

ABSTRACT

The formation of rennet gels made with native or dephosphorylated (40 and 93%) whole CN was monitored using a dynamic oscillatory shear spectrometer with a Couette geometry sample holder. Casein solutions were prepared with different Ca^{2+} :CN ratios and treated with rennet prior to loading into the sample holder. Gels formed when all the individual CN had either all or none of their phosphate groups, but only weak localized gels formed when the CN were at multiple levels of dephosphorylation. The rheological properties of gels with the highest storage (elastic) and loss (viscous) moduli 1 h after gel formation were compared [Ca^{2+} :CN ratios of .6 (native) or .3 (dephosphorylated)]. Rennet gels of maximally (93%) dephosphorylated CN had similar coagulation times and gel strengths, had lower $\tan \delta$ (the viscous modulus divided by elastic modulus), and formed at lower Ca^{2+} :CN ratios over a narrower range than did native CN. Partially (40%) dephosphorylated CN rennet gels were similar to maximally dephosphorylated casein gels in $\tan \delta$ and Ca^{2+} :CN ratios of formation but, compared with both native and maximally dephosphorylated CN gels, were much weaker and had much longer coagulation times. Electron microscopy showed that native CN micelles had fused together to form smooth columns but that the dephosphorylated CN had formed smaller dis-

crete aggregates that clustered together to form a more compact matrix.

(**Key words:** casein, gel, dephosphorylated, microstructure)

Abbreviation key: DP = dephosphorylated, G' = storage (elastic) modulus, G'' = loss (viscous) modulus, η^* = complex viscosity, $\tan \delta$ = viscous modulus divided by elastic modulus.

INTRODUCTION

The gelation of bovine CN with rennet (chymosin) is an essential step in the production of some dairy foods. The formation and strength of the gel are affected by many parameters that are still not fully understood (i.e., the roles of phosphate and phosphate- Ca^{2+} interactions in gel formation). Removal of phosphate groups from the CN alters Ca^{2+} binding (2, 21, 24), stability to Ca^{2+} (13, 18, 21, 24, 25), and micelle formation (1, 2, 12, 13, 16). Qualitative rennet coagulation studies report lower curd tensions for gels of dephosphorylated (DP) CN (11, 14, 22, 26) and longer coagulation times for DP CN micelles (7, 12, 14), artificial micelles made with DP CN (12), and DP whole CN (22). The microstructures of DP CN micelles (8, 23) and acid curds (9) show major differences from the microstructure of native CN micelles and acid curds.

In earlier studies, curd tension meters were used to measure the strength of DP CN curds (11, 22, 26). Oscillatory rheometers, utilizing a Couette type cup and bob, have been used for detailed study of the rheological properties of fragile milk gels (4, 20, 27, 28, 29). Little basic, detailed information is available on rheological properties of gels made with CN of different degrees of phosphorylation with different ratios of Ca^{2+} :CN. The purpose of this research was to examine the effects of dephosphorylation on the rheological proper-

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ties and microstructure of rennet gels at different Ca^{2+} :CN ratios.

MATERIALS AND METHODS

DP CN

Raw bovine skim milk was adjusted to pH 4.6 using 1 *N* HCl to precipitate the CN. The isolated acid CN was washed with water and stored frozen. Thawed acid CN was solubilized in water at pH 7 with 1 *M* NaOH and lyophilized to make sodium caseinate. Batches of aqueous sodium caseinate (2.5 mg/ml) at pH 6.5 were treated with potato acid phosphatase (Calbiochem, La Jolla, CA) according to the procedure of Bingham et al. (3). Partially DP whole CN was obtained by addition of .065 units of enzyme/ml of CN solution to aqueous CN solution, incubated 1 h in a shaking water bath (100 rpm) at 37°C, heated to 80°C for 5 min to inactivate the enzyme, and dialyzed against deionized water at 4°C to remove phosphates. Maximally DP CN was obtained by addition of .13 units of enzyme/ml of CN solution to aqueous CN solution, incubated for 2 h, and dialyzed extensively. Caseins were lyophilized, and samples were digested in sulfuric acid to determine total P content (17). Native whole CN had a P content of .230 mmol of P/g of CN, and the modified CN had .139 mmol of P/g of CN (40% or partially DP) and .016 mmol of P/g of CN (93% or maximally DP).

Urea-PAGE

Urea-PAGE was used to confirm the degree of CN modification. Profiles of DP individual and whole CN were obtained using the Phast-System® (Pharmacia, Uppsala, Sweden) as described by Van Hekken and Thompson (19). A 6.6 *M* urea, .112 *M* Tris-HCl, .112 *M* acetate buffer, pH 6.4, was used to modify the 8 to 25% gradient ultrathin gel prior to use and to solubilize the CN samples before mercaptoethanol (1%) and bromophenol blue tracking dye (.025%) were added.

