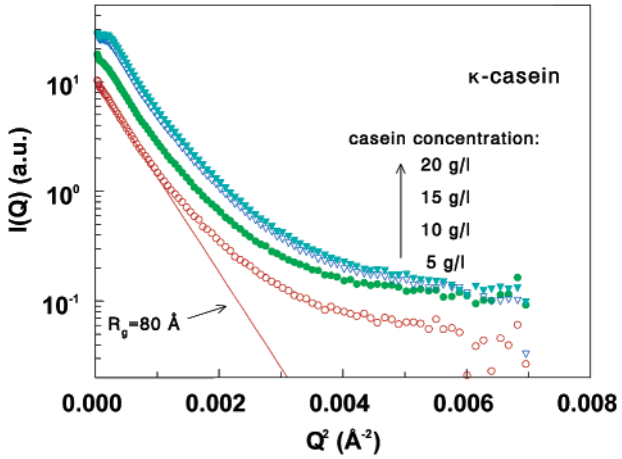

Figure 1. Guinier plot of SANS intensities of κ-casein dispersions.

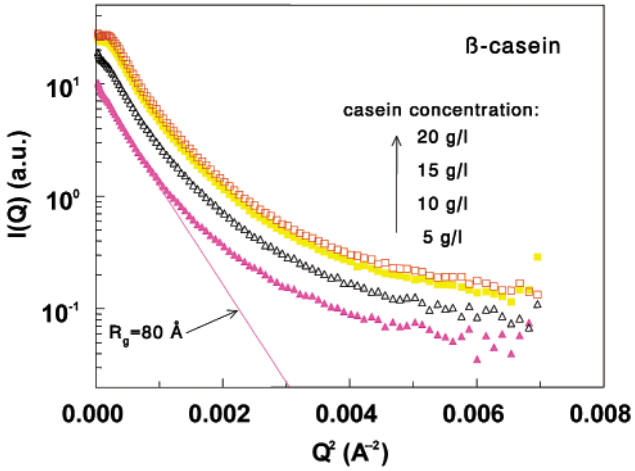

Figure 2. Guinier plot of SANS intensities of β-casein dispersions.

4. Results and Discussion

4.1. Small-Angle Neutron Scattering Experiments. SANS measurements were performed to show that the TGase treatment made the casein micelles much more stable. Therefore, the scattering curves of β- and κ-casein solutions and the effect of adding urea on both TGase-treated and untreated samples were compared.

κ-Casein Micelles. The scattering intensity was measured as a function of Q at the four concentrations, c, of 5, 10, 15, and 20 g L^{-1}, and the results are shown as a Guinier plot in Figure 1. Since the interparticle structure factor of the lowest concentration of 5 g L^{-1} will be close to 1 over the whole Q-range, we fitted the data in the lowest Q-range to the Guinier approximation, which gave a radius of gyration of 8.0 ± 1.0 nm. An equivalent homogeneous sphere has a radius of 10.3 ± 1.1 nm. The deviation from the Guinier slope is due to the limited range of the Guinier region, up to QRg = 1, but also due to size polydispersity. From the $I(Q)$ results, we calculated the interparticle structure factor at some arbitrary concentration by assuming that the interparticle structure factor at 5 g L^{-1} is 1. Thus, the results at 5 g L^{-1} were used for $P(Q)$. Further, all curves were constrained so that $S(Q)$ went to unity at high Q. The resulting interparticle structure factors are plotted as a function of Q in Figure 3. The first

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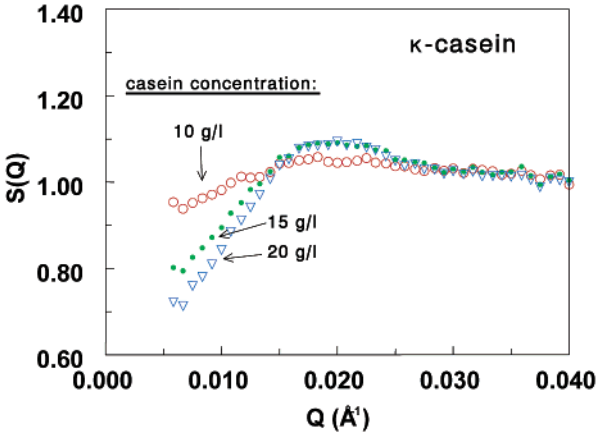

Figure 3. Structure factor of κ-casein dispersions; equivalent volume fractions are 0.035, 0.052, and 0.07.

