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## DEPHOSPHORYLATED BOVINE CASEINS: FUNCTIONAL AND RHEOLOGICAL PROPERTIES.

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### ABSTRACT

Caseins, the phosphoproteins of milk, have functional and rheological properties that make them a suitable protein additive in foods. The purpose of this research is to expand the food uses of casein by enzymatic dephosphorylation with concomitant alteration of their functional and rheological properties. Compared to native caseins, partially (40%) and maximally (93%) dephosphorylated caseins have higher isoelectric points and greater solubility in sodium chloride at pH 3 and in the presence of calcium ion. They have equivalent emulsion activities, but have lower emulsion and foaming capacities and stabilities. Rennet gels made with maximally dephosphorylated caseins have similar coagulation times and gel strengths, are more elastic, and form at lower and narrower calcium:casein ratios when compared to native casein. Partially dephosphorylated casein rennet gels are similar to maximally dephosphorylated gels in elasticity and calcium:casein ratios of formation but, compared to both native and maximally dephosphorylated casein gels, are much weaker and have much longer coagulation times. Removal of the negatively charged phosphate groups from whole casein produces modified caseins with unique functional and rheological properties that may be of value in formulating foods such as infant formula and novel soft cheeses.

### INTRODUCTION

Casein (CN), the major protein in bovine milk, is a blend of four phosphoproteins that are very important to the food industry due to high nutritional value and versatile functional and rheological properties. The four proteins,  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN, are found in a ratio of 3:1:3:1 and have 8 and 9, 10 to 13, 5, and 1 phosphate group/molecule, respectively; contain 169 ( $\kappa$ -CN) to 209 ( $\beta$ -CN) amino acid residues, and have molecular weights between 19,000 ( $\kappa$ -CN) and 25,200 ( $\alpha_{S2}$ -CN) Daltons (Eigel et al, 1984). The  $\alpha_{S2}$ -CN and  $\kappa$ -CN contain two cysteines each and  $\kappa$ -CN are glycosylated. Caseins have charged and hydrophobic areas and are flexible. In calcium containing solutions such as milk, caseins associate through protein-protein and protein-calcium interactions as submicelles and are stabilized by colloidal calcium phosphate in larger micelles.

## N-2

Phosphate groups have been removed from the caseins using acid phosphatases (Bingham et. al., 1976, Li-Chan and Nakai, 1989), alkaline phosphatases (Li-Chan and Nakai, 1989, Pearse et. al, 1986), and phosphoprotein phosphatases (Aoki et. al., 1987, Pepper and Thompson, 1963, Yamauchi et. al., 1967, Yoshikawa et. al., 1975, Yun et. al., 1982, Yamauchi and Yoneda, 1978). Dephosphorylation alters the calcium binding (Yamauchi et. al., 1967, Yoshikawa et. al., 1975, Yamauchi and Yoneda, 1978), artificial micelle formation (Aoki et. al., 1987, Bingham et. al., 1972, Pepper and Thompson, 1963, Schmidt and Poll, 1989), and electrophoretic mobility (Bingham et. al., 1976, Li-Chan and Nakai, 1989, Yamauchi et. al., 1967, Van Hekken and Thompson, 1992). Others have shown that dephosphorylated (DP) caseins are less heat and alcohol stable (Schmidt and Poll, 1989), take longer to coagulate when treated with rennet (Pearse et. al., 1986, Yamauchi and Yoneda, 1978), and have lower curd tension (Yamauchi and Yoneda, 1978, Yun et. al., 1982) than native casein. The acid curd of bovine dephosphorylated casein is more readily hydrolyzed by pepsin and has a microstructure that resembles human milk curd more closely than unmodified bovine curd (Li-Chan and Nakai, 1989). Information on other functional properties is lacking. This paper reviews our research on functional and rheological properties of dephosphorylated caseins.

## MATERIALS AND METHODS

### Dephosphorylation of Casein

Raw bovine skim milk obtained locally was adjusted to pH 4.6 using 1 N HCl to precipitate the casein. This acid casein was washed with water and stored frozen. Thawed acid casein was solubilized in water at pH 7 with 1 N NaOH and lyophilized to make sodium caseinate. Potato acid phosphatase (Calbiochem, La Jolla, CA) was used to remove phosphate groups from the caseinate according to Bingham et al (1976) as modified by Van Hekken and Strange (1991). Batches of aqueous casein (2.5 mg/ml) at pH 6.5 were treated with enzyme (0.13 or 0.065 units of enzyme/ml casein solution) and incubated for 1 or 2 hours in a shaking water bath at 37°C (150 rpm). Partially dephosphorylated whole casein was obtained by heat inactivating the enzyme (80°C for 5 min) after 1 hour incubation and before dialysis at 4°C to remove phosphates. Maximally dephosphorylated casein was obtained by incubating for 2 hours before dialyzing. Caseins were lyophilized and samples digested in sulfuric acid to determine total phosphorus content (Sumner, 1944).

