Comparison of Hydration Behavior of Bovine and Caprine Caseins As Determined by Oxygen-17 Nuclear Magnetic Resonance: Effects of Salt

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Oxygen-17 nuclear magnetic resonance (NMR) transverse relaxation measurements at 4.7 T were used to study the hydration properties of bovine and caprine casein solutions with and without NaCl. Measurements were carried out at 21 °C and pD 6.95. Nonlinear protein concentration dependences were observed for the oxygen-17 NMR transverse relaxation rates, but the fitting of the data did not require any higher order virial coefficients; only B_0 was needed. This indicates that long-range, charge–charge repulsive interactions dominate entirely the protein activity in solution. Estimates of the bovine casein average "net" charge were obtained from the virial coefficient (B_0) and the partial specific volume; these are in accord with published amino acid sequence data. For bovine casein, the α_{s1}- and β-components occur in nearly equal amounts, whereas in caprine casein, β-casein is the predominant species and α_{s1}-casein varies from high to low values, suggesting altered protein–protein interactions. Hydration levels of caprine casein high in the α_{s1}-casein component when compared with those of bovine casein (intermediate hydration) and the low α_{s1}-caprine casein (low hydration) are interpreted in terms of "trapped water" and "preferential interactions" with water on the basis of quantitative differences in casein monomer contents.

Keywords: *NMR, ^{17}O; water binding; salt binding; bovine casein; caprine casein; caprine α_{s1}-casein*

1. INTRODUCTION

Caseins and caseinates are widely used as food ingredients, as a consequence of their water-binding, emulsifying, foaming, gel-forming, and thickening capacities. Water plays a very important role in influencing the physical and functional properties of these milk proteins in food systems (Kinsella and Fox, 1986). Several major factors determine the special nature of water binding by the caseins: the hydrophobicity of the casein subunits, a loose packing density (or high voluminosity), and the extent of phosphate hydration in the caseins. The individual casein components of caprine milk have primary structures similar to those of bovine milk (Mercier et al., 1976; Brignon et al., 1989), but these proteins differ slightly in their physical properties (Mora-Gutierrez et al., 1993a,b). The distinguishing feature of caprine milk is that β-casein is the predominant protein, while α_{s1}- and α_{s2}-caseins vary in content (Grosclaude et al., 1987). In contrast, the β- and α_{s1}-caseins occur in nearly equal concentrations in bovine milk (Davies and Law, 1980). The variation of α_{s1}- and α_{s2}-casein contents is considered to affect the properties of the resultant caprine milk products (Ambrosoli et al., 1988); the nature and/or content of the casein components in caprine caseins is, therefore, an important consideration in relating structure to applications in solution.

Deuterium, oxygen-17, and proton nuclear magnetic resonance (NMR) relaxation studies can provide direct information on "binding" and mobility of water and serve as a probe of protein–solvent and protein–protein interactions in solution. However, oxygen-17 NMR relaxation measurements provide more reliable means for probing the hydration of proteins and foods than deuterium and proton water NMR relaxation; of major consequence is that "except for a narrow pH range around neutral, the oxygen-17 relaxation is not influenced by proton (deuterium) exchange with prototropic residues on the protein" (Halle et al., 1981).

Much of the previous work aimed at characterization of the hydration properties of casein and casein micelles has involved standard water sorption techniques (Berlin et al., 1968; Thompson et al., 1969; Tarodo de la Fuente and Alais, 1975; Rüegg and Moor, 1984) or more recently deuterium NMR (Kumosinski et al., 1987; Farrell et al., 1989), and investigations were carried out primarily on bovine milk casein. There has been little work done on the hydration of caprine milk caseins (Thompson et al., 1969; Richardson et al., 1974). These early studies suggested that the casein micelles of caprine milk are much less solvated than bovine micelles, resulting from compositional differences. Because of the scarcity of data for caprine casein, as well as the discovery of the variability in α_{s1}-casein content, this study was undertaken to determine quantitatively by oxygen-17 NMR relaxation techniques the extent of protein–water and protein–protein interactions for bovine and selected caprine caseins and to assess the effects of NaCl on these interactions.