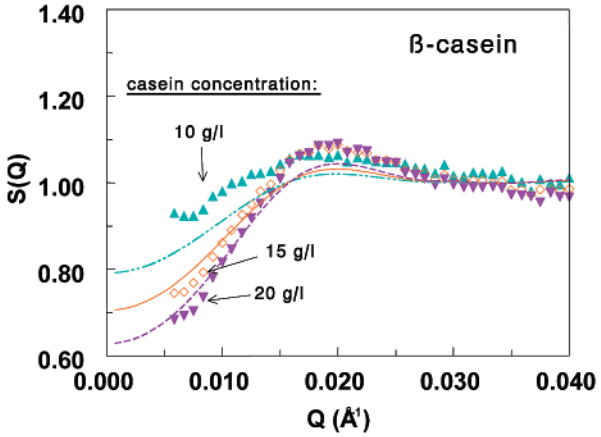

Figure 4. Structure factor of β-casein dispersions; equivalent volume fractions are 0.035, 0.052, and 0.07. The drawn lines are calculated for a polydisperse (10%) hard-sphere dispersion.

peak in the interparticle structure factor is found at $Q \approx 0.20 \pm 0.02$ nm^{-1}, which corresponds to a diameter of approximately 30.0 ± 3.0 nm and thus to a radius of 15.0 ± 1.5 nm. The interparticle structure factors of hard-sphere suspensions were calculated using the program of Robertus[21] and a model comprising hard spheres with an average radius of 15.0 nm and a Schulz−Flory size distribution with a standard deviation of 10%. The trend in the theoretical predictions matched that of the experimental results when the specific volume was 3.5 mL g^{-1}, so that $\phi = 0.0035c$. Implicitly, it is thus assumed that the form factor does not change with concentration. We think that the results justify this assumption.

TGase-Treated κ-Casein Micelles and the Effect of Urea Addition. The results for the four samples after treatment with TGase are shown as a Guinier plot in Figure 5. A comparison with the non-cross-linked results in Figure 1 shows that the TGase-treated samples are qualitatively comparable with non-cross-linked κ-casein although the upward curvature seen at the lowest Q values in the two lower concentrations is an indication of greater aggregation as a result of a small number of intermicellar links. Differences in scattering intensities seem to be mainly due to experimental uncertainties and protein concentration differences. In Figure 7, the results at 5 and 20 g L^{-1} without TGase treatment are compared to scattering curves obtained after addition of the urea. The scattering intensities were decreased by about a factor of 5 at small Q, indicating that the κ-casein micelles were broken down to monomers by the urea.

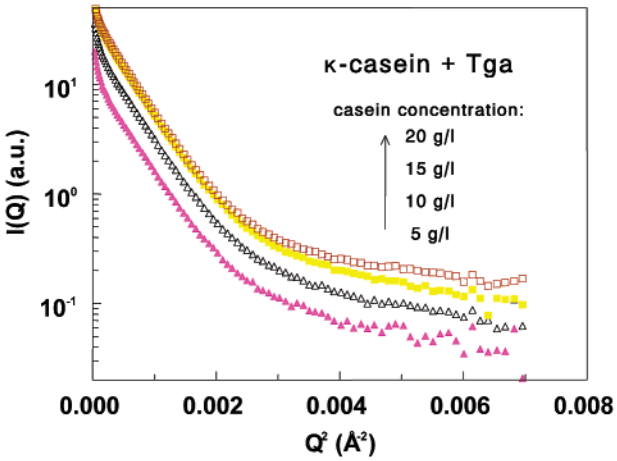

Figure 5. Guinier plot of κ-casein dispersions treated with transglutaminase.

