Heat-Induced, pH-Dependent Dissociation of Casein Micelles on Heating Reconstituted Skim Milk at Temperatures below 100 °C

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The effect of pH (6.3−7.1) and temperature (20−90 °C) on the dissociation of casein from the micelles in reconstituted skim milk was investigated. These pH conditions encompass those naturally found, whereas heat treatments below 100 °C are commonly encountered during the processing of milk and milk products. Low levels of casein were rendered soluble at pH below 6.7 regardless of heating temperature, whereas increasing levels of casein were solubilized as the pH was increased from 6.7 to 7.1. This pH-dependent dissociation of the casein micelles showed an unusual dependence on temperature. Low levels of casein were dissociated at 20 °C at all pH values. The quantity of casein solubilized increased with temperature to a maximum dissociation at about 70 °C and then decreased at higher temperatures. The dissociation behavior of α_s-casein and β-casein at pH ≥ 6.7 showed dependence on temperature similar to that of the total casein. In contrast, above pH 6.5, the dissociation of κ-casein increased essentially linearly with increasing temperature over the entire temperature range studied. The proportion of β-casein in the soluble casein was essentially constant regardless of the temperature and pH, whereas the proportions of α_s-casein and κ-casein varied with both temperature and pH. The results of this study have indicated that, at certain pH, a marked dissociation of protein from the casein micelles occurs on heating at temperatures below 100 °C; this phenomenom has not previously been reported to occur under such mild heating conditions.

Keywords: *Casein micelles; dissociation; heat treatment; reconstituted skim milk*

INTRODUCTION

The casein micelles of bovine milk are macromolecular assemblies of the four casein proteins complexed with mineral components, primarily calcium and phosphate. The latter, often referred to as colloidal calcium phosphate (CCP), is largely responsible for maintaining micellar integrity. Despite extensive research efforts, the definitive structure of casein micelles remains unresolved; however, several models have been proposed (Rose, 1969; Waugh, 1971; Slattery and Evard, 1973; Schmidt, 1980, 1982; Holt, 1992). These models have been progressively refined as more information on micellar structure becomes available. Most of the experimental data to date can be explained by a porous structure composed of small sub-micellar units which are held together by the CCP. κ-Casein is not spread uniformly through the micelle but occupies a key surface position (Kirchmeier, 1973; Schmidt, 1982; Walstra, 1990).

The casein micelles constitute a colloidal system of remarkable stability, capable of withstanding the severe treatments commonly associated with modern commercial dairy processing. However, under certain conditions, micellar dissociation can be induced with the formation of soluble casein proteins. Extensive dissociation occurs on the addition of chaotropes such as urea (McGann and Fox, 1974; Aoki et al., 1986), through the chelation of calcium (Lin et al., 1972; Holt et al., 1986; Griffin et al., 1988) or through the application of high hydrostatic pressures (Schmidt and Buchheim, 1970). Varying degrees of micellar dissociation can also be induced through the alteration of milk pH and temperature (Kudo, 1980; Singh and Fox, 1985; Dalgleish and Law, 1988). A reduction in the pH of milk solubilizes the CCP. As CCP is involved in maintaining micellar structure, its solubilization is accompanied by the solubilization of the individual caseins. However, this dissociation is markedly temperature dependent. At low temperatures (4 °C), a significant proportion of the casein dissociates even at the natural pH of the milk (about pH 6.7), whereas at 30 °C, only a limited dissociation occurs even when the pH is reduced (Rose, 1968; Reimerdes and Klostermeyer, 1976; Dalgleish and Law, 1988). The predominant protein dissociated at these low temperatures is β-casein; however, significant levels of α_s-casein and κ-casein can be solubilized, particularly at lower pH (Dalgleish and Law, 1988).

Heating milk or whey-protein-free milk at temperatures above 110 °C caused a substantial increase in the level of soluble casein, of which about 40% was found to be κ-casein (Fox et al., 1967; Aoki et al., 1974). Kudo (1980) showed that this heat-induced dissociation of casein from the micelles in milk was pH dependent. At pH values below 6.8, the denatured whey proteins associated with the casein micelles and little casein was rendered soluble. At higher pH values, increasing levels of casein, along with denatured whey proteins, were dissociated from the casein micelles. This observation led Kudo to postulate that the minimum in the heat coagulation time−pH profile of milk, as first described by Rose (1961), was a consequence of the dissociation of κ-casein, complexed with whey proteins, from the micelles at pH 6.8 or above. Subsequent studies (Singh and Fox, 1985, 1986; Nieuwenhuijse et al., 1991; Singh and Creamer, 1991; Anema et al., 1993) have confirmed

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that a pH-dependent dissociation of κ-casein-rich protein from the casein micelles occurs on heating unconcentrated milk at temperatures of ≥90 °C.

Milk samples are routinely exposed to temperatures in the range between 4 and 140 °C; however, in contrast to the studies on the pH-dependent dissociation of casein micelles in milk at low temperatures (≤30 °C) or at very high temperatures (≥90 °C), no similar studies on micellar dissociation at intermediate temperatures have been reported. This communication reports the results of a study on the influence of heating at temperatures between 20 and 90 °C on the pH-dependent dissociation of protein from the casein micelles in reconstituted skim milk.