Rennet Gels

Aqueous stock solutions (5.5% CN, .02% sodium azide, pH 7.0) were prepared for native

and for partially and maximally DP CN and kept frozen until needed. Stock solution (7.3 g) was weighed into a small vial and stirred continuously with addition of 5 *M* CaCl_2 to a final concentration of .2 to 2 mmol of Ca^{2+} /g of CN. The Ca^{2+} :CN solution was adjusted to pH 6.3 with either HCl or NaOH, brought to a final 5% CN concentration, capped, and stirred overnight at room temperature (22°C). A 7.0-ml aliquot of Ca^{2+} :CN solution was treated with 70 μl of 1% rennet (number 01034 single strength rennet; Chr. Hansen's Lab., Inc., Milwaukee, WI), mixed, and pipetted immediately into a sample holder.

Rheological Properties

Formation of gels made of native and partially and maximally DP CN was monitored using a dynamic oscillatory shear spectrometer (RDA-700; Rheometrics, Inc., Piscataway, NJ) set at 2% strain, a frequency of 1 rad/s, and 22°C and fitted with a sample holder of Couette geometry (cup radius, 18.5 mm; bob radius, 17.5 mm; bob length, 37.08 mm). Rhios software (Rheometrics Inc.) was used to program the RDA-700 for time sweeps with a maximum of 6 h, to collect data, and to calculate moduli. Coagulation time was taken as the time after rennet addition at which the storage (elastic) modulus (G') exceeded the loss (viscous) modulus (G'') ($G''/G' < 1.0$). One hour after gel formation, the G' , G'' , and G''/G' ($\tan \delta$) of rennet gels of native and modified CN were calculated for samples that registered torque values above 1×10^{-1} g.cm. Immediately after the time sweeps, frequency sweeps were run using 2% strain and frequencies of .1 to 10 rad/s. Rheological data for each sample, run in triplicate, were analyzed using ANOVA and Bonferroni *t* test ($P = .05$) (15).

Microstructure

Samples for electron microscopy studies were taken from 2 ml of renneted solutions, prepared as described, of native and of partially and maximally DP CN at Ca^{2+} :CN (millimoles per gram) ratios of .6, .3, and .3, respectively. Native and maximally DP CN formed gels throughout the entire 2 ml, and the partially DP solution formed a loose gel-like pellet that settled to the bottom of the test tube.

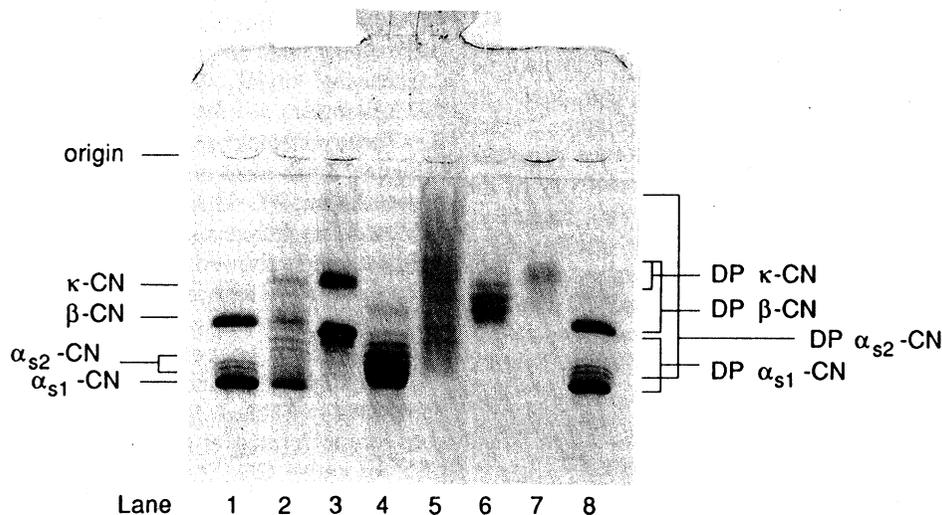


Figure 1. Urea-PAGE profiles of bovine CN using 8 to 25% gradient gel and the PhastSystem® (Pharmacia, Uppsala, Sweden). Samples by lanes are lanes 1 and 8, native whole CN; lane 2, partially dephosphorylated (DP) whole CN; lane 3, maximally DP whole CN; lane 4, partially DP α_{s1} -CN; lane 5, partially DP α_{s2} -CN; lane 6, partially DP β -CN; and lane 7, partially DP κ -CN. Labels for native casein bands are to the left, and ranges for DP casein bands are to the right.

One hour after gel formation (determined visually), undisturbed CN gels were overlaid with 10 ml of 1% glutaraldehyde in .1 M sodium cacodylate buffer at pH 6.3. Samples were fixed at room temperature for 3 h and stored overnight at 4°C. Samples to be examined using a JSM-840A scanning electron microscope (JEOL USA, Peabody, MA) were dehydrated in a graded series of ethanol solutions, freeze-fractured in liquid N, dried in CO₂ to critical point, and sputter-coated with gold according to a modified method of Modler et al. (10). Mean diameters of CN units that formed the protein matrix were calculated from micrographs at a magnification of 10,000 ($n = 107$), which included a nominal thickness of gold coating of 20 nm. Samples to be examined using a scanning transmission electron microscope (CM-12; Philip Electronics Institute Co., Eindhoven, The Netherlands) were postfixed in 2% osmium tetroxide in the cacodylate buffer, washed, dehydrated in a graded series of ethanol solutions, and embedded in an epoxy resin mixture. Thin sections were cut with diamond knives and stained with aqueous solutions of uranyl acetate and lead citrate.