### Urea-PAGE

Urea-PAGE was used to confirm the degree of casein modification. Profiles of dephosphorylated individual and whole casein were obtained using the PhastSystem as described by Van Hekken and Thompson (1992). A 6.6 M urea, 0.112 M Tris-HCl, 0.112 M acetate buffer at pH 6.4 was used to modify the 8-25%

gradient ultra-thin gel prior to use and to solubilize the casein samples before mercaptoethanol (1%) and bromophenol blue tracking dye (0.025%) were added.

### **Solubility**

Solubility of 0 (native), 40 (partially), and 93% (maximally) dephosphorylated whole casein was determined in 0.15 M sodium chloride at pH 3 to 8 or in 0.01 M imidazole, pH 7 at different calcium concentrations as described by Van Hekken and Strange (1991). Samples in sodium chloride solutions were centrifuged at 12,000xg for 15 min at 25°C and samples in calcium chloride solutions were ultracentrifuged at a minimum of 100,000xg for 37 min at 37°C. The absorbance of the supernates was measured at 280 nm and the protein concentration was calculated based on standard curves made from diluted protein stock solutions.

### **Foaming**

The foaming capacity and stability of solutions containing 0.1% native or modified (40 or 93% dephosphorylated) caseins, 0.15 M sodium chloride, and 0.02% sodium azide at pH 7, were evaluated as described by Van Hekken and Strange (1991). A 15 ml aliquot of the casein solution was placed in a calibrated column and nitrogen gas sparged from the bottom of the column for 2 min (flow rate of 30 ml/min). Foam height was recorded initially and after every min until volume had collapsed 50%. Capacity was measured as initial foam volume/nitrogen flow rate. The time required for volume to collapse 50% was used as a stability index.

### **Emulsions**

Emulsion properties (capacity, formation ability, and stability) of solutions containing 0.1% native or modified (40 or 93% dephosphorylated) caseins, 0.15 M sodium chloride, and 0.02% sodium azide at pH 3 to 8, were evaluated as described by Van Hekken and Strange (1991). Emulsion capacity, expressed in ml oil/mg casein, was determined by adding corn oil to the 0.1% casein solution (pH adjusted) while homogenizing (Polytron homogenizer with PTA 10 shaft and at setting 6, Brinkman, Westbury) until phase inversion occurred. Emulsion formation and emulsion stability were determined from turbidity measurements (Pearce and Kinsella, 1978) on emulsions made with 3 parts 0.1% casein (adjusted to pH 3 to 8 and centrifuged) and 1 part corn oil and emulsified using a Polytron homogenizer (setting 6 for 30 sec). Emulsion activity index (EAI), the area of interface stabilized per unit weight of protein, was calculated for all samples containing at least 0.06% protein/ml using the following equation (Akita and Nakai, 1990):

$$EAI = \frac{(2.303) (2) (ABS_{500}) (dilution\ factor)}{(oil\ volume) (c) (10^4)}$$

where  $ABS_{500}$ =turbidity, oil volume=0.25 and c=g protein/ml in the aqueous pH

adjusted centrifuged solution. Stability of the emulsions was determined by placing the emulsified samples in a boiling water bath and measuring the time required for turbidity values to decrease 50%.

### **Rennet Gels**

The rheological properties [coagulation time, storage modulus ( $G'$ ), loss modulus ( $G''$ ),  $\tan \delta(G''/G')$ ] of rennet gels of native and modified caseins were evaluated using a dynamic oscillatory analyzer (RDA-700, Rheometrics Inc., Piscataway, NJ) as described by Van Hekken and Strange (1992). Solutions containing 5% casein, 0.02% sodium azide, and the appropriate amount of calcium chloride (0.2 to 2 mmoles calcium/g casein) were adjusted to pH 6.3, and stirred overnight at room temperature. Rennet (final dilution  $10^{-4}$  v/v) (Chris Hansen Lab., Inc., Milwaukee, WI) was added immediately before the solution was pipetted into the sample holder [Couette geometry (cup radius, 18.5 mm; bob radius, 17.5 mm; bob length, 37.08 mm)]. The dynamic analyzer was programmed for a time sweep experiment using 2% strain and a frequency of 1 rad/sec at 22°C. Coagulation time was determined when  $\tan \delta$  was less than 1.0. The  $G'$ ,  $G''$ , and  $\tan \delta$  of the gels were compared one hour after gelation.

Samples for electron microscopy studies were taken from 2 ml of renneted solutions, prepared as described above, of native, partially, and maximally dephosphorylated casein at calcium:casein (mmoles/g) ratios of 0.6, 0.3, and 0.3, respectively. Native and maximally dephosphorylated caseins formed gels of the entire 2 ml, while the partially dephosphorylated solution formed a loose gel pellet that settled to the bottom of the test tube. One hour after gelation (determined visually), undisturbed casein gels were overlaid with 10 ml of 1% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 6.3. Samples were fixed at room temperature for three hours and stored overnight at 4°C. Samples were dehydrated in alcohol, freeze fractured in liquid nitrogen, thawed in alcohol, critical point dried in CO<sub>2</sub> and sputter coated with gold. A JEOL JSM-840A scanning electron microscope (JEOL USA, Peabody, MA) was used to obtain micrographs.