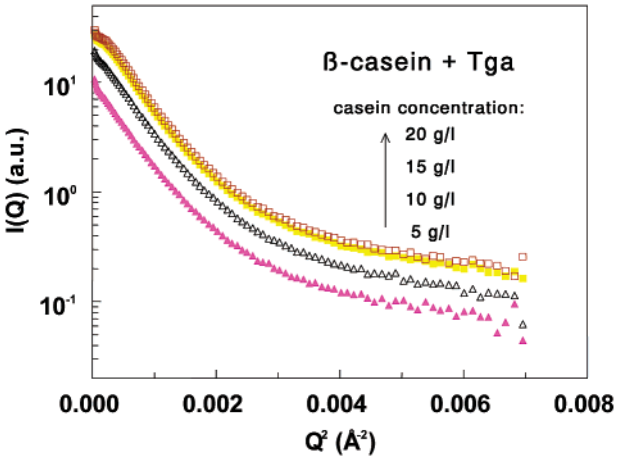

Figure 6. Guinier plot of β-casein dispersions treated with transglutaminase.

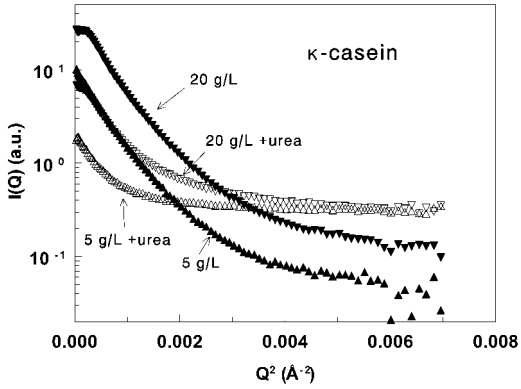

Figure 7. Guinier plot of κ-casein dispersions and κ-casein dispersions to which urea is added showing the dissociation of the micelles.

The scattering intensities of TGase-treated samples both with and without urea are plotted in Figure 9. The effect of the urea on the intensities at low Q is much less than for the samples not treated with the cross-linking reagent. Using dynamic light scattering, O'Connell and De Kruif[25,26] showed that TGase treatment made the hydrodynamic radius independent of temperature or urea treatment.

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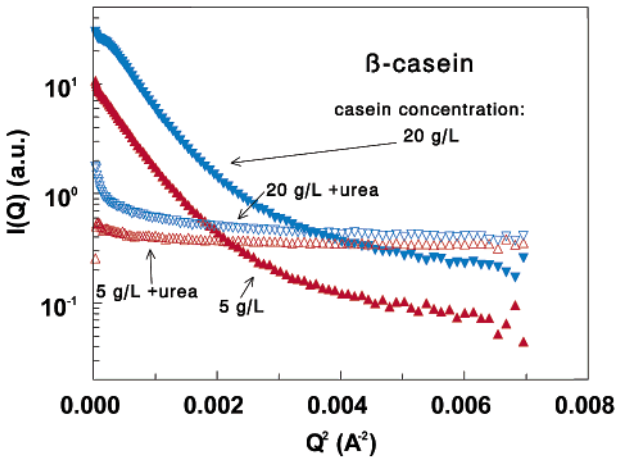

Figure 8. Guinier plot of β-casein dispersions and κ-casein dispersions to which urea is added showing the dissociation of the micelles.

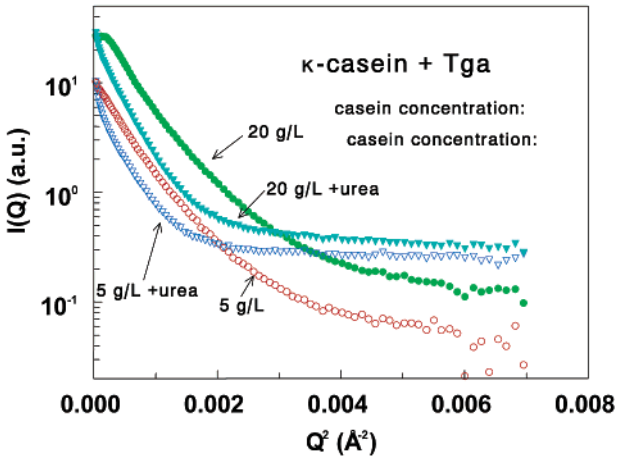

Figure 9. Guinier plot of κ-casein dispersions treated with TGase and κ-casein dispersions treated with TGase to which urea is added showing that the micelles remain largely intact.