2. THEORY

The majority of the NMR hydration data has been interpreted in terms of a two-state model with fast exchange (Pessen and Kumosinski, 1985). If there are

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no additional contributions to relaxation, the observed relaxation rate (R_{obs}) is the weighted average of the "bound" and "free" water relaxation rates, R_B and R_F, respectively

$$R_{obs} = P_B R_B + P_F R_F \quad (1)$$

where P_B and P_F represent the corresponding fractions of bound and free water in the sample. If C_p is the protein concentration in grams of protein per gram of water and n_H is the protein hydration in grams of bound water per gram of protein then

$$P_B = n_H C_p \quad (2)$$

and, since $P_F = 1 - P_B$, eq 2 may be rewritten as

$$R_{obs} = (R_B - R_F)P_B + R_F = n_H(R_B - R_F)C_p + R_F \quad (3)$$

with R_{obs}, R_B, and R_F being either the longitudinal (R_1) or transverse (R_2) NMR relaxation rates.

Equation 4 predicts a linear relationship between R_{obs} and protein concentration C_p (Pessen and Kumosinski, 1985):

$$R_{obs} = R_F + n_H(\Delta R)C_p \quad (4)$$

R_{obs} is the observed NMR relaxation rate corrected for inhomogeineity broadening, R_F is the relaxation rate of the free, liquid water, n_H is the protein hydration, and $\Delta R = R_B - R_F$. However, the observed nonlinear behavior of the NMR relaxation rates with concentration has been attributed to significant protein–protein interactions, such as charge repulsion, or to charge fluctuations as predicted by the Kirkwood–Shumaker theory (Kirkwood and Shumaker, 1952). These nonidealities can be accounted for if the protein concentration C_p in eq 4 is replaced by the protein activity a_p, where

$$a_p = C_p \exp(2B_0C_p + 2B_{0.5}C_p^{0.5} + 0.667B_{1.5}C_p^{1.5} + 1.5B_2C_p^{2} + \ldots) \quad (5)$$

All terms are as described above. The B_0 virial coefficient reflects the repulsive or attractive forces arising from the "net" protein charge (Z), the protein excluded volume ($\bar{v}_p$), and a preferential interaction term (Arakawa and Timasheff, 1982; Kumosinski et al., 1987)

$$2B_0 = Z^2/(4m_sM_p) + \bar{v}_p/1000 - (\partial g_s/\partial g_p)^2\,(1/m_s) \quad (6)$$

where m_s is the molarity of salt used, M_p is the protein molecular weight, $\bar{v}_p$ is the average partial specific volume of the caseins, and $\partial g_s/\partial g_p$ is the preferential binding term (grams of preferentially bound salt per gram of protein); this $\partial g_s/\partial g_p$ must be included to take into account the preferential interaction of Na^+, Cl^-, and water at the protein interface. The B_2 virial coefficient of eq 5 represents attractive forces arising from fluctuating multipoles. Under salt-free (isoionic) conditions, the $B_{0.5}$ and $B_{1.5}$ terms must be included in eq 5 to account for attractive effects due to charge fluctuations (Kirkwood and Shumaker, 1952; Timasheff et al., 1957; Pessen and Kumosinski, 1985). Essentially, the exponential term of eq 5 represents the activity coefficient; therefore, determination of these virial coefficients will allow for quantitation of the protein–protein interactions or the protein activity.

3. MATERIALS AND METHODS

3.1. Materials. All reagents used were of analytical grade or ACS certified. Deuterium oxide (99.8 atom % D) was obtained from Sigma (St. Louis, MO).

3.2. Preparation of Bovine and Caprine Caseins. Caseins were obtained from the milk of a Jersey cow and French-Alpine goats. The caprine milk caseins were selected as yielding high and low levels of the α_{s1}-casein component as determined by reversed-phase high-performance liquid chromatography (RP-HPLC) (Mora-Gutierrez et al., 1991). Caseins were isolated from 2 L of fresh, uncooled milk to which phenylmethanesulfonyl fluoride (0.1 g/L) was added immediately to retard proteolysis. The milk was centrifuged at 4000g for 10 min at room temperature to remove the cream fraction. Skim milk (500 mL) was diluted with an equal volume of distilled water and warmed to 37 °C. Casein was precipitated by careful addition of 1 N HCl to pH 4.6. The precipitate was homogenized with a Biospec homogenizer at low speed and dissolved by addition of NaOH to yield a solution of pH 7.0. The casein was reprecipitated, washed, and then resuspended. The sodium caseinate was subsequently cooled to 4 °C and centrifuged at 100000g for 30 min to remove residual fat. Finally, the casein suspensions were dialyzed exhaustively versus cold deionized water at 4 °C for 72 h, with three changes, adjusted to pH 7.0, and then lyophilized. The integrity of the samples was confirmed by RP-HPLC.