RESULTS

Urea-PAGE

Urea-PAGE separates proteins according to their charge to mass ratio. As reported in earlier studies (3, 9, 19, 21), the removal of the negatively charged phosphate groups caused the DP CN to migrate more slowly than the native CN. The whole CN used in the functional and rheological studies are shown in Figure 1. Native whole CN (lanes 1 and 8) had one major band each for α_{s1} -CN and β -CN, a minor doublet for α_{s2} -CN, and a very faint doublet for κ -CN. When compared with the partially DP α_{s1} -CN, α_{s2} -CN, β -CN, and κ -CN (lanes 4, 5, 6, and 7, respectively), the partially DP whole CN (lane 2) showed multiple DP bands for α_{s1} -CN and β -CN, indicating different P content. The bands for DP α_{s2} -CN and κ -CN were either too faint or were masked by the α_{s1} -CN and β -CN bands. Maximally DP CN (lane 3) showed most of the α_{s1} -CN and all of the β -CN migrating in the same manner as bands of fully DP α_{s1} -CN and β -CN. Only faint indications of protein bands for DP α_{s2} -CN and κ -CN were noted.

Rheological Properties

Rheological data for a typical sample collected during the time sweeps are shown in Figure 2. The initial G' (elastic modulus), G'' (viscous modulus), and $\tan \delta$ (phase angle) for native and maximally DP CN (Figure 2, a and b) were scattered because the torque generated was $<.2$ g·cm, the minimum sensitivity of the transducer. At the initiation of gel formation, the G' and G'' rose rapidly, while $\tan \delta$ decreased to a fairly constant value <1.0 . The G' and G'' for native CN leveled off within .5

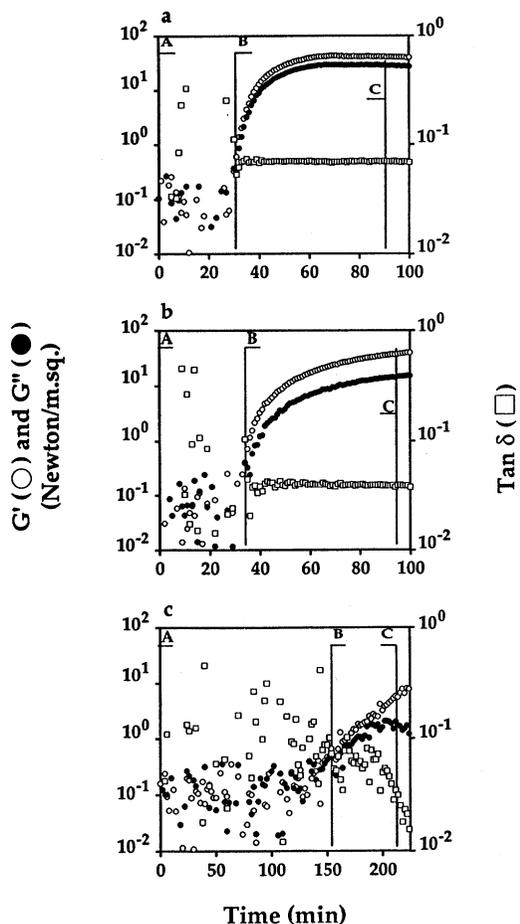


Figure 2. Example of data collected from time sweeps for renneted a) native CN at Ca^{2+} :CN of .6, b) maximally dephosphorylated (DP) CN at Ca^{2+} :CN of .3, and c) partially DP CN at Ca^{2+} :CN of .3. A = Addition of rennet, B = gel formation, and C = 1 h after gel formation, G' = elastic modulus, G'' = viscous modulus, and $\tan \delta = G''/G'$.

h, while maximally DP CN continued to increase slowly. In the partially DP CN samples 1 h after gel formation (Figure 2c), the G' was still rising, the G'' had leveled off and was decreasing slightly, and the $\tan \delta$ was still decreasing.

The mechanical spectra (frequency sweeps) (Figure 3) showed that the G' values were greater than G'' values at all frequencies and increased as frequency increased; the complex viscosity (η^*) showed an inverse relationship. This behavior is consistent with the frequency-dependent behavior of a solid lightly cross-linked viscoelastic polymer (5).

Rheological properties of whole CN are presented in Table 1. Stable gels with measurable torque formed at Ca^{2+} :CN ratios of .4 to 1.5 for native CN, .2 to .6 for maximally DP CN, and at .3 only for partially DP CN, although coagulation of CN was noted at higher Ca^{2+} :CN ratios. The rheological properties of the gels that had the highest G' and G'' (Ca^{2+} :CN ratios of .6 for native and .3 for the modified CN) were compared. Coagulation times, the time from addition of rennet to when $\tan \delta$ (G''/G') decreased to <1.0 , were the same for native and maximally DP CN (32 to 33 min) but were significantly longer (over 163 min) for partially DP CN. Elastic moduli (G') of native and maximally DP CN gels were not significantly different, although the native CN gels had larger viscous moduli (G'') than maximally DP CN gels. Partially DP CN formed significantly weaker gels; both G' and G'' were smaller than those of native and maximally DP CN gels. The $\tan \delta$ values for the modified CN gels were similar to each other and lower than those for native CN, indicating that the properties of the modified CN gels were more elastic and less viscous than those of the native CN gels.