## **RESULTS AND DISCUSSION**

### **Urea-PAGE**

In the urea-PAGE system, where proteins separate according to their charge to mass ratio, the removal of the negatively charged phosphate groups cause the dephosphorylated caseins to migrate more slowly than the native caseins (Bingham et. al., 1976, Li-Chan and Nakai, 1989, Yamauchi et. al., 1967, Van Hekken and Thompson, 1992). The whole caseins used in the functional and rheological studies are shown in Figure 1. Native whole casein (lanes 1 and 8), with a phosphorus (P) content of 230 nmol P/mg CN, has major bands for  $\alpha_{S1}$ -CN and  $\beta$ -CN, a minor doublet for  $\alpha_{S2}$ -CN, and a very faint doublet for  $\kappa$ -CN. When

compared to the partially dephosphorylated  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN (lanes 4, 5, 6, and 7, respectively), partially dephosphorylated whole casein (lane 2), with 139 nmol P/mg CN, shows multiple bands for  $\alpha_{S1}$ -CN and  $\beta$ -CN at all levels of dephosphorylation. The bands for dephosphorylated  $\alpha_{S2}$ -CN and  $\kappa$ -CN are either too faint or masked by the other caseins. Maximally dephosphorylated casein (lane 3), with 16 nmol P/mg CN, shows most of the  $\alpha_{S1}$ -CN and all the  $\beta$ -CN migrating in the same manner as bands of fully dephosphorylated  $\alpha_{S1}$ -CN and  $\beta$ -CN. Only faint indications of protein bands for dephosphorylated  $\alpha_{S2}$ -CN and  $\kappa$ -CN are noted.

### **Solubility**

The solubilities of whole caseins (modified and unmodified) in 0.15 M sodium chloride at pH 3 to 8 are shown in Figure 2. Dephosphorylation of caseins caused a basic shift in isoelectric point that increased as degree of dephosphorylation increased (0.5 pH unit for maximally dephosphorylated casein). Above their respective isoelectric points, no difference was noted in the solubility of the caseins. At pH 3, the solubility increased as the degree of dephosphorylation increased.

The solubilities of whole caseins (modified and unmodified) in pH 7 imidazole buffer at different calcium levels are shown in Figure 3. The dephosphorylated caseins were more soluble in the presence of calcium and solubility increased as degree of dephosphorylation increased. Removal of the phosphate groups reduces calcium binding sites on the caseins and disrupts their ability to form micelles (Aoki et. al., 1987, Schmidt and Poll, 1989).

The solubility of a protein is of major importance to the food industry. Solubility affects the ability of the protein to interact and associate with other components in the solution. The dephosphorylated caseins are as soluble (pH 6-8) or more soluble (pH 3) than native casein in a sodium chloride solution (a common food ingredient) and are more soluble in the presence of calcium than native caseins. In summary, we found that removal of the negatively charged phosphate groups improves casein solubility.

### **Interfacial Protein Films**

Foaming properties (initial foam volume and foam stability) of modified and unmodified caseins are presented in Figure 4. Native whole casein has excellent foaming properties (Townsend and Nakai, 1983). In our study, native casein had a foam capacity value of 1.7 and stability of 5.5 min. Compared to native casein, the foams made of partially and maximally dephosphorylated caseins had lower capacity values (1.3 and 1.2, respectively), and were very unstable.

Emulsion properties of native and modified caseins are presented in Table 1. Initial emulsion formation as seen by turbidity and emulsion activity index

(surface area of emulsion covered by unit amount of protein) showed no significant ( $P < 0.05$ ) difference for caseins above their isoelectric points. The EAI values were not calculated for samples that had less than 0.06% soluble protein as data were not reliable. Emulsion capacity (amount of oil a unit weight of casein could support in an emulsion) and stability (length of time for the turbidity of heat stressed emulsion to decrease 50%) of modified caseins were lower than those of the native caseins.

At phase interfaces, native caseins are flexible and have the ability to form stable monolayers, critical properties for emulsions and foams. Removal of negatively charged phosphate groups changes the distribution of charges within the protein resulting in destabilization. This alters the proteins' tertiary structure and its ability to bridge the interfaces between two phases which may lead to rupture of the emulsion or foam.

### **Rennet Gels**

Rheological properties of whole caseins are presented in Table 2. Gels formed at calcium:casein ratios of 0.4 to 1.5 for native casein, 0.2 to 0.6 for maximally dephosphorylated casein, and at 0.3 only for partially dephosphorylated casein. The rheological properties of the gels that had the highest  $G'$  and  $G''$  (calcium:casein ratios of 0.6 for native and 0.3 for the modified caseins) were compared. Coagulation times, the time from addition of rennet to when  $\tan \delta$  ( $G''/G'$ ) decreased to less than 1.0, were similar for native and maximally dephosphorylated caseins (32-33 min) but were longer (over 163 min) for partially dephosphorylated casein. Native and maximally dephosphorylated casein gels had similar storage moduli ( $G'$ ) and the native casein gels had larger loss moduli ( $G''$ ) than maximally dephosphorylated casein gels. Partially dephosphorylated casein formed significantly weaker gels; both  $G'$  and  $G''$  were smaller than those of native and maximally dephosphorylated casein gels. The  $\tan \delta$  values for the modified casein gels were similar to each other and lower than native casein, indicating that the properties of the modified casein gels were more elastic and less viscous than those of the native casein gels. Even though the types of gels were different, maximally dephosphorylated casein gels were comparable in strength to native casein gels. Partially dephosphorylated caseins have heterogenous phosphate content resulting in heterogenous conformation. The heterogeneity of conformation may result in decreased intermolecular associations leading to the formation of an insufficient number of stable colloidal casein particles from which the gel matrix is formed.