3.3. Preparation of Samples for NMR Measurements. A set of samples containing caseins was prepared by adding a measured volume of D_2O to a preweighed amount of powder and stirring until all protein dissolved. Samples were prepared with D_2O instead of H_2O so that oxygen-17 NMR measurements could be made without proton-exchange broadening (Lioutas et al., 1986). A second set of samples was prepared by adding incremental amounts of lyophilized caseins from 0.1 to 8.5% (w/v) to a constant ratio of salt (NaCl) to D_2O, of 0.21 M. All measurements were completed within 12 h after sample preparation.

The pD of the protein solutions was 6.95. Conversion to pD values was made according to the relation pD = pH + 0.4 (Covington et al., 1968), where pH is the pH meter reading for a D_2O solution with the electrode calibrated in standard H_2O buffers.

3.4. NMR Measurements. Natural abundance oxygen-17 (27.1 MHz) single-pulse NMR measurements were carried out with a Varian XL-200 spectrometer (Varian Associates, Palo Alto, CA) equipped with a high-resolution, narrow-bore (54 mm) 4.7 T superconducting magnet, a Varian 4000 series data system computer with Pascal software (v. 6.3), and a 10 mm broadband series 3 probe.

The oxygen-17 NMR spin–spin (T_2) relaxation parameter was determined by measuring the line widths at half-height for each spectrum at each concentration. To correct for any residual magnetic field inhomogeneity, the net line broadening ($\Delta\upsilon_B$) was calculated by subtracting the line width of liquid D_2O ($\Delta\upsilon_{free}$) from that of the sample ($\Delta\upsilon_{obs}$). The net or differential transverse relaxation rates (ΔR_2, s^{-1}) were then calculated from the line widths according to the standard formula

$$\Delta R_2\,(s^{-1}) = \pi(\Delta\upsilon_B)\,(s^{-1}) = \Delta T_2^{-1}\,(s) \quad (7)$$

About 4 mL of well-dispersed and thoroughly mixed protein in D_2O solutions (not pH-adjusted; pD 6.95) were run in 10 mm high-resolution NMR tubes (Wilmad, Buena, NJ). In all experiments two independent series of NMR measurements were conducted at 21 ± 1 °C. The oxygen-17 NMR 90° pulse width for D_2O was 19 μs, and 1024 scans were sufficient for a signal-to-noise ratio of 200:1 (with 5 Hz exponential line broadening applied). The spectral width was 5 kHz, the acquisition time was 0.50 s, and an 8K point–time–domain array was used for storing the data with adequate resolution.

3.5. Nonlinear Regression Analysis. The protein concentration dependences of bovine and caprine caseins were fitted with an iterative nonlinear regression program (Systat version 5.1) which employed the Quasi-Newton algorithm on a Macintosh II microcomputer (Apple Computer Inc., Cupertino, CA). This program minimizes the standard deviation of the experimental points from the curve, also known as the root

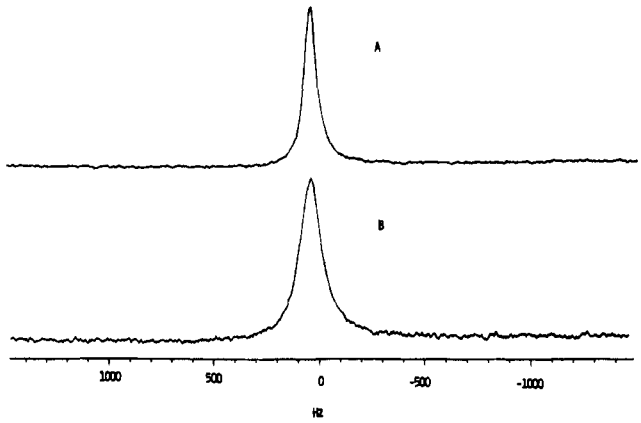

Figure 1. Oxygen-17 FT NMR spectra of caprine casein high in α_{s1}-casein at 21 ± 1 °C and pD 6.95: (A) 0.07% (w/v); (B) 4.62% (w/v) in D_2O.