Microstructure

Gel microstructures of native and DP CN at Ca^{2+} :CN of .6 and .3, respectively, 1 h after gel formation were examined. Scanning electron microscopy showed that native CN gels (Figure 4a) had a typical open spongy appearance in which the CN micelles fused together to form an open network of large smooth CN strands. The diameter of the native whole CN

subunit was 930 ± 240 nm. The modified CN gels (Figure 4b, partially DP CN, and 4c, maximally DP CN) had smaller interstitial space, and the strands were made of smaller subunits (diameter of 190 ± 70 nm for partially DP CN and 200 ± 70 nm for maximally DP CN), which had only aggregated together and not fused into smooth strands.

Transmission electron microscopy showed that the strands of native and maximally DP CN gels (Figure 5, a and b, respectively) had a solid and uniform distribution of electron dense material (stained protein). In the strands

of partially DP CN gels (Figure 5c), the individual CN aggregates were visible, suggesting that, even after rennet destabilization, the protein aggregates still had strong repulsive charges and maintained small spaces between the individual aggregates.

DISCUSSION

Dephosphorylation reduces the net negative charge of the CN and alters the charge distribution along the protein chain, which influences the way the protein associates with itself and other proteins. In Ca^{2+} solutions, native CN associate through protein-protein and protein- Ca^{2+} interactions to form sub-micelles (mean diameter of 10 to 15 nm) and are stabilized by colloidal calcium phosphate in larger micelles (mean diameter of 100 nm) (6). The DP CN still form aggregates of CN in the presence of Ca^{2+} , although dephosphorylation removes the majority of Ca^{2+} -binding sites (2, 21) and leaves only the weaker binding sites of the glutamic and aspartic acid residues (2). The loss of phosphate groups reduces the number of bridging points for calcium phosphate (1) and results in abnormal micelles, which are less stable in the presence of Ca^{2+} (13) and smaller (12, 13, 16). Dephosphorylation can occur naturally. An enzyme active in soured milk (acid phosphatase) dephosphorylates the CN in existing micelles, which causes the disruption of the micelle into smaller sub-micelles (8).

The matrix of a rennet gel is made up of CN micelles associated together. In our study, the native CN fused together in units about nine times larger in diameter than typical micelles, and the DP CN associated into units about one-fourth the size of the native casein units but still about twice the diameter of micelles in fluid milk. The DP CN (regardless of degree of DP) associate differently and in smaller units than the native CN.

Enzyme coagulation of milk proteins occurs in two phases: enzymatic (cleavage of κ -CN to form para κ -CN and glycomacropeptide) and nonenzymatic (coagulation of destabilized milk proteins). Dephosphorylated CN coagulates when treated with rennet. The loss of phosphate groups on the CN does not inhibit the enzymatic phase (22). However, the phosphate groups are very important in the nonenzymatic

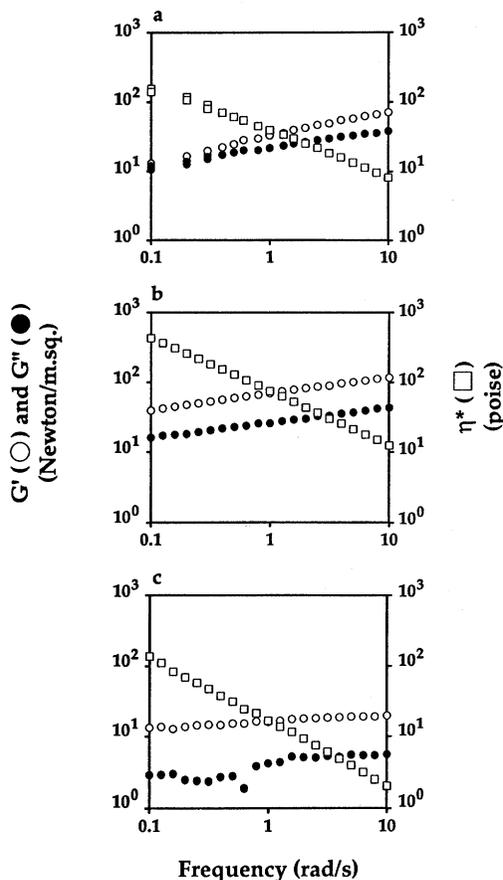


Figure 3. Example of data collected from frequency sweeps for renneted a) native CN at Ca^{2+} :CN of .6, b) maximally dephosphorylated (DP) CN at Ca^{2+} :CN of .3, and c) partially DP CN at Ca^{2+} :CN of .3. G' = Elastic modulus; G'' = viscous modulus, and η^* = complex viscosity.

phase because longer coagulation times have been reported for DP CN (14, 22).