EXPERIMENTAL PROCEDURES

Milk Supply. Low-heat skim milk powder (whey protein nitrogen index above 6; Sanderson, 1970) was obtained from Tui Milk Products, Pahiatua, New Zealand. Experimental skim milk samples were prepared by reconstituting the skim milk powder to 10% (w/w) total solids (TS) in water purified by deionization followed by filtration through a Milli-Q apparatus (Millipore Corp., Bedford, MA). The reconstituted skim milk samples were allowed to equilibrate at ambient temperature (about 20 °C) with gentle stirring for 2 h before further treatment.

Adjustment of pH and Heat Treatments. Reconstituted skim milk samples were adjusted to pH values in the range from 6.3 to 7.1 by the slow addition of 1 M HCl or 1 M NaOH to well-stirred solutions. The pH-adjusted solutions were allowed to equilibrate for 3 h. Subsamples of milk (6 mL) at each pH were transferred to glass vials and heated, with continuous rocking, for 15 min in a thermostatically controlled water bath preset to temperatures in the range from 20 to 90 °C. After heat treatment, the milk samples were cooled to room temperature by immersion of the glass vials in cold running water.

Turbidity Measurements. The turbidity of heated skim milk samples was measured at 900 nm using a Kontron Uvikon 941 spectophotometer and a 2 mm quartz cell. The average of duplicate turbidity measurements was recorded.

Ultracentrifugation. Soluble caseins and whey proteins were defined as those that did not sediment from the milk during ultracentrifugation at 30 000 rpm (63000*g* average) for 60 min at 20 °C in a Beckman L2-65 ultracentrifuge and the associated Beckman Ti-80 rotor (Beckman Instruments Inc., Palo Alto, CA). Weighed aliquots of the heated milk samples (approximately 15 mL) were transferred to tared centrifuge tubes and ultracentrifuged. The clear supernatant was carefully removed from the pellets and weighed, and the protein content was determined by gel electrophoresis and laser densitometry.

Gel Electrophoresis and Laser Densitometry. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Bio-Rad mini-gel slab electrophoresis unit (Bio-Rad Laboratories, Richmond, CA). The resolving gel contained 15% acrylamide (2.6% Bis) and 0.1% SDS in 1.5 M Tris/HCl buffer at pH 8.8, and the stacking gel contained 4% acrylamide and 0.1% SDS in 0.5 M Tris/HCl buffer at pH 6.8. Samples were dispersed in 0.5 M Tris/HCl buffer at pH 6.6 containing 2% SDS, 0.05% β-mercaptoethanol, and 0.01% bromophenol blue and heated at 100 °C for 4 min. Ultracentrifugal supernatants were diluted 1:12, and the untreated milk samples were diluted 1:40 with the sample buffer. The gels were run at 200 V for 1 h and stained with Coomassie Blue (R) in 25% (v/v) 2-propanol and 10% (v/v) acetic acid for 1 h. After staining, the gels were destained with two changes of a 10% 2-propanol and 10% acetic acid solution for a total period of 24 h.

Isoelectric-focusing polyacrylamide gel electrophoresis (IEF-PAGE) was performed using a Pharmacia Multiphor II apparatus (Pharmacia-LKB, Freiburg, Germany) and the method described by Braun et al. (1990). The gel was composed of 4.5% (w/v) acrylamide (0.14% Bis), 45.2% (w/v) urea, 14% (w/v) glycerol, and 5.9% (v/v) of a prepared ampholyte solution (16% (v/v) ampholine, pH 2.5−4.5, 13% (v/v) pharmalyte, pH 4.2−4.9, 32% (v/v) servalyte, pH 4.5−5.0, 21% (v/v) servalyte, pH 5.0−5.5, 10% (v/v) pharmalyte, pH 5.0−6.0, and 8% (v/v) ampholine, pH 5.0−8.0). Samples were dispersed in a buffer composed of 8 M urea and 30 mM dithiothreitol. Ultracentrifugal supernatants were diluted 1:5, the milk samples were diluted 1:10 with the sample buffer, and 10 μL of the diluted sample was loaded on to the gel. The anode solution was 0.5 M H_3PO_4, and the cathode solution was 0.5 M NaOH. The running conditions involved a 30 min pre-focus step at 4 W (2500 V maximum) before sample loading. This was followed by a 60 min sample focusing step at 4 W (2500 V maximum) and a 100 min final focus step at 3000 V (20 W maximum). After electrophoresis, the protein bands were fixed by immersing the gel in 15% trichloroacetic acid solution before staining for 40 min with a solution containing 0.15% (w/v) Coomassie Blue (G) and 5% $CuSO_4$ in 45% methanol and 10% acetic acid. The gels were destained using 25% methanol and 10% acetic acid until a clear background was obtained.