The microstructures of the renneted casein gels one hour after gelation are shown in Figure 5. Native casein gels have a typical open spongy appearance in which the casein micelles have fused together to form an open network of large smooth casein pillars. The dephosphorylated gels have smaller interstitial space and the pillars are made of smaller subunits stuck together (have not fused into smooth columns). Earlier studies have shown that dephosphorylated caseins

form abnormal and smaller micelles (Aoki et. al., 1987, Pepper and Thompson, 1963, Schmidt and Poll, 1989). The rennet gels shown here appear to be made up smaller subunits that do not coalesce.

Removal of the negatively charged phosphate groups alters the ability of the dephosphorylated caseins to interact with each other. In the presence of calcium, small aggregates of dephosphorylated caseins form (close to submicelles in size) and, when treated with rennet, associate together but do not form the large, fused matrix seen for native casein. The  $\tan \delta$  values and microstructures show that the gels of native and modified caseins are different. Rheological data ( $G'$  and  $G''$ ) indicate that native and maximally dephosphorylated caseins form gels of similar strength, although the microstructure indicates they are not constructed in the same manner. The gel formed of partially dephosphorylated casein did not involve the entire solution and consisted of small casein particles that associated similarly to maximally dephosphorylated casein and settled out of the solution. Whole casein, made up of the differently charged  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CNs, can associate together to form a gel when the caseins have either all or none of their phosphate groups. When the four caseins are at multiple levels of dephosphorylation (0 to 100% DP), the proteins can only undergo limited associations that result in weak, localized gels.

## CONCLUSIONS

Removal of the phosphate groups from casein alters the protein by reducing net negative charge and changing the charge distribution. It also changes the tertiary structure and alters interactions and associations between self and other components. Dephosphorylation produces modified caseins with combinations of functional and rheological properties that may be of value in the food industry. Foods that may benefit from using dephosphorylated casein as an ingredient include: infant formulas which require good solubility when calcium fortified, good initial emulsion, and minimal foaming; acid food systems which require good solubility at low pHs or in the presence of NaCl; bakery goods which require discrete layers of fat in the batter; and novel cheeses which require soft textures. The use of these modified caseins is limited only by the imagination.

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Table 1. Effect of pH on the emulsion properties of native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins in 0.15 M sodium chloride.

pH	INITIAL TURBIDITY (500 nm)			EMULSION ACTIVITY INDEX (m <sup>2</sup> /g)			EMULSION STABILITY (min)			EMULSION CAPACITY (ml oil/mg casein)		
	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP
3.0	0.018 <sup>ef</sup>	0.051 <sup>de</sup>	0.094 <sup>cd</sup>	*	*	*	3 <sup>f</sup>	4 <sup>f</sup>	4 <sup>f</sup>	1.77 <sup>ghi</sup>	1.08 <sup>klm</sup>	1.85 <sup>gh</sup>
4.0	0.007 <sup>f</sup>	0.007 <sup>f</sup>	0.006 <sup>f</sup>	*	*	*	3 <sup>f</sup>	3 <sup>f</sup>	3 <sup>f</sup>	1.44 <sup>hij</sup>	0.74 <sup>m</sup>	1.10 <sup>klm</sup>
4.5	0.008 <sup>ef</sup>	0.007 <sup>f</sup>	0.021 <sup>ef</sup>	*	*	*	6 <sup>f</sup>	5 <sup>f</sup>	3 <sup>f</sup>	1.30 <sup>kl</sup>	0.88 <sup>lm</sup>	0.98 <sup>klm</sup>
5.0	0.110 <sup>bc</sup>	0.038 <sup>ef</sup>	0.043 <sup>ef</sup>	146 <sup>ab</sup>	*	*	68 <sup>ef</sup>	6 <sup>f</sup>	3 <sup>f</sup>	3.08 <sup>o</sup>	1.81 <sup>ghi</sup>	1.40 <sup>ijk</sup>
5.5	0.161 <sup>a</sup>	0.156 <sup>a</sup>	0.155 <sup>a</sup>	139 <sup>ab</sup>	134 <sup>ab</sup>	179 <sup>a</sup>	209 <sup>bc</sup>	127 <sup>de</sup>	62 <sup>ef</sup>	3.39 <sup>bc</sup>	2.28 <sup>de</sup>	2.08 <sup>efg</sup>
6.0	0.149 <sup>ab</sup>	0.168 <sup>a</sup>	0.167 <sup>a</sup>	134 <sup>ab</sup>	156 <sup>ab</sup>	158 <sup>ab</sup>	212 <sup>bc</sup>	152 <sup>cd</sup>	188 <sup>bcd</sup>	3.27 <sup>bc</sup>	2.26 <sup>def</sup>	2.38 <sup>de</sup>
7.0	0.184 <sup>a</sup>	0.172 <sup>a</sup>	0.182 <sup>a</sup>	169 <sup>ab</sup>	153 <sup>ab</sup>	155 <sup>ab</sup>	294 <sup>a</sup>	205 <sup>bc</sup>	235 <sup>ab</sup>	3.60 <sup>ab</sup>	2.48 <sup>de</sup>	2.50 <sup>de</sup>
8.0	0.181 <sup>a</sup>	0.186 <sup>a</sup>	0.156 <sup>a</sup>	174 <sup>ab</sup>	171 <sup>ab</sup>	132 <sup>b</sup>	298 <sup>a</sup>	248 <sup>ab</sup>	187 <sup>bcd</sup>	3.94 <sup>a</sup>	2.48 <sup>de</sup>	2.60 <sup>d</sup>