In our study, the DP CN gels are made up of small CN aggregates that required less Ca^{2+} than native CN to form. The Ca^{2+} :CN ratios at which gel formation occurs correspond well to the Ca^{2+} concentration needed to initiate CN (native and DP) precipitation (18). A pH of 6.3 was selected as the pH for the gelling experiments because pilot studies showed that partially DP CN would not gel pH >6.3, DP CN were not soluble pH <6.0, and highest G' were obtained for aged gels at pH 6.15 (29). Native caseinate (2.8%) solutions form rennet gels at pH 6.6 if at least 13 mM Ca^{2+} are present (28).

In our study, as in past studies (7, 12), partial dephosphorylation had more effect on the coagulation time than did maximal dephosphorylation. Pearse et al. (12) report that maximally DP β -CN has less effect on the micelle surface properties and coagulation than the native or partially DP β -CN. Hsu et al. (7)

found partially DP CN difficult to coagulate; he was unable to coagulate partially (25 to 55%) DP CN with .011 M CaCl_2 at pH 6.8, but did see the appearance of graininess in his milk film at .015 and .020 M CaCl_2 (Ca^{2+} :CN approximately .4, .6, and .8, respectively). In our study, partially DP CN formed a stable gel with measurable torque only at a Ca^{2+} :CN ratio of .3 and at pH 6.3.

The phosphate groups affected the strength of the curd. In studies using curd tension meters, softer and more fragile curds are formed in test milk (estimated Ca^{2+} :CN of .3) that has been partially DP (11, 26). Compared with milks of native CN, test milks of maximally DP whole CN (estimated Ca^{2+} :CN of .45) have similar coagulation times but zero curd tension (measured 10 min after the addition of rennet).

In most curd strength studies, the native and DP CN were compared at the same Ca^{2+} :CN ratios. In our study, comparison of native and

TABLE 1. Effect of Ca^{2+} :CN ratio on the rheological properties of native [0% dephosphorylated (DP)] and modified [partially (40% DP) and maximally (93% DP)] whole CN.

	Ca^{2+} :CN						
	.2	.3	.4	.6	1.0	1.5	2.0
Coagulation time, ¹ min							
Native	124bcd	32 ^e	32 ^e	61 ^{cde}	127 ^{bc}
Partially DP	* ²	155 ^{ab}	156 ^{ab}	**	**
Maximally DP	54 ^{cde}	33 ^e	43 ^{de}	39 ^e	83 ^{bcde}
Elastic modulus ³ (G'), N/m ²							
Native	10.9 ^{de}	34.5 ^{ab}	33.4 ^{ab}	8.4 ^e	**
Partially DP	*	3.6 ^e	***	**	**
Maximally DP	21.0 ^{cd}	38.0 ^a	25.2 ^{bc}	11.4 ^{de}	**
Viscous modulus ⁴ (G''), N/m ²							
Native	7.0 ^{de}	24.2 ^a	18.7 ^{ab}	4.3 ^{de}	**
Partially DP	*	1.9 ^e	***	**	**
Maximally DP	8.5 ^{cd}	14.7 ^{bc}	9.9 ^{cd}	4.5 ^{de}	**
Tan δ ⁵ (G''/G')							
Native651 ^{ab}	.703 ^a	.560 ^{abc}	.505 ^{bc}	**
Partially DP	*	.454 ^c	***	**	**
Maximally DP	.404 ^c	.388 ^c	.391 ^c	.395 ^c	**

^{a,b,c,d,e}Data were analyzed using ANOVA and Bonferroni t test. Within each category (coagulation time, G' , G'' , and tan δ), means with no superscript letter in common are significantly different ($P < .05$).

¹SEM = 27.

²* = No gel formed, ** = gel had insufficient torque for measurement, and *** = gel broke within 30 min after gelation.

³SEM = 3.9.

⁴SEM = 2.2.

⁵SEM = .064.

maximally DP CN at the same Ca^{2+} :CN ratio (6) showed that the G' of maximally DP CN was significantly less than that of the native CN. Gels of similar G' were obtained if the Ca^{2+} :CN ratio was decreased to .3 for maximally DP CN. Therefore, our study compared gels at optimal Ca^{2+} :CN ratios.

A substance responds to an applied stress in an elastic (G' ; measures the energy stored and

then released) and viscous (G'' ; measures the energy lost during a cycle of deformation) manner. Zoon et al. (27, 28, 29) described G' and G'' as proportional to the number of effective bonds (intermolecular crosslinkages) within a substance and described gels with higher G''/G' ($\tan \delta$, indicates the dynamic character of the substance) as having more mobile bonds and more viscous, less elastic