SDS-PAGE gels and IEF-PAGE gels were scanned using laser densitometry (either an Ultroscan XL densitometer (Pharmacia-LKB, Freiburg, Germany) or a Molecular Dynamics Model P.D. computing densitometer (Molecular Dynamics Inc., Sunnyvale, CA)). The integrated intensities of the major protein bands were determined. The quantity of each protein in the ultracentrifugal supernatants was determined as a percentage of that in the milk sample, with corrections applied for the change in volume induced by centrifugation and dilution differences.

Less detailed experiments on four separate reconstituted skim milk samples from different milk powder supplies were conducted to confirm the findings of this study. In these latter experiments, the pH was adjusted to 6.5 and 7.1 and the samples were heated at temperatures of 20, 60, and 90 °C only.

RESULTS AND DISCUSSION

The effect of pH on the turbidity of skim milk samples heated at temperatures in the range from 20 to 90 °C for 15 min is shown in Figure 1a. At all temperatures, the turbidity decreased as the pH of the milk was increased. A decrease in turbidity with pH for milk samples at ambient temperature has also been reported by Rajput et al. (1983) and El-Abbassy (1987) and is possibly a consequence of the increased voluminosity of the micellar complex with increasing pH (Creamer, 1985; Anema and Creamer, 1993). At each pH, the percentage change in turbidity of the milk samples relative to the samples at 20 °C was determined. This allowed the turbidity change with heating temperature for the milk samples at the various pH values to be compared (Figure 1b). The effect of temperature on milk turbidity was markedly dependent on pH. At pH 6.3, the turbidity of the milk increased slightly with increasing heating temperature up to 60 °C. This may have been due to the partial aggregation of the casein micelles at this low pH. Above 60 °C, the turbidity increased markedly and essentially linearly with increasing temperature. This increase in turbidity at temperatures above 60 °C has been reported to be due primarily to the association of denatured whey proteins with κ-casein at the micelle surface (Jeurnink, 1992). At pH 6.5, there was virtually no change in the turbidity of the milk with temperature up to 60 °C; however, above 60 °C, the turbidity again increased essentially linearly with increasing temperature. For the milk samples at pH 6.7 or above, the turbidity decreased with increasing temperature to a minimum at about 60 °C and then increased again at higher temperatures. As the pH was increased from 6.7 to 7.1, the magnitude of

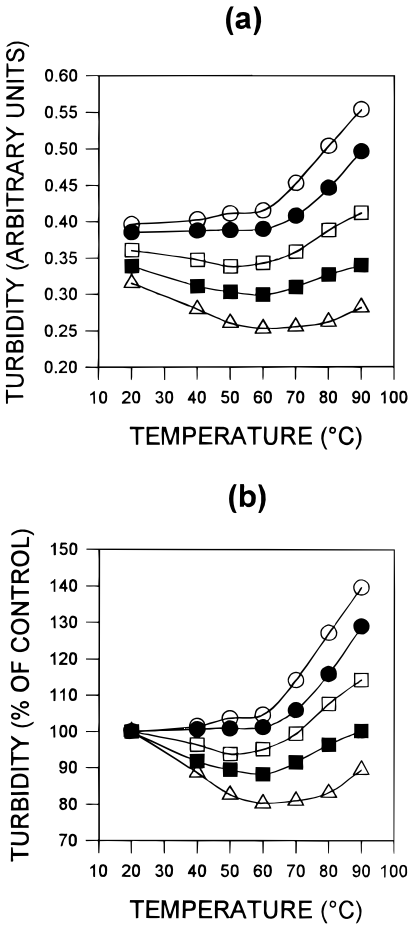

Figure 1. Effect of temperature and pH on the turbidity of reconstituted skim milk samples. (a) Absolute values; (b) percentage change from control (20 °C) samples. ○, pH 6.3; ●, pH 6.5; □, pH 6.7; ■, pH 6.9; △, pH 7.1.

the minimum in turbidity at 60 °C was greater and the increase in turbidity at the higher temperatures was less pronounced (Figure 1b).

The turbidity of a colloidal suspension is related to the size and the scattering properties of the dispersed particles. The unusual behavior of the turbidity of the heated skim milks with pH, as shown in Figure 1, may have been due to a temperature-dependent dissociation of protein from the casein micelles as the pH of the system was increased. This supposition was confirmed by an SDS-PAGE analysis of ultracentrifugal supernatants obtained from the heated skim milk samples, which showed that increasing levels of casein were rendered soluble as the pH of the milk was increased (Figure 2). A densitometric evaluation of the major protein bands (total casein, α_s-casein (α_{s1}-casein and α_{s2}-casein combined), β-casein, κ-casein, β-lactoglobulin (β-lg), and α-lactalbumin (α-lac)) yielded the results shown in Figure 3. At 20 °C, the level of total casein in the supernatants was low, although this level increased slightly as the pH of the milk was increased (Figure 3a). For the milk samples at pH 7.1, the proportion of the casein liberated into the serum increased almost linearly with increasing temperature, up to a maximum dissociation at about 70 °C, and then decreased as the temperature was increased further. A similar temperature dependence of micellar dissociation was observed at pH 6.9 and 6.7; however, markedly less casein was liberated at each heating temperature as the pH was decreased. Low levels of casein were found in the