Data analyzed using ANOVA and Bonferroni t-test. Within each category (turbidity, activity index, stability, or capacity), values with the same superscript letter are not significantly different (P < .05).

\* - Emulsion activity index values were not calculated because sample had less than 0.6 % soluble protein/ml.

Table 2. Effect of calcium:casein (CN) (mmol:g) ratio on the rheological properties of native [0% dephosphorylated (DP)] and modified (partially (40% DP) and maximally (93% DP)) whole caseins.

Ca:CN	COAGULATION TIME (min)			STORAGE MODULUS (G') (dyne/cm <sup>2</sup> )			LOSS MODULUS (G'') (dyne/cm <sup>2</sup> )			TAN $\delta$ (G''/G')		
	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP	0% DP	40% DP	93% DP
0.2	--	a	54 <sup>cde</sup>	--	a	210 <sup>cd</sup>	--	a	85 <sup>cd</sup>	--	a	0.404 <sup>c</sup>
0.3	--	155 <sup>ab</sup>	33 <sup>e</sup>	--	36 <sup>e</sup>	380 <sup>a</sup>	--	19 <sup>e</sup>	147 <sup>bc</sup>	--	0.454 <sup>c</sup>	0.388 <sup>c</sup>
0.4	124 <sup>bcd</sup>	156 <sup>ab</sup>	43 <sup>de</sup>	109 <sup>de</sup>	c	252 <sup>bc</sup>	70 <sup>de</sup>	c	99 <sup>cd</sup>	0.651 <sup>ab</sup>	c	0.391 <sup>c</sup>
0.6	32 <sup>e</sup>	b	39 <sup>e</sup>	345 <sup>ab</sup>	b	114 <sup>de</sup>	242 <sup>a</sup>	b	45 <sup>de</sup>	0.703 <sup>a</sup>	b	0.395 <sup>c</sup>
1.0	32 <sup>e</sup>	b	83 <sup>bcdde</sup>	334 <sup>ab</sup>	b	b	187 <sup>ab</sup>	b	b	0.560 <sup>abc</sup>	b	b
1.5	61 <sup>cde</sup>	--	--	84 <sup>e</sup>	--	--	43 <sup>de</sup>	--	--	0.505 <sup>bc</sup>	--	--
2.0	127 <sup>bc</sup>	--	--	b	--	--	b	--	--	b	--	--

Data analyzed using ANOVA and Bonferroni t-test. Within each category (coagulation time, G', G'', and tan  $\delta$ ), values with the same superscript letter are not significantly different (P < .05).

a-no gel formed; b-gel had insufficient torque for measurement; c-gel broke within 30 minutes after gelation

Figure 1. Urea-PAGE profiles of bovine caseins using 8 to 25% gradient gel and the Phastsystem. Samples in lanes are 1) and 8) native whole casein (CN), 2) partially dephosphorylated (DP) whole CN, 3) maximally DP whole CN, 4) partially DP  $\alpha_{s1}$ -CN, 5) partially DP  $\alpha_{s2}$ -CN, 6) partially DP  $\beta$ -CN, and 7) partially DP  $\kappa$ -CN.