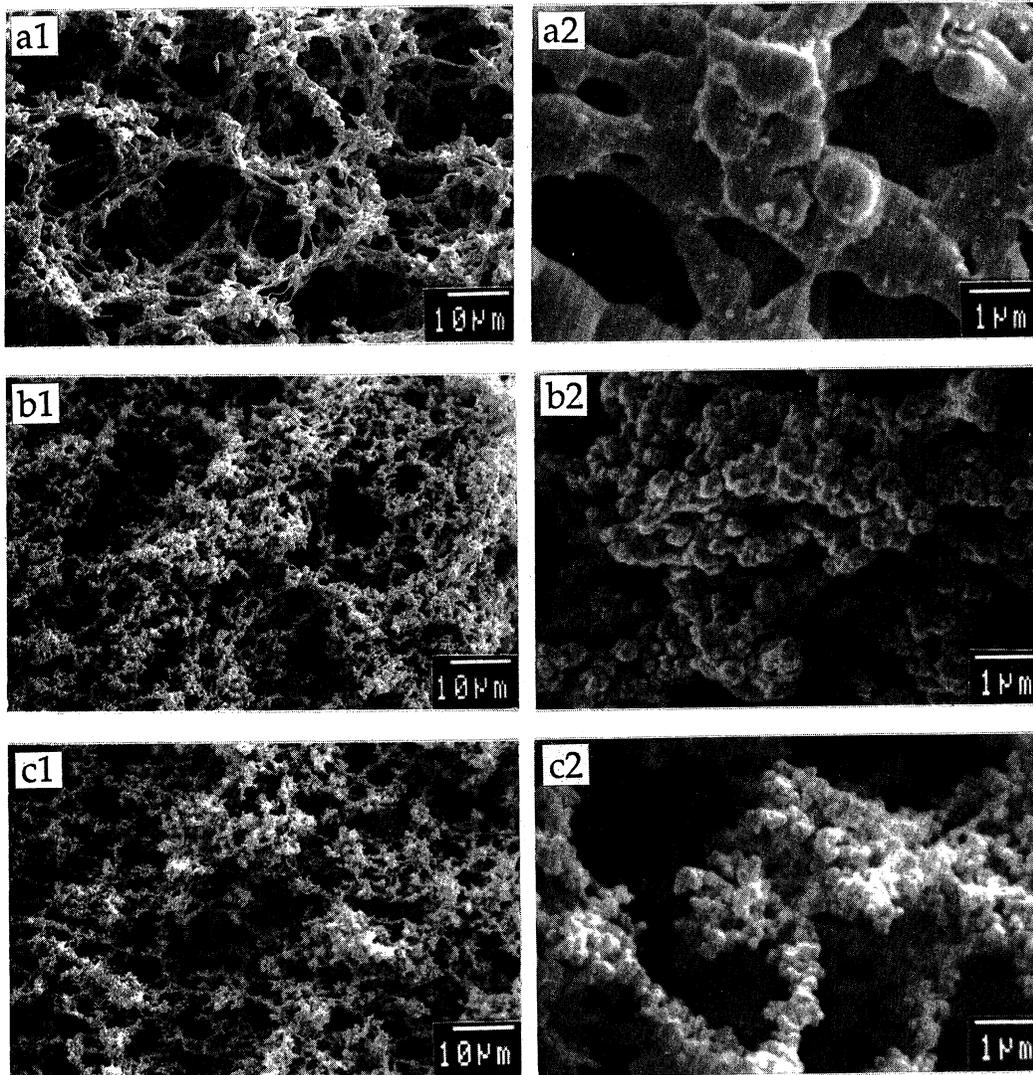


Figure 4. Scanning electron micrographs of renneted whole CN gels preserved 1 h after gel formation in glutaraldehyde. Samples are a) native, b) maximally dephosphorylated, and c) partially dephosphorylated CN. Scale bars are 1 = 10 μm and 2 = 1 μm .