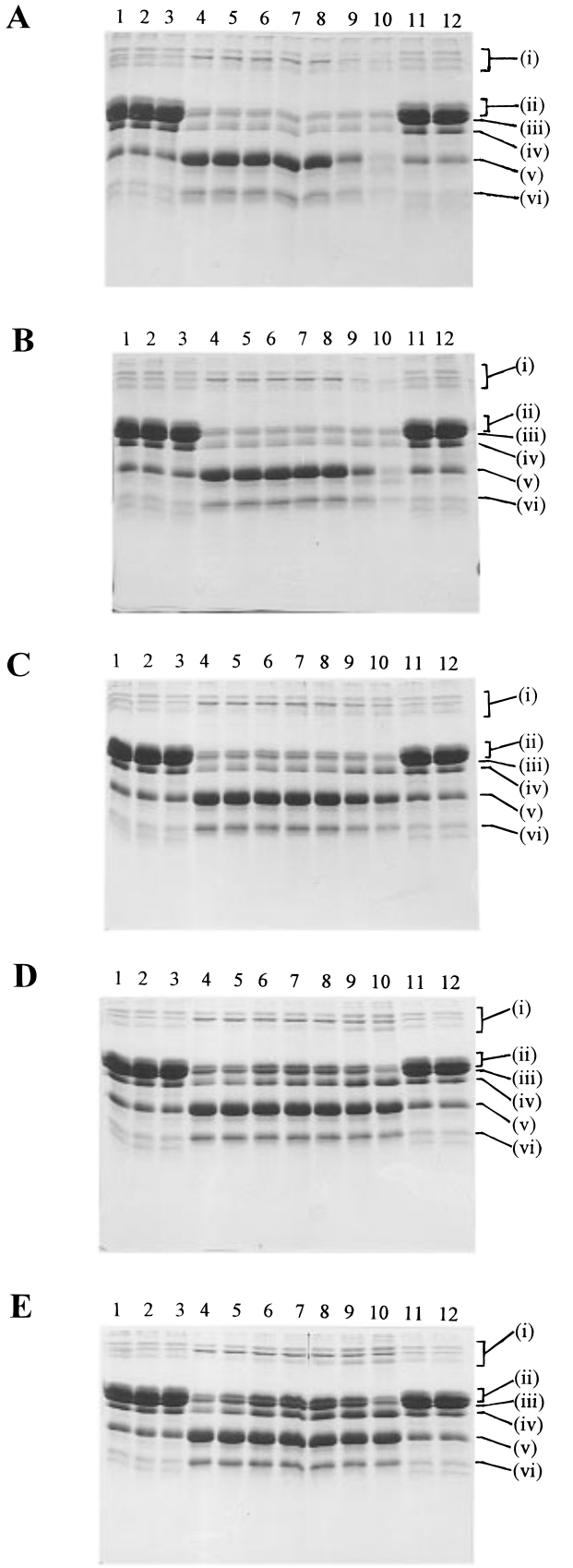

Figure 2. SDS-PAGE patterns of milk samples and supernatants obtained from 10% TS reconstituted skim milk samples at (A) pH 6.3, (B) pH 6.5, (C) pH 6.7, (D) pH 6.9, and (E) pH 7.1. Lanes 1, 11, and 12, unheated milk; lane 2, milk heated at 60; lane 3, milk heated at 90 °C; lanes 4−10, supernatants from milk heated at 20, 40, 50, 60, 70, 80, and 90 °C, respectively. (i) Immunoglobulin-G, lactoferrin, and bovine serum albumin; (ii) α_s-casein; (iii) β-casein; (iv) κ-casein; (v) β-lg; (vi) α-lac.