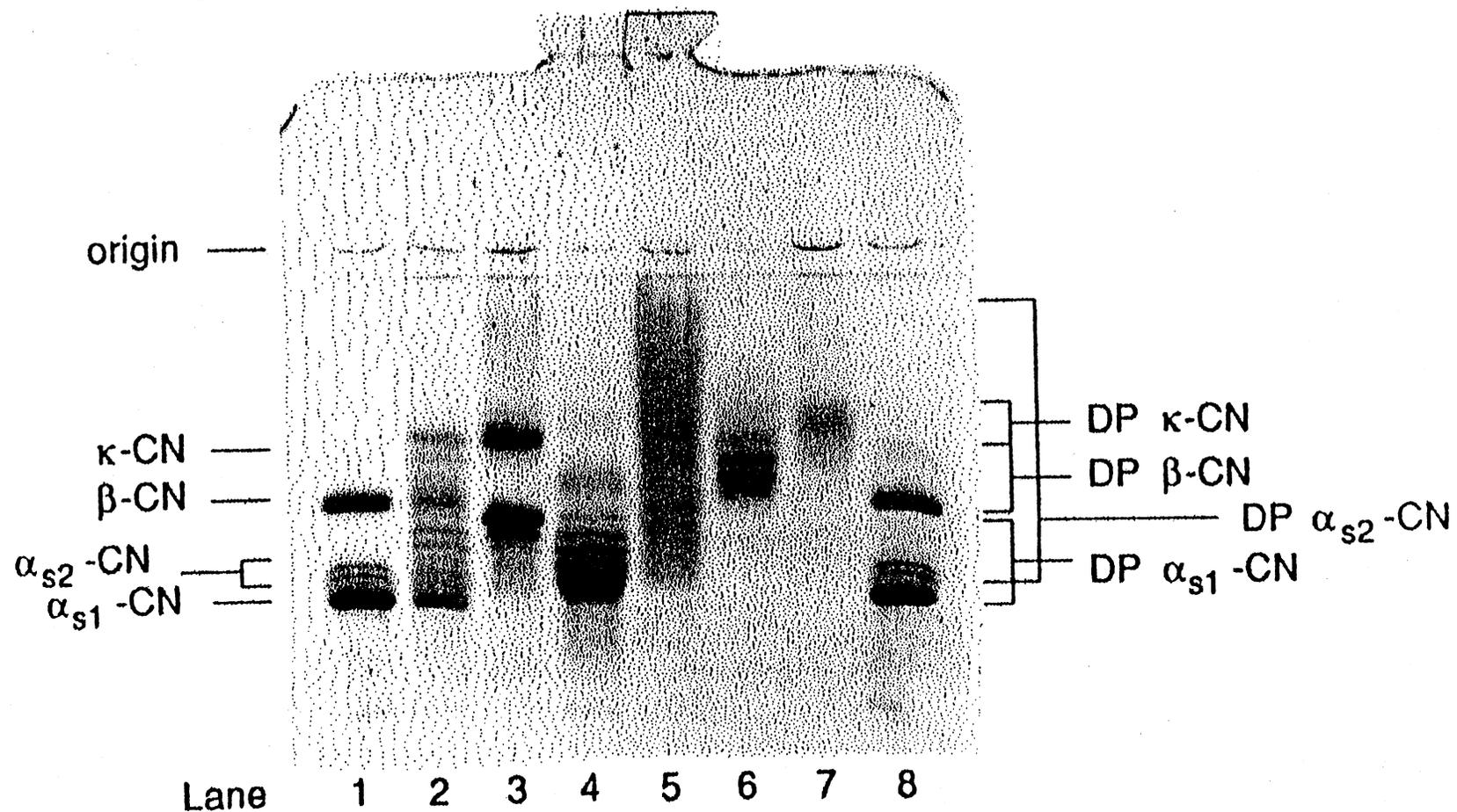


Figure 2. Effect of pH on the solubility of 0.1% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins in 0.15 M sodium chloride.