mean square (RMS) where the RMS is defined as

$$\text{RMS} = \{[\Sigma(R_{2obs} - R_{2calc})^2]/(\text{no. of data points} - \text{no. of fitting parameters})\}^{1/2} \quad (8)$$

Here R_{2obs} and R_{2calc} are the observed and calculated transverse relaxation rates, respectively. RMS values were normalized to be within at least 5% error of the fit for all the data.

4. RESULTS AND DISCUSSION

4.1. Oxygen-17 NMR and Protein Hydration. Nuclei with spin $I > {}^1/_2$ possess electric quadrupole moments, and their relaxation behavior is usually dominated by the interaction of the nuclear quadrupole with the electric field gradient at the nucleus (Halle and Wennerström, 1981). This is the case for deuterium ($I = 1$) and oxygen-17 ($I = {}^5/_2$). Both the deuterium and oxygen-17 nuclei avoid the problem of cross-relaxation encountered with proton NMR of water in the presence of macromolecules (Pessen and Kumosinski, 1985). However, the deuterium nuclei are also affected by chemical exchange with the macromolecule, whereas the water oxygen-17 relaxation is not. The oxygen-17 nuclear spin relaxation can be affected only by the exchange of entire water molecules (or OH^- anions depending on the pH) and, therefore, it is the nucleus of choice for water–protein studies.

If the exchange of water molecules is fast on an NMR scale, which means that a water molecule has only a very short residence time on the protein before exchanging back into the bulk phase, the relaxation time observed will be a weighted average of bound and free water populations. Under these conditions, a single Lorentzian oxygen-17 NMR peak would be observed for water in a protein solution. The oxygen-17 NMR spectra of bovine and caprine caseins showed a single Lorentzian peak for protein concentrations up to 8.5% w/v (Figure 1), in agreement with a fast-exchange model for protein hydration (Derbyshire, 1982).

4.2. Protein Activity Effects in Bovine and Caprine Casein Solutions. The ratios of α_{s2}-, α_{s1}-, β-, and κ-caseins, for the samples used in this study, have been given (Mora-Gutierrez et al., 1993b). Oxygen-17 NMR transverse relaxation rates were determined as a function of protein concentration for bovine and caprine caseins at pD 6.95 in the absence of salt (Figure 2); there appears to be only a relatively small deviation from ideality (linearity) in the protein concentration range investigated (0–8.5% w/v) for solutions of caprine casein high in α_{s1}-casein (Figure 2B). Under the same experimental conditions a more marked deviation from