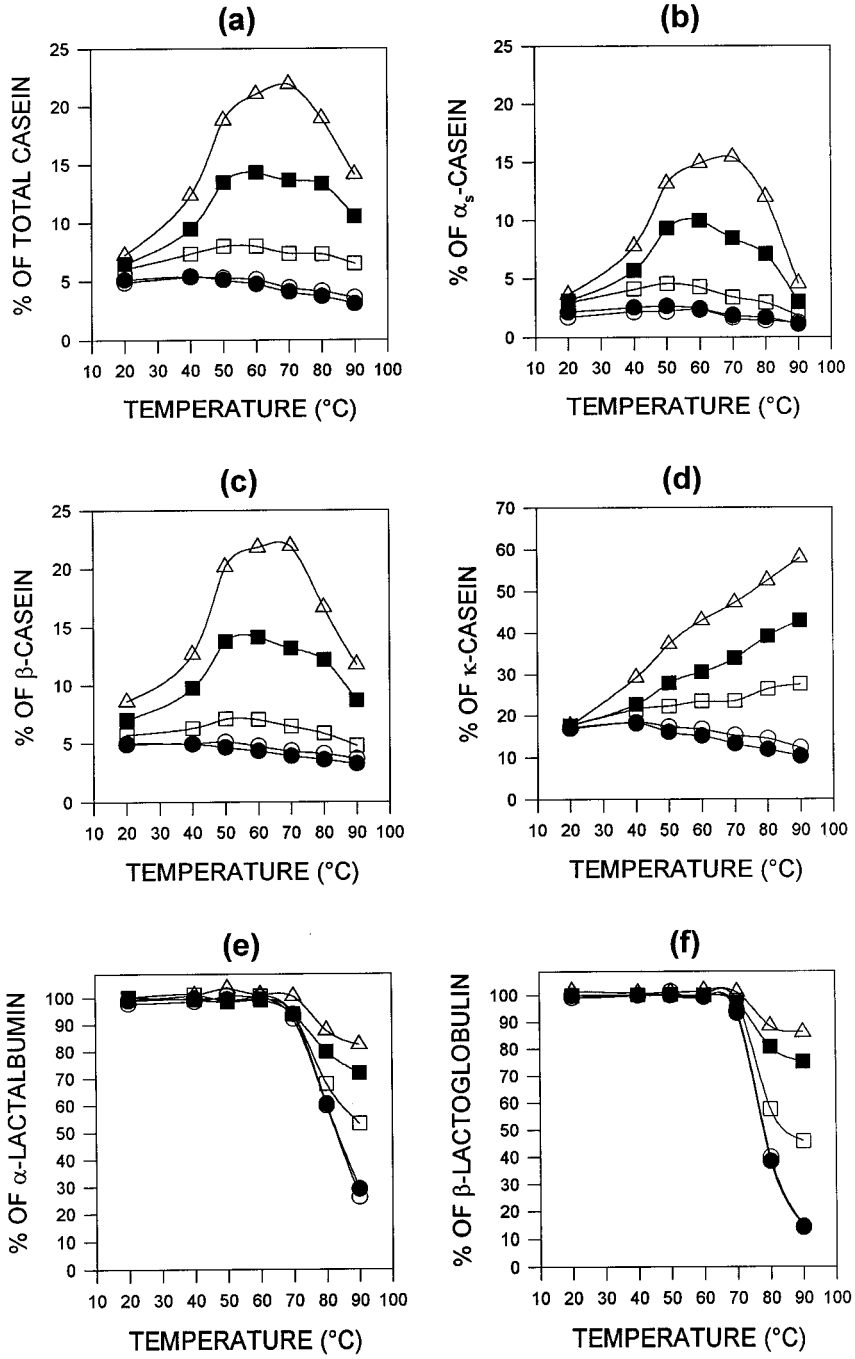

Figure 3. Effect of temperature and pH on the level of protein in the supernatants obtained from 10% TS reconstituted skim milk samples. (a) Total casein; (b) α_s-casein; (c) β-casein; (d) κ-casein; (e) α-lac; (f) β-lg. ○, pH 6.3; ●, pH 6.5; □, pH 6.7; ■, pH 6.9; △, pH 7.1.

supernatants from the samples at pH 6.3 and 6.5, and this level decreased as the temperature at heating was increased.

The dependence of α_s-casein and β-casein dissociation on pH and temperature (Figure 3, parts b and c, respectively) showed similar trends to that observed for the total casein. At pH 6.3 and 6.5, low levels of these caseins were dissociated regardless of the heating temperature. At higher pH, the level of α_s-casein and β-casein dissociating increased with increasing temperature to a maximum dissociation at about 70 °C, and then decreased as the temperature was raised further. At each heating temperature, the level of dissociated α_s-casein and β-casein increased as the pH was raised from 6.7 to 7.1. The dependence of κ-casein dissociation on temperature and pH displayed a different behavior from that of α_s-casein or β-casein (Figure 3d). At pH 6.3 and 6.5, low levels of κ-casein were dissociated from the micelles and this level decreased as the heating temperature was increased up to 90 °C. At higher pH, the level of κ-casein dissociating from the micelles increased essentially linearly with increasing temperature throughout the range investigated. At any particular temperature or pH, a higher level of κ-casein than β-casein and a higher level of β-casein than α_s-casein was dissociated from the micelles.

At temperatures up to 60 °C, virtually all of the α-lac and β-lg remained soluble regardless of the pH of the milk at heating (Figure 3, parts e and f, respectively). This suggested that no denaturation of these proteins had occurred under these heating conditions, as expected from the known temperature dependence of the