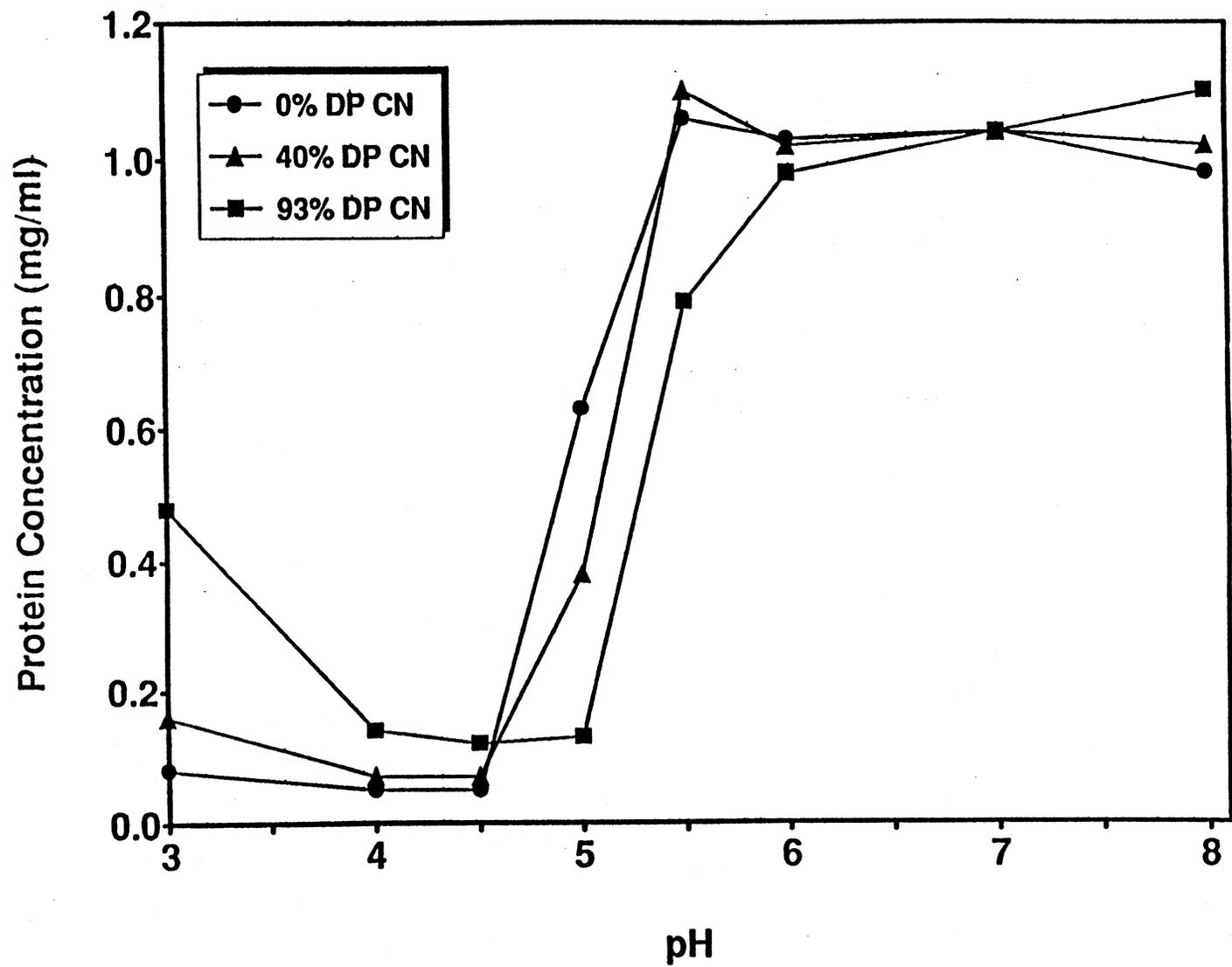


Figure 3. Effect of calcium concentration on the solubility of 0.2% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins in 0.01 M imidazole, pH 7.0.