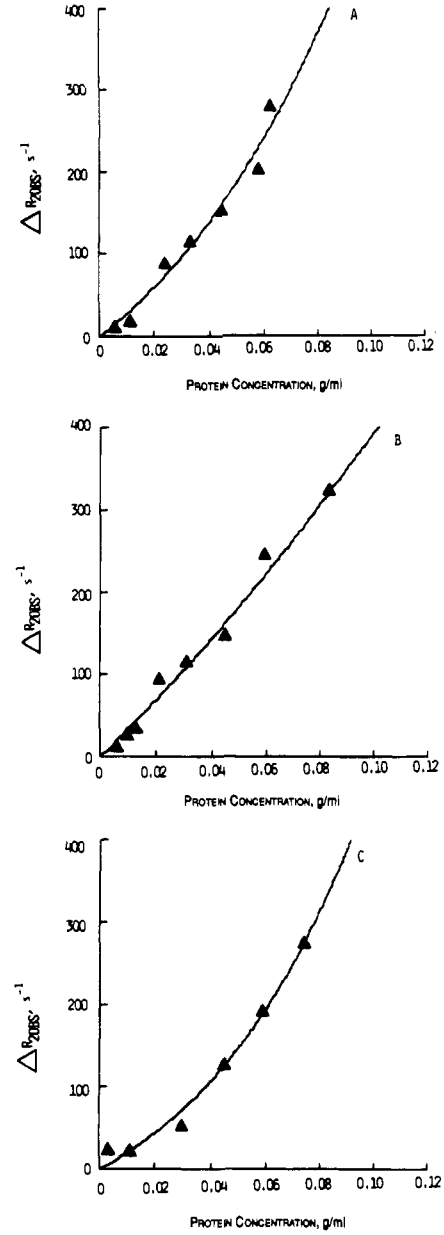

Figure 2. Dependence of the oxygen-17 NMR transverse relaxation rates, ΔR_2 (s^{-1}), on protein concentration (g/mL) for (A) bovine casein, (B) caprine casein high in α_{s1}-casein, and (C) caprine casein low in α_{s1}-casein in the absence of salt at 21 ± 1 °C and pD 6.95.

ideality was observed for solutions of the bovine and the caprine casein low in α_{s1}-casein (parts A and C of Figure 2, respectively). In all three cases there is an indication that protein–protein interactions are present (Pessen and Kumosinski, 1985). The results obtained from the nonlinear regression analysis of the data according to eqs 4 and 5 are presented in Table 1. In the protein concentration range from 0.1 to 8.5% (w/v) no other virial coefficient other than B_0 was necessary to fit the oxygen-17 NMR data as judged by no significant improvement in RMS.

Several investigators have fitted protein NMR data as a function of concentration to quantitate the extent of protein–protein and protein–solvent interactions (Kumosinski et al., 1987; Kakalis et al., 1990a). The virial coefficient for bovine casein from deuterium NMR (B_0 = 3.2 mL/g; Kumosinski et al., 1987) is somewhat in agreement with the virial coefficients obtained from oxygen-17 NMR (B_0 = 4.3 mL/g with 0.21 M salt and 3.6 mL/g without salt). The differences arise in part because these nuclei monitor different relaxation and

Table 1. Calculated Hydration Products $n_H\Delta R^a$ and Virial Coefficients B_0^b from Nonlinear Regression Analysis of Oxygen-17 NMR Transverse Relaxation Data for Bovine and Caprine Casein Solutions at 21 ± 1 °C and pD 6.95 According to Equations 4 and 5

	bovine	caprine, high in α_{s1}-casein	caprine, low in α_{s1}-casein
	A. No Added Salt		
$n_H\Delta R$	2595.3 ± 22.6	3358.9 ± 17.9	1806.27 ± 22.2
B_0	3.6 ± 0.2	0.8 ± 0.1	4.8 ± 0.2
	B. 0.21 M NaCl		
$n_H\Delta R$	2487.0 ± 23.4	3069.5 ± 18.4	1828.3 ± 22.0
B_0	4.3 ± 0.2	1.5 ± 0.1	3.8 ± 0.2

[a] mL g^{-1} s^{-1}. The protein concentration was in g of protein/mL of solvent. [b] mL/g.

exchange processes, and there are differences in the buffers as well. In this context, one advantage of probing the oxygen-17 nucleus is that the intramolecular origin of the electric field gradient at the water oxygen nucleus makes the quadrupolar interaction independent of the molecular environment (Halle et al., 1981). This not only greatly facilitates interpretation of the relaxation data but also allows a more reliable measure of protein hydration.

Timasheff et al. (1957) have shown that the B_0 values (obtained from light scattering data for isoionic BSA) are negative at very dilute salt concentrations (~1 mM NaCl), increase with the concentration of salt added, pass through zero at about 1.7 mM NaCl, and then become increasingly positive upon addition of more than 0.1 M NaCl. This was attributed to the progressive binding of chloride ions, resulting in an increasingly net negative charge on the protein and an electrostatic repulsion leading to increasingly positive values of B_0.