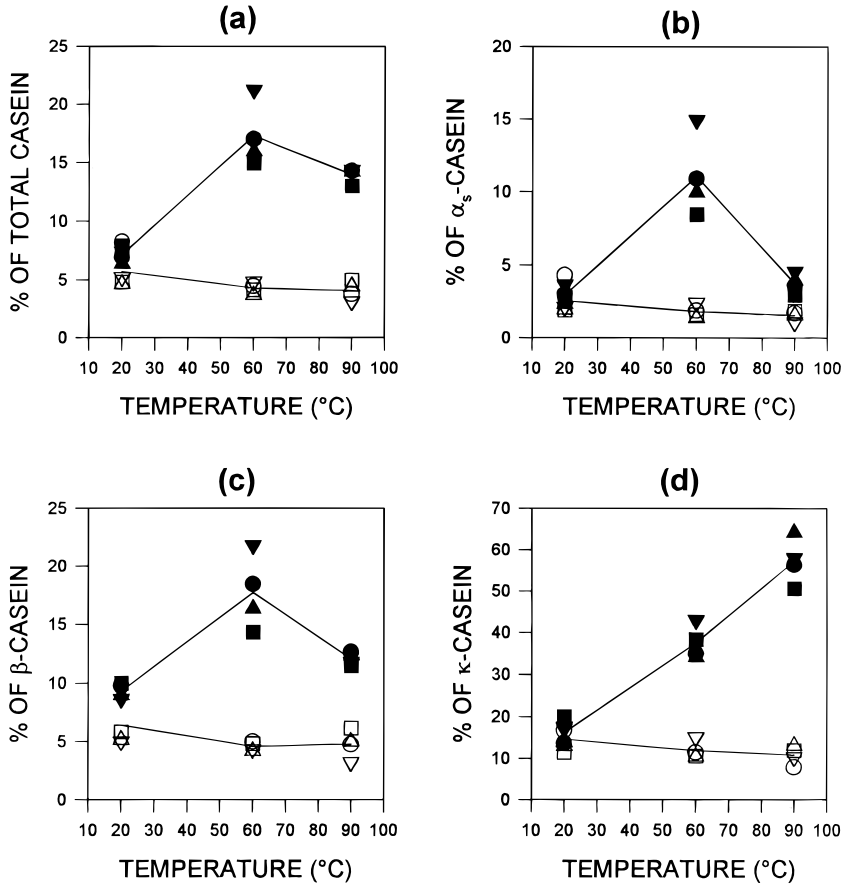

Figure 4. Comparison of the dissociation behavior with pH and temperature for four reconstituted skim milk samples. ○●, milk 1; □■, milk 2; △▲, milk 3; ▽▼, milk 4. ○□△▽, pH 6.5; ●■▲▼, pH 7.1.

thermal denaturation for α-lac and *β*-lg (Dannenberg and Kessler, 1988). At temperatures above 60 °C, the level of α-lac and *β*-lg remaining in the supernatants decreased with increasing temperature; however, the quantity remaining soluble was markedly dependent on the pH of the milk before heating. At pH 6.3 and 6.5, the level of α-lac and *β*-lg decreased markedly as the temperature of heating increased above 60 °C, indicating that a proportion of these proteins was thermally denatured and these denatured whey proteins cosedimented with the casein micelles on ultracentrifugation, probably by forming complexes with *κ*-casein via sulfhydryl−disulfide interchange reactions (Sawyer, 1969). As the pH of the milk was increased, a greater proportion of the α-lac and *β*-lg remained in the ultracentrifugal supernatants at each heating temperature. The rate of α-lac and *β*-lg denaturation is not markedly affected by pH over the pH range from 6 to 9 (Hillier et al., 1979); therefore, a similar level of these proteins would be expected to be denatured at all pH values used in this study and these denatured whey proteins would still form complexes with *κ*-casein. However, as a significant proportion of the *κ*-casein is dissociated from the micelles at higher pH, the denatured whey proteins complexed with the dissociated *κ*-casein would also remain soluble.

Four separate reconstituted skim milk samples, adjusted to pH 6.5 and 7.1, were treated at 20, 60, and 90 °C, and the levels of casein present in the ultracentrifugal supernatants were determined by SDS-PAGE and laser densitometry. All four reconstituted skim milk samples behaved similarly, although there was some

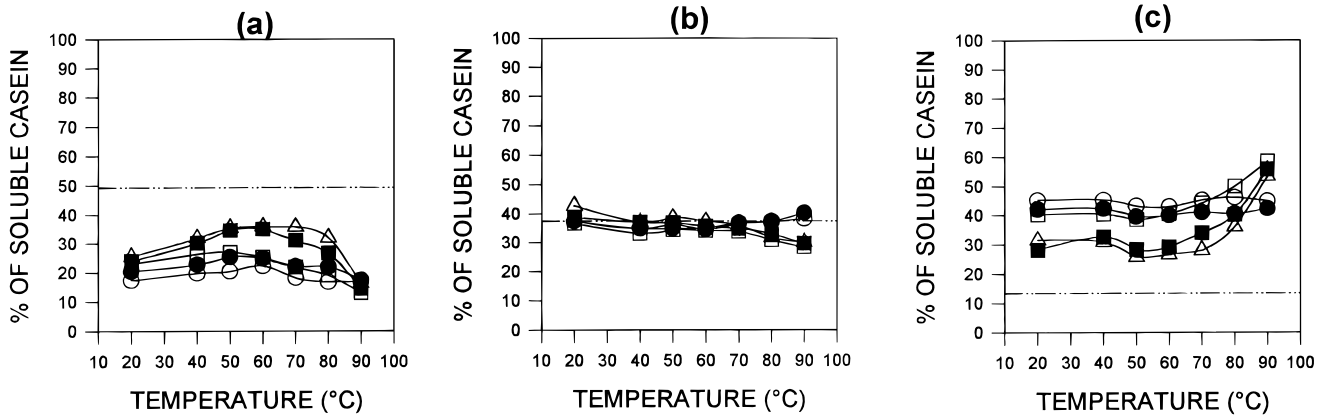

Figure 5. Composition of the soluble casein. (a) α_s-Casein; (b) *β*-casein; (c) *κ*-casein. ○, pH 6.3; ●, pH 6.5; □, pH 6.7; ■, pH 6.9; △, pH 7.1; − · · −, level of individual casein in the milk sample.