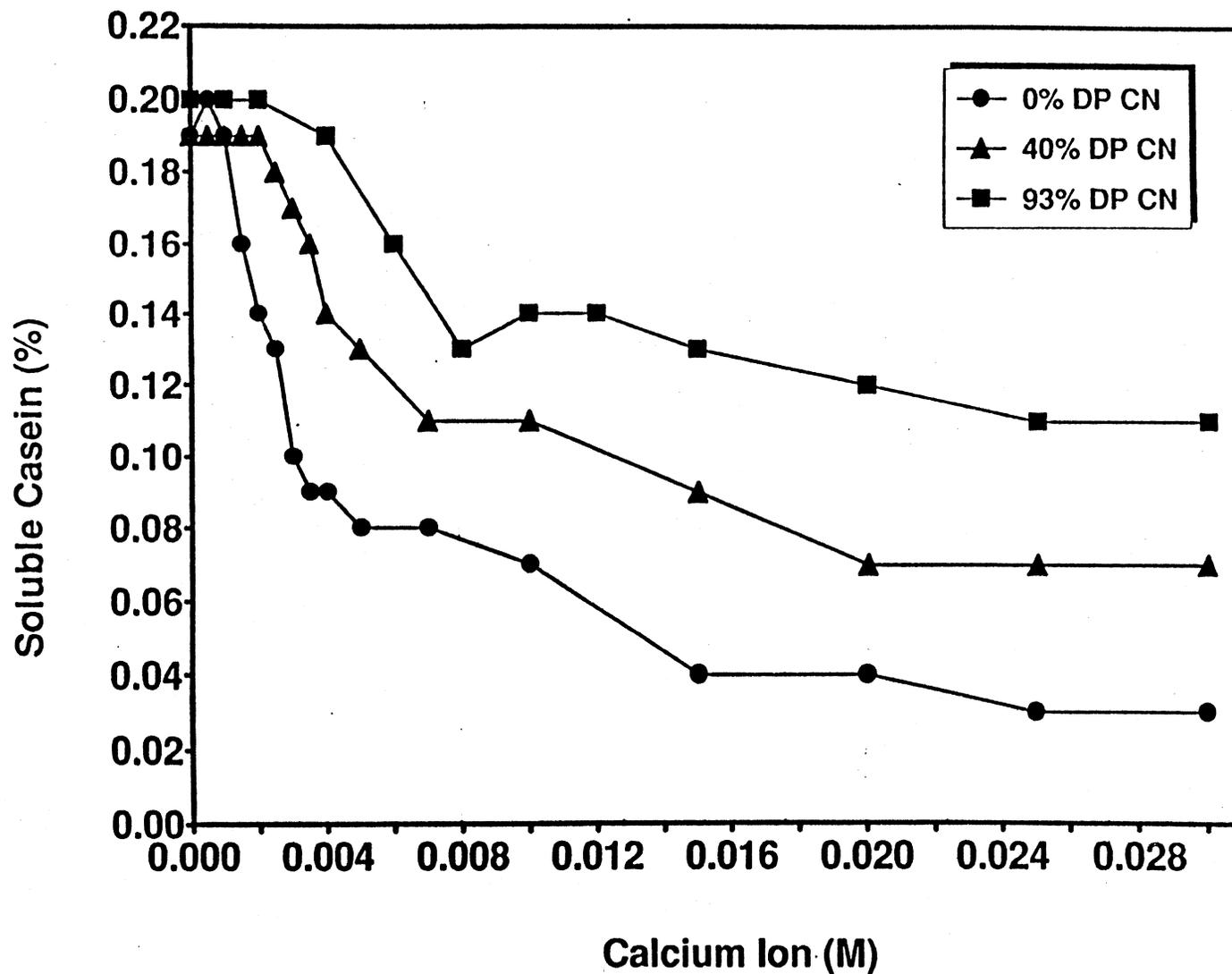


Figure 4. Foaming properties of 0.1% native [0% dephosphorylated (DP)] and modified (40 and 93% DP) whole caseins in 0.15 M sodium chloride, pH 7.

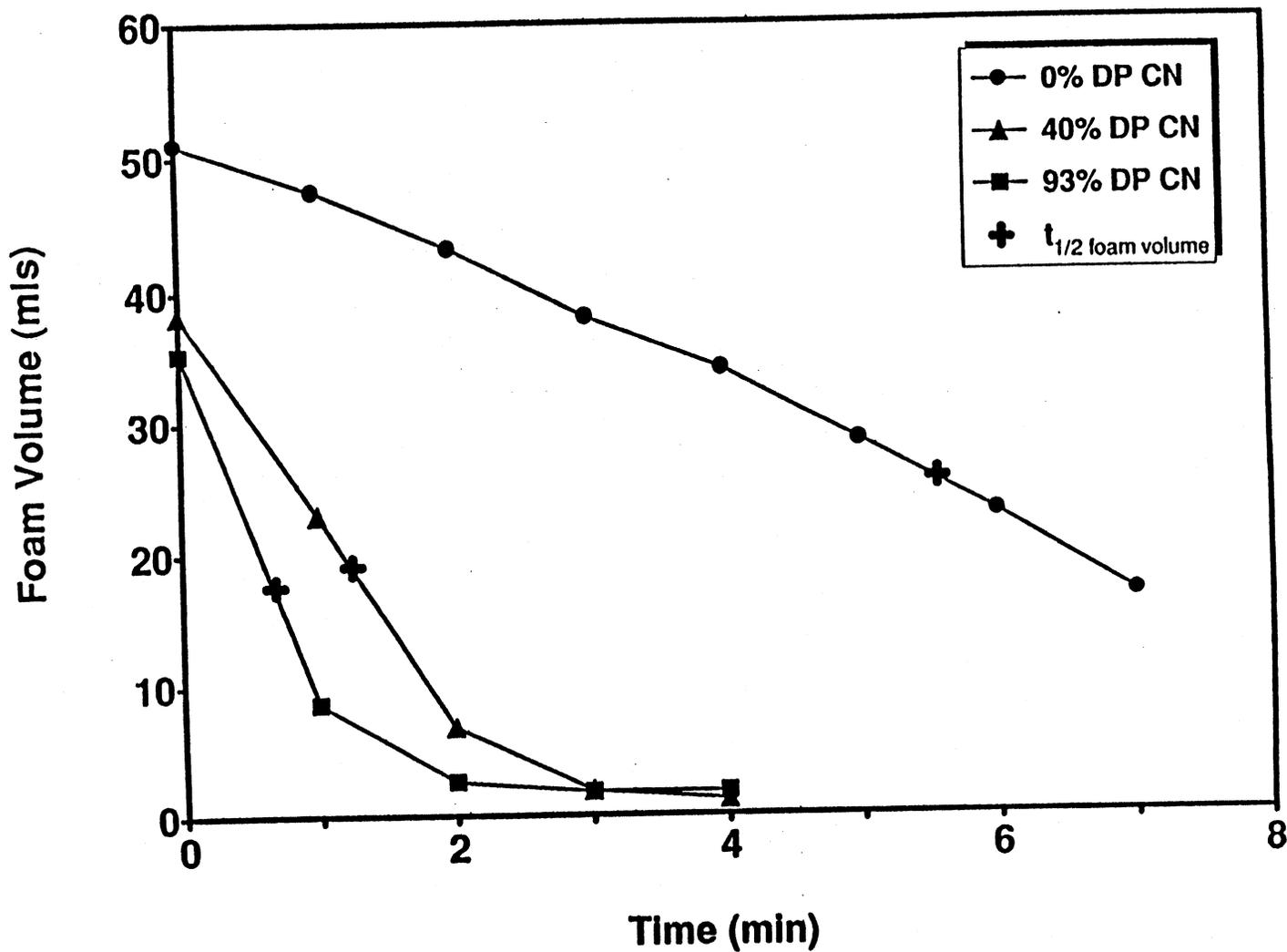


Figure 5. Scanning electron micrographs of renneted whole casein gels preserved one hour after gelation in glutaraldehyde. Samples are a) native, b) partially dephosphorylated, and c) maximally dephosphorylated caseins at either 1) 1,000 or 2) 10,000 magnification.