So, in conclusion, the association of κ-casein leads to micelles of a distinct integrity and size which can be "fixed" by TGase.

β-Casein Micelles. The same series of SANS experiments was carried out with β-casein as was done with κ-casein. The scattering curves for the two types of micelle were quite comparable as can be seen from the Guinier plots in Figures 1 and 2. The radius of gyration calculated from the scattering curve of the lowest protein concentration used (5 g L^{-1}) was also found to be 8.0 ± 1.0 nm. The calculated interparticle structure factors are given in Figure 4. The major peak at about $Q = 0.02$ nm^{-1} corresponds to a hard-sphere radius of 15.0 ± 1.5 nm. We found that we could describe the interparticle structure factors for 10, 15, and 20 g L^{-1} β-casein using the same model as for κ-casein. The specific volume was thus the same (3.5 mL g^{-1}). The scattering curves shown in Figure 8 for β-casein micelles treated with the TGase are almost identical to those shown in Figure 6 indicating that the TGase treatment did not affect the scattering of the β-caseins. Figure 8 shows that β-casein micelles can be broken down by adding urea, and in Figure 10 it is shown that the TGase treatment partly protects the β-casein micelles against dissociation by urea.

4.2. Nuclear Magnetic Resonance. Figures 11 and 12 show the effect of TGase treatment on the ^{1}H-NMR spectra of κ- and β-casein. TGase treatment of κ-casein

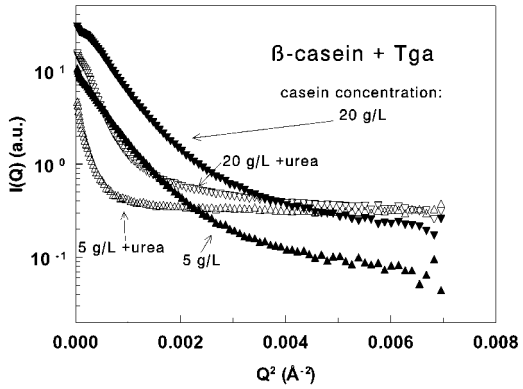

Figure 10. Guinier plot of β-casein dispersions treated with TGase and β-casein dispersions treated with TGase to which urea is added showing that the micelles remain partly intact.