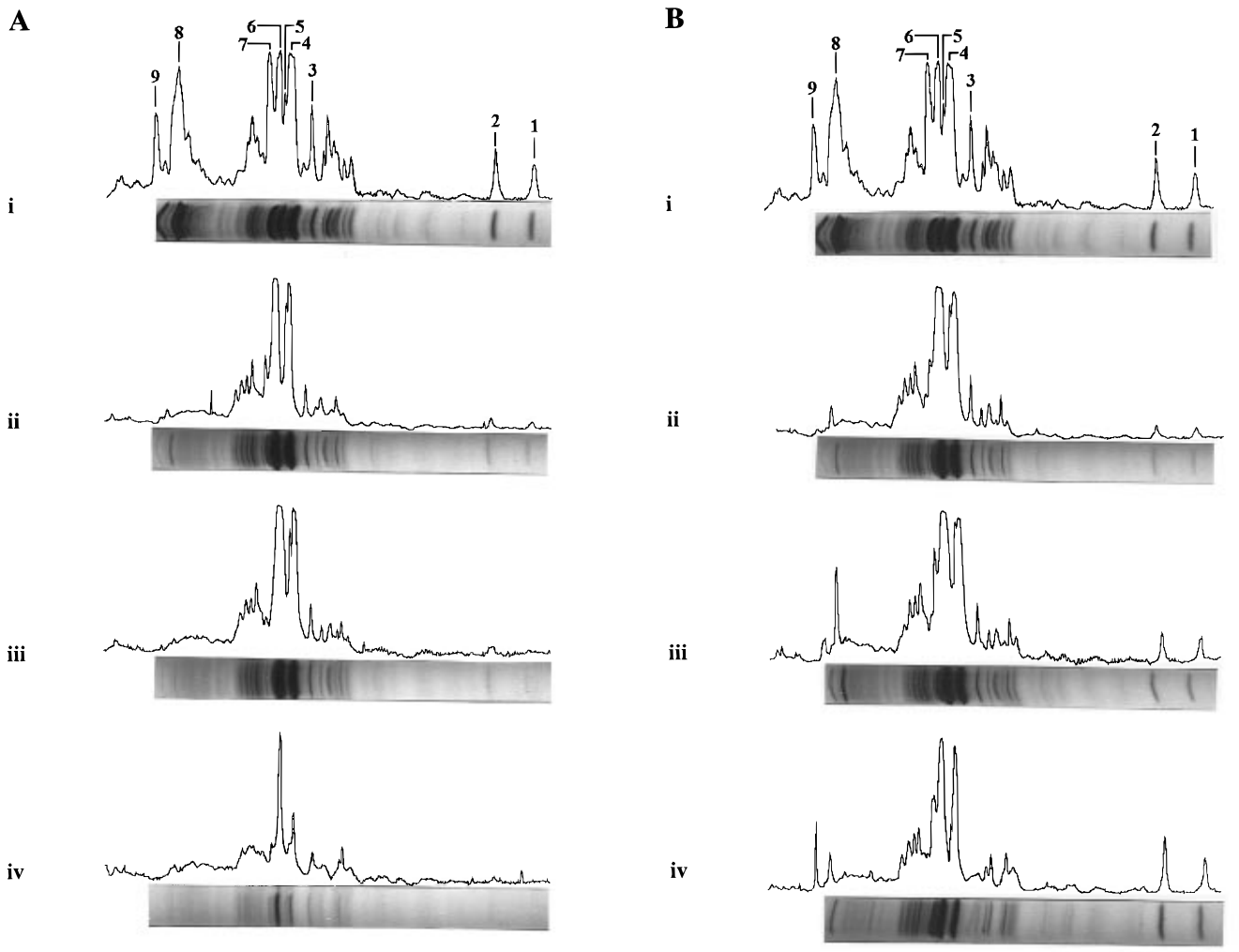

Figure 6. IEF-PAGE patterns and densitometric traces of milk samples and supernatants obtained from 10% TS reconstituted skim milk samples at pH 6.5 (A) and pH 7.1 (B). (i) Standard skim milk sample; (ii) supernatant from milk heated at 20 °C; (iii) supernatant from milk heated at 60 °C; (iv) supernatant from milk heated at 90 °C. Numbers correspond to the following milk proteins: (1) κ-casein B; (2) κ-casein A; (3) β-casein B; (4) β-lg B; (5) β-casein A^1; (6) β-lg A and α-lac; (7) β-casein A^2; (8) α_{s1}-casein B; (9) α_{s0}-casein.

variation in the level of dissociated casein between samples (Figure 4). Low levels of casein were dissociated in all samples at 20 °C regardless of the pH, and in all samples at pH 6.5 regardless of the temperature. In contrast, an average of about 17% of the total casein, 11% of the α_s-casein, 18% of the β-casein, and 37% of the κ-casein was solubilized at 60 °C and pH 7.1. At 90 °C, the level of dissociated κ-casein increased to about 57% whereas the levels of α_s-casein and β-casein decreased to about 4 and 12%, respectively.