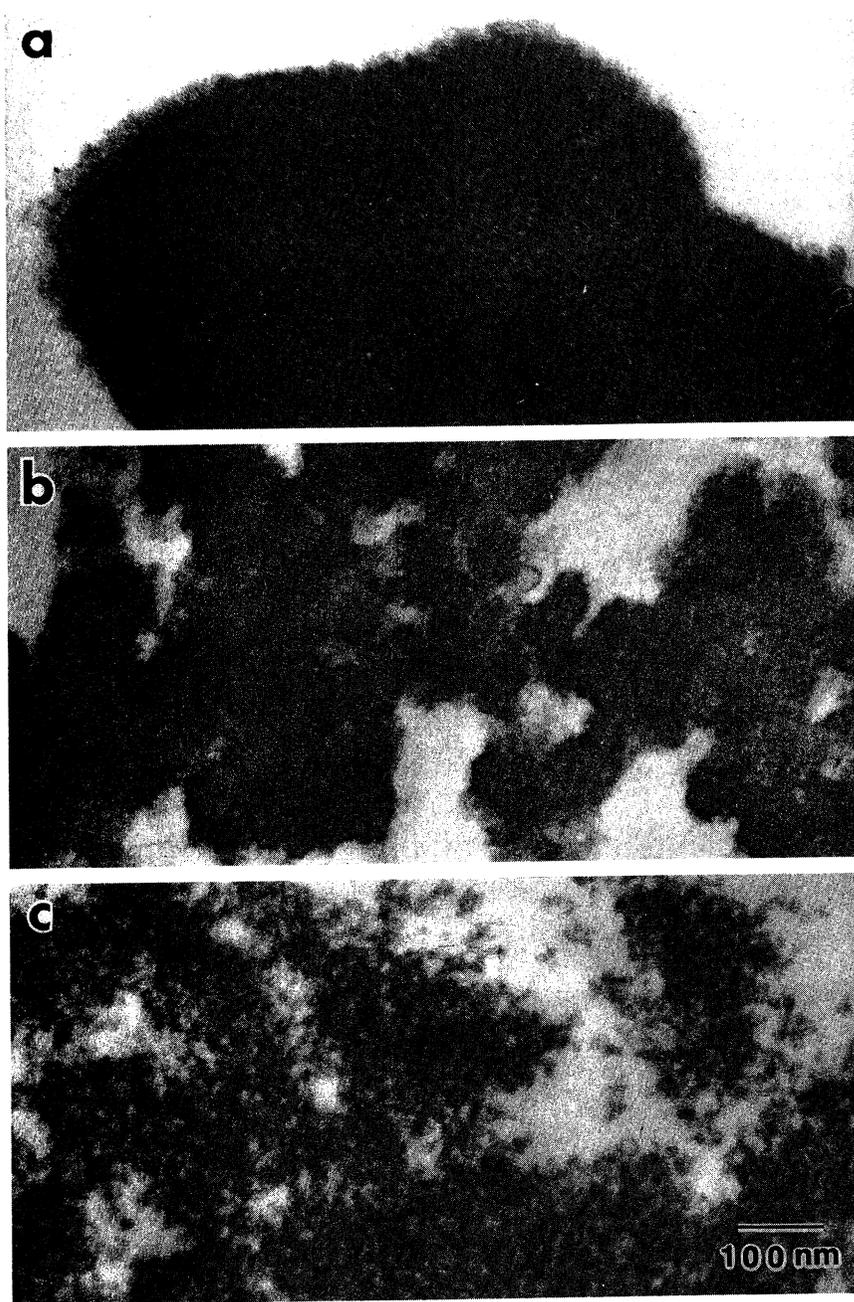


Figure 5. Transmission electron micrographs of renneted whole CN gels preserved 1 h after gel formation in glutaraldehyde. Samples are a) native, b) maximally dephosphorylated, and c) partially dephosphorylated CN. Scale bar is 100 nm.

properties. In acid gels of renneted artificial CN micelles, slight reductions in the concentration of colloidal calcium phosphate (essential bridging material) reduced moduli but did not change $\tan \delta$, and 50% reductions increased $\tan \delta$, indicating a more mobile structure.

In our study, the DP CN rennet gels had lower $\tan \delta$ than native casein rennet gels, indicating a more elastic behavior. Although both DP CN gels had similar $\tan \delta$, the distribution of protein in the gel matrix columns was much denser (Figure 5) for maximally DP CN than for partially DP CN, which have lower G' and G'' values.

Although the microstructures of gels were different, maximally DP CN gels were comparable in strength to native CN gels. Transmission electron microscopy showed that the matrix strands for both native and maximally DP CN consisted of electron dense material. The proteins had associated together to give a solid support although the native CN strand was smooth and the maximally DP CN strand was very rough. The partially DP CN had heterogeneous phosphate content, resulting in heterogeneous conformation. The gel formed of partially DP CN did not involve the entire solution (estimated 20 to 30% of protein involved in gel) but consisted of small CN particles that associated similarly to maximally DP CN and settled out of the solution. The heterogeneity of composition of the partially DP CN may have resulted in decreased intermolecular associations, leading to the formation of an insufficient number of stable colloidal CN particles from which the gel matrix was formed. Therefore, even with extended coagulation time, the partially DP CN were unable to associate into dense, uniform strands (as seen by spaces between CN subunits in the scanning transmission electron microscope) and could form only a weak, localized gel.

Whole CN, made up of the differently charged α_{s1} -CN, α_{s2} -CN, β -CN and κ -CN, associates together to form a gel when the CN have either all or none of their phosphate groups. When the four CN are at multiple dephosphorylation (0 to 100%), the proteins can only undergo limited associations that result in weak, localized gels.

CONCLUSIONS

Removal of the phosphate groups from CN alters the protein by reducing net negative charge and changing the charge distribution. This removal also changes the tertiary structure and alters interactions and associations between self and other components. Compared with native CN gels, maximally DP CN at appropriate Ca^{2+} :CN form strong, stable gels with more elastic behavior. Dephosphorylation produces modified CN, which, upon treatment with rennet, forms gels that are more elastic and unique in microstructure. These rheological properties may be desirable in the production of novel dairy foods.