The net average charge of the caseins and the preferential binding of ions to these proteins make first-order contributions to the value of B_0, whereas the effect of the protein excluded volume makes a second-order contribution to the value of B_0. The observed increase in magnitude of the B_0 values in the presence of 0.21 M NaCl for bovine and caprine casein high in α_{s1}-casein is entirely consistent with Timasheff's theory (Figure 3; Table 1). The differences in magnitude between the B_0 values of the bovine and the caprine casein high in α_{s1}-casein in the absence and presence of salt (Table 1) might appear to suggest qualitatively that a lower proportion of charged sites for water and salt binding are present in that caprine casein. In contrast for the caprine casein low in α_{s1}-casein, the B_0 value decreases with added salt. Note that the hydration products ($n_H\Delta R$) for the caseins follow patterns which are inverse to those of the virial coefficients (B_0) for the caseins in the absence and presence of salt (Table 1). Preferential interactions between water and the charged sites of the caseins could be the cause of the observed differences in $n_H\Delta R$. The preferential binding term ($\partial g_s/\partial g_p$) calculated for caprine casein high in α_{s1}-casein is larger than those of the bovine casein and the caprine casein low in α_{s1}-casein (Table 2). This indicates that there is preferential binding of salt for the caprine casein high in the α_{s1}-component. If preferential salt binding occurs, the calculated extent of hydration should be greater for this caprine casein than for their bovine and caprine casein low α_{s1}-casein counterparts; from the hydration product $n_H\Delta R$ of Table 1, it is evident that there is an "excess" of water associated with the caprine casein high in α_{s1}-casein, although its net charge is not significantly different from that of the caprine casein low in the α_{s1}-component (Mora-Gutierrez et al., 1993b).

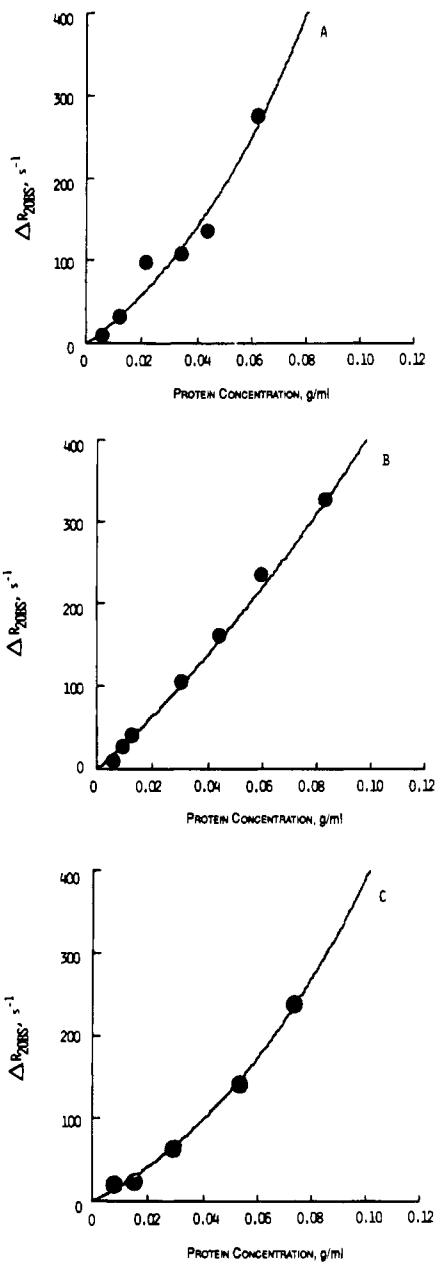

Figure 3. Dependence of the oxygen-17 NMR transverse relaxation rates, ΔR_2 (s^{-1}), on protein concentration (g/mL) for (A) bovine casein, (B) caprine casein high in α_{s1}-casein, and (C) caprine casein low in α_{s1}-casein in the presence of 0.21 M NaCl at 21 ± 1 °C and pD 6.95.