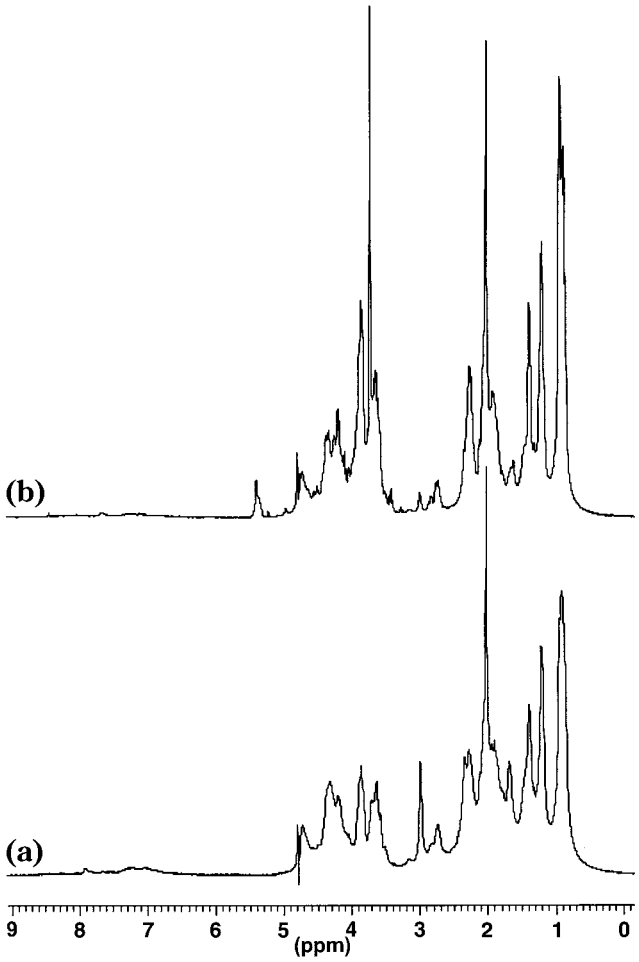

Figure 11. ^{1}H-NMR spectrum of 3% (w/w) κ-casein dissolved in 50 mM phosphate buffer (pH 7.1) in D_2O. (a) Spectrum of native κ-casein. (b) Spectrum of κ-casein treated with transglutaminase. The sharp peak at 3.7 ppm in the transglutaminase-treated sample is due to residual Tris buffer.

decreased the intensity of the resonance of the lysine ε-methylene protons at 3.0 ppm[27] and led to the appearance of a new resonance at 5.4 ppm. The latter resonance originates, very probably, from the ε-methylene protons of lysine residues covalently linked to a glutamine. The strong decrease in intensity of the resonance at 3.0 ppm (approximately 80%) is indicative of a relatively high

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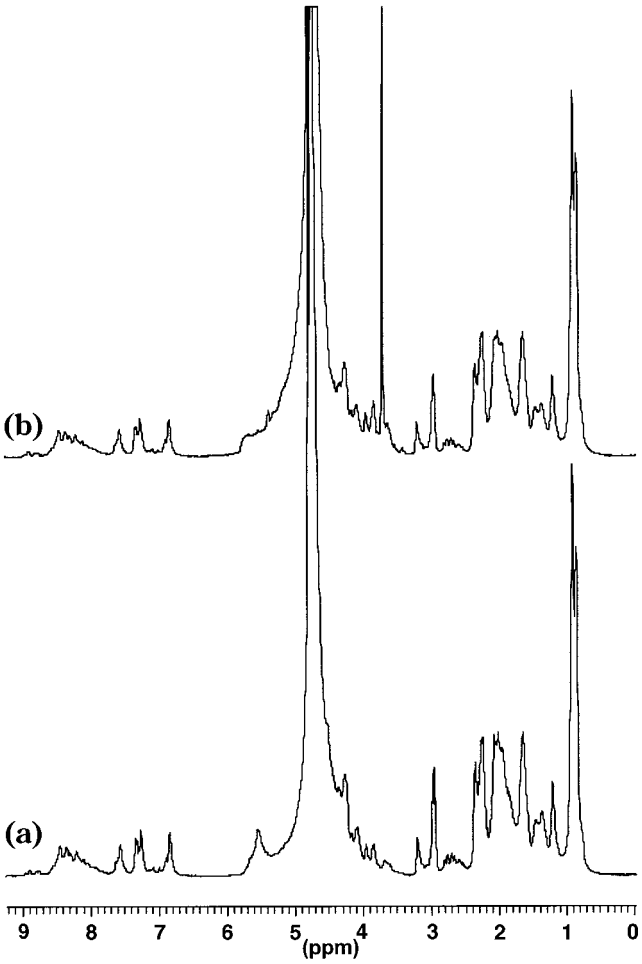

Figure 12. ^{1}H-NMR spectrum of 2% (w/w) β-casein dissolved in 50 mM phosphate buffer (pH 7.1) in 90% H_2O/10% D_2O. (a) Spectrum of native β-casein. (b) Spectrum of β-casein treated with transglutaminase. The sharp peak at 3.7 ppm in the transglutaminase-treated sample is due to residual Tris buffer.

degree of cross-linking in this preparation. In the case of β-casein, these effects are much less pronounced, indicating a relatively low degree of cross-linking.