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REFERENCES

- 1 Aoki, T., N. Yamada, I. Tomita, Y. Kako, and T. Imamura. 1987. Caseins are cross-linked through their ester phosphate groups by colloidal calcium phosphate. *Biochim. Biophys. Acta* 911:238.
- 2 Bingham, E. W., H. M. Farrell, Jr., and R. J. Carroll. 1972. Properties of dephosphorylated α_{s1} -casein. Precipitation by calcium ions and micelle formation. *Biochemistry* 11:2450.
- 3 Bingham, E. W., H. M. Farrell, Jr., and K. J. Dahl. 1976. Removal of phosphate groups from casein with potato acid phosphatase. *Biochim. Biophys. Acta* 429:448.
- 4 Dejmek, P. 1987. Dynamic rheology of rennet curd. *J. Dairy Sci.* 70:1325.
- 5 Ferry, J. D. 1980. Illustrations of viscoelastic behavior of polymeric systems. Page 33 in *Viscoelastic Properties of Polymers*. 3rd ed. John Wiley & Sons, Inc., New York, NY.
- 6 Fox, P. F. 1989. The milk protein system. Page 1 in *Developments in Dairy Chemistry—4. Functional Milk Proteins*. Elsevier Appl. Sci., New York, NY.
- 7 Hsu, R.Y.H., L. Anderson, R. L. Baldwin, C. A. Ernstrom, and A. M. Swanson. 1958. Rennin coagulation of enzymatically dephosphorylated casein. *Nature (Lond.)* 182:798.
- 8 Knoop, A. M., and K. H. Peters. 1975. Phosphatase activity in acidified milk. *Milchwissenschaft* 30:674.
- 9 Li-Chan, E., and S. Nakai. 1989. Enzymic dephosphorylation of bovine casein to improve acid clotting properties and digestibility for infant formula. *J. Dairy Res.* 56:381.
- 10 Modler, H. W., S. H. Ylu, U. K. Bollinger, and M. Kalab. 1989. Grittiness in a pasteurized cheese spread. *Food Microstruct.* 8:201.

- 11 Ohmiya, K., S. Sugano, S.-E. Yun, and S. Shimizu. 1983. Immobilization of acid phosphatase and its use for dephosphorylation of casein. *Agric. Biol. Chem.* 47:535.
- 12 Pearse, M. J., P. M. Linklater, R. J. Hall, and A. G. Mackinlay. 1986. Effect of casein micelle composition and casein dephosphorylation on coagulation and syn-eresis. *J. Dairy Res.* 53:381.
- 13 Pepper, L., and M. P. Thompson. 1963. Dephosphorylation of α_s - and κ -caseins and its effect on micelle stability in the κ - α_s -casein system. *J. Dairy Sci.* 46:764.
- 14 Reimerdes, E. H., and G. Roggenbuck. 1980. *Chemie und technologie der milchproteine. 1. Die modifizierung von β -casein und caseinmicellen durch saure phosphatase aus kartoffeln.* *Milchwissenschaft* 35:195.
- 15 SAS/STAT® Guide for Personal Computers, Version 6 Edition. 1987. SAS Inst. Inc., Cary, NC.
- 16 Schmidt, D. G., and J. K. Poll. 1989. Properties of artificial casein micelles. 4. Influence of dephosphorylation and phosphorylation of the casein. *Neth. Milk Dairy J.* 43:53.
- 17 Sumner, J. B. 1944. Method for the colorimetric determination of phosphorous. *Science* (Washington, DC) 100:413.
- 18 Van Hekken, D. L., and E. D. Strange. 1993. Functional properties of dephosphorylated bovine whole casein. *J. Dairy Sci.* 76:3384.
- 19 Van Hekken, D. L., and M. P. Thompson. 1992. Application of PhastSystem® to the resolution of bovine milk proteins on urea-polyacrylamide gel electrophoresis. *J. Dairy Sci.* 75:1204.
- 20 Van Vliet, T., S.P.F.M. Roefs, P. Zoon, and P. Walstra. 1989. Rheological properties of casein gels. *J. Dairy Res.* 56:529.
- 21 Yamauchi, K., S. Takemoto, and T. Tsugo. 1967. Calcium-binding property of dephosphorylated caseins. *Agric. Biol. Chem.* 31:54.
- 22 Yamauchi, K., and Y. Yoneda. 1978. Effect of dephosphorylation of casein on its coagulation and proteolysis by chymosin. *Agric. Biol. Chem.* 42:1031.
- 23 Yoshikawa, M., H. Samejima, M. Takeuchi, R. Sasaki, and H. Chiba. 1981. Conversion of β -casein to a stabilizer for α_{s1} -casein by enzymatic and chemical modifications. *Agric. Biol. Chem.* 45:333.
- 24 Yoshikawa, M., E. Sugimoto, and H. Chiba. 1975. Studies on the interaction between α_{s1} - and β -caseins. *Agric. Biol. Chem.* 39:1843.
- 25 Yoshikawa, M., M. Tamaki, E. Sugimoto, and H. Chiba. 1974. Effect of dephosphorylation on the self-association and the precipitation of β -casein. *Agric. Biol. Chem.* 38:2051.
- 26 Yun, S., K. Ohmiya, and S. Shimizu. 1982. Role of the phosphoryl group of β -casein in milk curdling. *Agric. Biol. Chem.* 46:1505.
- 27 Zoon, P., T. van Vliet, and P. Walstra. 1988. Rheological properties of rennet-induced skim milk gels. 1. Introduction. *Neth. Milk Dairy J.* 42:249.
- 28 Zoon, P., T. van Vliet, and P. Walstra. 1988. Rheological properties of rennet-induced skim milk gels. 3. The effect of calcium and phosphate. *Neth. Milk Dairy J.* 42:295.
- 29 Zoon, P., T. van Vliet, and P. Walstra. 1989. Rheological properties of rennet-induced skim milk gels. 4. The effect of pH and NaCl. *Neth. Milk Dairy J.* 43:17.