Table 2. Calculated Protein Net Charge Z and Preferential Binding Term ($\partial g_s/\partial g_p$) of Bovine and Caprine Caseins in D_2O at 21 ± 1 °C and pD 6.95 in 0.21 M NaCl Using Equation 6

casein	Z^a	$(\partial g_s/\partial g_p)^b$	$(\partial g_s/\partial g_p)^c$
bovine	−16.1	0.0336	
caprine			
high in α_{s1}-casein	−16.5	0.0480	0.0327
low in α_{s1}-casein	−16.1	0.0366	0.0102

[a] esu. [b] g of salt/g of protein. Calculated with B_0 values from Table 1; the average charge, Z, was chosen as −16.1 (Eigel, 1984), and a $\bar{v}_p$ of 0.736 (Kumosinski et al., 1987) was taken for an average partial specific volume of the caseins. [c] g of salt/g of protein. Calculated with B_0 values from Table 1, as above, except Z was chosen as −12.0 for the caprine caseins on the basis of their reported composition (Mora-Gutierrez et al., 1993b).

This suggests a different spatial orientation of net charge resulting from compositionally related protein–protein interactions.

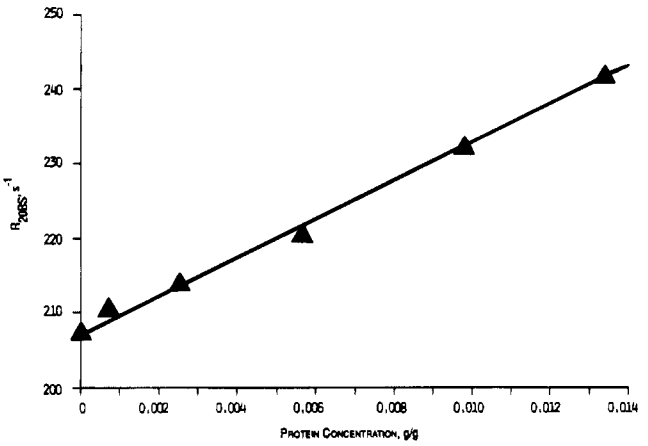

Figure 4. Dependence of the oxygen-17 NMR transverse relaxation rates, R_2 (s^{-1}), on protein concentration (g/g) at 21 ± 1 °C and pD 6.95 for the dilute concentration range of caprine casein high in α_{s1}-casein in the absence of salt. The straight line is $R_2 = 2411.17C_p + 208.09$ ($r = 0.9991$). The good linear fit in the protein concentration range from 0.00 to 0.014 g/g of water implies that in this range the protein activity does not differ greatly from the protein concentration.

Table 3. Hydration Estimates of Bovine and Caprine Caseins[a]

	hydration,[b] g of water/g of protein	
casein	no added salt	0.21 M NaCl
bovine	0.00356	0.00404
caprine		
high in α_{s1}-casein	0.00510	0.00572
low in α_{s1}-casein	0.00255	0.00257

[a] From oxygen-17 NMR data at 21 ± 1 °C and at pD 6.95, according to a two-state, isotropic model. [b] Assuming $\tau_c = 56$ ns for bovine casein submicelles (Kakalis et al., 1990b).

4.3. Calculation of the Hydration Number (n_H). We can use the oxygen-17 NMR data of bovine and caprine caseins in the protein concentration range from 0.00 to 0.02 g/mL (Figures 2 and 3) to calculate the amount of water bound (n_H). On the basis of the two-state, isotropic hydration model, the transverse relaxation rate of bound water, R_{2B}, can be calculated according to

$$R_{2B} = \tau_c[K(e^2qQ/h)^2(1 + (\eta^2/3) - S^2)] \qquad (9)$$

where τ_c is the correlation time of the water molecules, $K = 12\pi^2/125$, e^2qQ/h is the nuclear quadrupole coupling constant (Abragam, 1961), η is the asymmetry parameter for the electric field gradient at the nucleus, and S is the order parameter that describes the motion of water molecules bound to the protein. The oxygen-17 quadrupole coupling constant e^2qQ/h for liquid water or "hexagonal" ice, I_h, is 6.67 MHz (Halle et al., 1981), whereas the asymmetry parameter is $\eta = 0.93$. A value of 1.0 for the order parameter S (isotropic tumbling) is used. A value for τ_c of 56 ns was assumed (Kakalis et al., 1990b). With R_{2B} known, the amount of bound water (n_H) can be obtained from the slope of the R_{2obs} vs C_p plot; an example is shown in Figure 4. The amount of bound water (n_H) detectable by oxygen-17 NMR (Table 3) seems to be directly related to the nature and/or the number of water binding sites on the caseins. However, it should be pointed out that the amount of bound water (n_H) in this isotropic model is more likely to be internal (trapped) water in the protein.