Both β- and κ-casein are known to exhibit self-association which was described by a polymer−monomer equilibrium.[4,5,6,28,29,31] κ-Casein forms 30-mers, nearly independently of solution conditions. The degree of self-association of β-casein, however, strongly depends on solution conditions such as ionic strength and temperature. Both caseins in the polymeric form give peaks in their NMR spectra with relatively small line widths, indicating that at least some of the polypeptide side chains in the polymer are relatively mobile. Moreover, both NMR patterns are typically random-coil patterns (no chemical shift dispersion of C_α protons, no ring current shifted methyl resonances). The spectrum of β-casein corresponds to its amino acid residue composition; the pattern of κ-casein shows very little or no intensity in the aromatic region of the spectrum. The results for β-casein are in accordance with those of Andrews et al.[7] who also observed

Table 1. Intensity of the Methyl Resonances at 0.9 ppm Expressed as a Percentage of the Intensity Observed in the Presence of 6 M Urea

sample	intensity (%)
κ-casein native, no urea	11
κ-casein after TGase treatment + urea	66
β-casein native, no urea	32
β-casein after TGase treatment + urea	33

a random coil-like pattern. In the case of κ-casein, it has been demonstrated that the NMR spectrum of the polymer originates from the C-terminal part of κ-casein;[30] the N-terminal part is rigid (the NMR signals of this part are not observable due to severe line broadening) and forms the core of the particle. The native polymers and the effect of cross-linking by TGase can be characterized by comparing the NMR intensity of the native (polymeric) proteins with the intensity observed in the presence of 6 M urea (inducing complete dissociation of non-cross-linked polymers). The results are presented in Table 1. Native κ-casein exhibits a relatively low NMR intensity, indicating that most of the protein is relatively immobile. Non-cross-linked β-casein at a concentration of 20 g L^{-1} shows 33% of the intensity observed in the presence of urea. Andrews et al.[7] report an intensity of 63% at a β-casein concentration of 5 g L^{-1}. The intensity of the NMR spectrum of TGase-treated κ-casein increased only slightly upon addition of urea: apparently the κ-casein monomers in the polymer are intermolecularly cross-linked inhibiting the dissociation of the polymer. For β-casein, the intensity of the NMR spectrum increased considerably, probably due to the relatively low degree of cross-linking in this particular preparation. It is our experience that cross-linking with TGase is effective only in (locally) concentrated systems, which is not surprising since the enzymatic reaction requires that two different and large molecules and an enzyme come into close proximity. Therefore, the results above indicate that the β-casein micelles are less compact and more dynamic in such a way that cross-linking is less effective than with κ-casein micelles.

4.3. Differential Scanning Calorimetry. Thermograms of β- and κ-casein dispersions at 5 g L^{-1} are shown in Figure 13. Essentially the same result was found at the higher concentration of 50 g L^{-1}. Both curves show almost no change of specific heat characteristic of the denaturation of a globular protein although the β-casein does exhibit an apparently cooperative endothermic transition at 13 °C, associated with the strongly temperature-dependent association properties of this protein. The transition is so close to the starting point of the thermogram that it is impossible to position a baseline or calculate the enthalpy change. The absence of any cooperative endothermic transition in the κ-casein thermogram is in agreement with the findings from earlier studies[5,6,32] that showed that micelles of this protein were constant in size, independent of temperature. Apart from the low-temperature transition in β-casein, the specific heat increases gradually, consistent with a progressive but noncooperative increase in vibrational and rotational degrees of freedom.