Moreover, the results presented in Table 3 are based on experiments without $CaCl_2$ (casein submicelles). The casein submicelles that survive exhaustive dialysis against distilled water consist of protein only (Swaisgood, 1983), and the hydration values for oxygen-17 NMR are in accord with those found by deuterium NMR (0.0065 g of H_2O/g of protein) for bovine casein in 0.08 M KCl (Kumosinski et al., 1987). However, the binding of water by the bovine casein submicelles as measured by deuterium NMR was quite different from that of micelles (Kumosinski et al., 1987); on average, bovine casein micelles were 2–3 times more hydrated than the submicelles. These authors postulated that the incorporation of submicelles into micelles by calcium phosphate salt bridges leads to an increase in internal hydration (trapped water). In contrast, salt (NaCl) exerts little influence on casein hydration properties (Table 3). Kuntz and Kauzmann (1974) suggested that the increased hydration of globular proteins in the presence of salts is due to binding of hydrated ions to protein charged groups, thus increasing the protein charge and the amount of bound water. However, the lack of substantial changes in hydration with added salt for each casein sample (Table 3) argues for the concept that the water disclosed by NMR relaxation techniques is internal trapped water and not bound to surface charges. Kumosinski et al. (1994) have suggested a mechanism to account for the nature of trapped water in casein submicelles by comparison of putative 3D models and small-angle X-ray scattering data. It could be that compositional differences reflect differences in protein–protein interactions which in turn affect the amount of water trapped by submicelle particles as viewed by oxygen-17 NMR.

4.4. Calculation of Bovine and Caprine Casein Net Average Charge. Using the average B_0 values obtained by nonlinear regression of the oxygen-17 NMR data for bovine and caprine caseins in D_2O at pD 6.95 with 0.21 M NaCl (Table 1), we were able to evaluate a net average charge, Z, per protein molecule of average molecular mass, M_p = 23 300 Da, using eq 6. The results are presented in Table 2. These calculated Z values are in close agreement with the average value of −16.1 derived from the amino acid sequences of bovine caseins (Eigel et al., 1984) and using the ratios of casein components of Davies and Law (1980). The values for the two caprine caseins differ significantly from approximations for caprine caseins (−12.0) using the compositional data of Mora-Gutierrez et al. (1993b) and the average net charge values of casein components of Swaisgood (1983). By assuming a net charge of −12.0 for the two caprine caseins (a reflection of their elevated β-casein contents), lower preferential interaction terms of 0.0327 and 0.0102 g of salt/g of protein can be calculated for the caprine caseins high and low in α_{s1}-casein, respectively. The actual effects that contribute to B_0 arise from a variety of kinds of molecular interactions (protein–protein, salt–water, salt–salt), and so these calculations must be considered at best approximations. However, the differences observed ultimately derive from the compositional differences. The low salt binding by the caprine casein low in the α_{s1}-component may reflect that cheeses made from this type of milk have a very soft texture (Ambrosoli et al., 1988). In contrast, the caprine casein high in α_{s1}-casein has a preferential interaction term closer to that of bovine casein.

5. CONCLUDING REMARKS

Oxygen-17 NMR transverse relaxation rates of bovine and caprine casein solutions at 21 °C and pD 6.95 exhibit complex nonlinear dependences on protein concentration; such variations are, however, modeled at all concentrations for the activity of proteins. Good fits of

the oxygen-17 NMR data for bovine and caprine caseins were obtained with eqs 4 and 5, which do not include any higher order virial coefficients than B_0, indicating that repulsive interactions between casein molecules dominate their hydration and ion binding properties. The major differences in hydration in caseins arise most likely from altered protein composition resulting in altered protein–protein interactions which affect hydration behavior and preferential interactions. All of this shows that composition can change or alter properties.

Additional oxygen-17 NMR studies of caseins with other salts (KCl, $CaCl_2$) are likely to yield further insight into the effects of electrolytes on the aggregation, activity, and hydration properties of bovine and caprine caseins.

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