Nevertheless, the essential finding is clear, that neither protein is folded into a stable, globular conformation, in agreement with the result from NMR studies reported here and elsewhere[29,30] that the proteins have an open structure with a considerable amount of side-chain flexibility. The degree of flexibility or the proportion of

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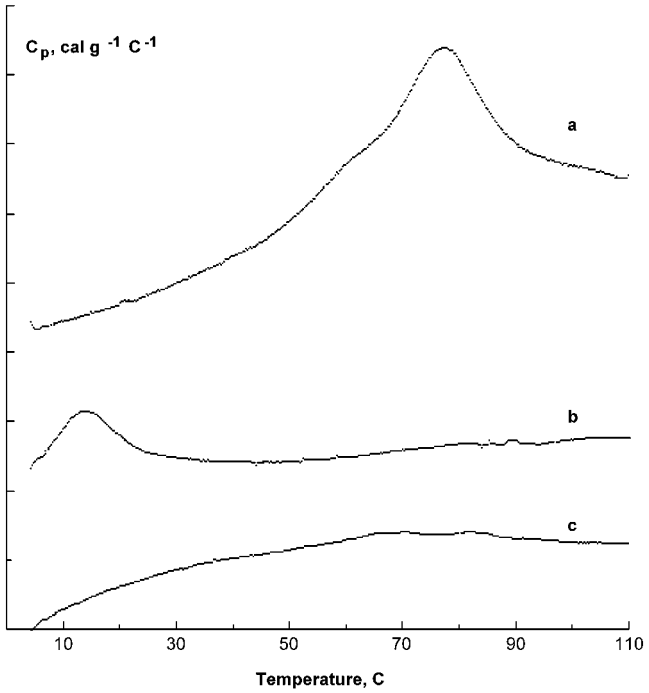

Figure 13. Differential scanning thermograms of (a) bovine β-lactoglobulin at 5 mg mL^{-1}, (b) β-casein at 10 mg mL^{-1}, and (c) κ-casein at 10 mg mL^{-1}. The ordinate axis divisions are 2000 cal g^{-1} °C^{-1}, and individual thermograms have been spaced for greater clarity by a vertical adjustment.

flexible side chains may increase gradually with temperature, as evidenced by the tendency of the sharper resonances to further narrow as the temperature increases.[28,30]

The observation of an open structure is supported by recent measurements of the secondary structure of caseins by Raman optical activity at 50 g L^{-1} and ambient temperature.[33] The predominant secondary structure in both proteins was of the poly-L-proline-II (PP-II) type which is expected to give a conformation rather similar to that of a stiff random coil.[33] The helix of the PP-II secondary structure is open to solvent hydration and may be further stabilized by through-space dipolar interactions along the backbone,[35] but the helix is not stabilized by any intramolecular H-bonds. Nevertheless, pairs of polypeptide chains in the PP-II conformation can form intermolecular H-bonds by forming a type of extended structure with a right-handed twist.[34] As temperature increases, PP-II in poly-L-lysine is gradually converted to a more random structure[36] but it is stabilized by high concentrations of chaotropic agents such as urea[36] and natively unfolded, or rheomorphic,[37] proteins such as caseins have a persistence length comparable to that of globular proteins fully denatured (and, if needed, fully S−S reduced) by 6 M urea.[33]

In summary, the calorimetric and spectroscopic measurements support a structure for the β- and κ-casein micelles in which there is a high specific volume. Intermolecular interactions are weak, comprising intermolecular backbone H-bonding and entropic, solvent-derived forces arising from the displacement of solvent from the backbone and side chains in the associated state.

5. Conclusions

Both β- and κ-casein form quasi spherical micelles with a high specific volume due to the unfolded nature of the polypeptide chains. At or above room temperature, the micelles have a distinct structure and size. The particle scattering factor of the micelles could be measured by SANS at 5 g L^{-1}. The radius of gyration was 8 nm, and the scattering radius, 10.3 nm. At the higher protein concentrations, an interparticle structure factor was measured, which was described by a polydisperse hard-sphere model. The "hard-sphere" interaction radius was 15 nm.

Incubation of β- and κ-casein solutions with TGase led to the formation of intramolecular cross-links in the casein micelles. Because of this, it was not possible to dissociate the micelles by adding 6 M urea. The cross-linking effect was more pronounced in the case of κ-casein, which might be due to a difference of conformation between the caseins. The molecular structure of the caseins is not understood in enough detail at present to identify the specific interactions or packing and symmetry considerations that could generate the well-defined characteristics of their micelles.

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