About 50% of the casein in the skim milk sample was α_s-casein, 37% was β-casein, and 13% was κ-casein; these values are in the normal range for milk samples (Swaisgood, 1982). The proportion of β-casein in the soluble casein was similar to that observed in the milk sample and remained relatively constant regardless of the temperature or pH (Figure 5b). In contrast, the proportion of α_s-casein in the soluble casein was markedly lower and that of κ-casein was markedly higher than that found in the milk sample. In addition, these proportions varied considerably with both temperature and pH (Figure 5, parts a and c, respectively). At pH 6.3 and 6.5, the proportion of κ-casein in the soluble casein remained essentially constant throughout the temperature range. At higher pH, the proportion of κ-casein remained relatively constant at temperatures up to about 60 °C and then increased markedly as the temperature was raised further. Apart from the samples heated at very high temperatures, the proportion of soluble κ-casein decreased with increasing pH. At pH 6.3 and 6.5, the proportion of α_s-casein in the soluble casein remained relatively constant throughout the temperature range; however, at higher pH, the proportion increased to a maximum at about 60 °C and then decreased at higher temperatures. In general, a higher proportion of the soluble casein was α_s-casein at higher pH.

It is well-known and widely reported that a pH-dependent dissociation of micellar casein occurs at temperatures below 30 °C (Rose, 1968; Holt et al., 1986; Dalgleish and Law, 1988) and at temperatures above 80 °C (Kudo, 1980; Singh and Fox, 1985; Nieuwenhuijse et al., 1991; Singh and Creamer, 1991; Anema et al., 1993). This study, however, provides the first experimental evidence that a pH-dependent micellar dissociation occurs between these two temperature extremes. Over this temperature range, maximum micellar dissociation occurs at a temperature of about 70 °C (Figure 1a). This is in marked contrast to the report of Singh and Fox (1986), in which it is suggested that no dissociation of micellar κ-casein occurs at temperatures below 90 °C. This conclusion appears to be based solely on the heat stability characteristics of ultracentrifugal pellets obtained from milk heated at a range of tem-

peratures and pH rather than from the direct measurement of dissociated casein. The dissociation at temperatures between 20 and 90 °C is similar to that reported for the high-temperature dissociation in that κ-casein is the major protein dissociated; however, in contrast to the dissociation at ≥90 °C, a substantial proportion of the soluble casein is α_s-casein and β-casein, particularly at temperatures below 80 °C (Figure 4). In contrast, β-casein is the predominant casein rendered soluble at low temperatures (Dalgleish and Law, 1988).

The process by which casein is dissociated from the micelles when the temperature and pH are raised remains unknown. Casein micellar structure is maintained by hydrophobic effects, electrostatic interactions, and the CCP. Hydrophobic effects reportedly increase with temperature up to about 80 °C, decrease at higher temperatures and apparently have little pH dependence (Tanford, 1980); therefore, these effects are unlikely to play a role in this dissociation behavior of casein micelles. The pH dependence of the dissociation suggests that electrostatic interactions may be involved. Singh and Creamer (1991) suggested that the charge on κ-casein is important in keeping this protein associated with the micelles and that the dissociation of κ-casein at high temperatures (≥90 °C) and moderately high pH is a consequence of the irreversible modification of the protein charge by heat-induced effects. IEF-PAGE, which separates proteins based on their isoelectric points, should indicate whether irreversible changes to the charge of any of the proteins has occurred on heat treatment. IEF-PAGE showed that the genetic combinations of the dissociated proteins were the same as that observed in the raw milk and that there was no apparent change to the overall charge of any of the dissociated casein proteins (Figure 6). This precluded the possibility that dissociation is induced through modifications to the protein charge distribution.

A possible explanation for this dissociation behavior of micellar casein is the occurrence of a pH- and temperature-dependent conversion of the native CCP to an alternative form of calcium phosphate which is less capable of maintaining micellar integrity. This conversion will weaken the CCP linkages so that at high pH, where protein charge is greater, the modified CCP can no longer maintain micellar structure and dissociation of casein occurs. Aoki et al. (1990) have reported that the linkage of casein to CCP is reduced after heat treatment and suggest that a heat-induced change to the native CCP may explain this behavior. Visser et al. (1986) have shown that the temperature at which amorphous calcium phosphate precipitates from heated simulated milk serum is markedly pH dependent, with a lower precipitation temperature at higher pH. In addition, Holt (1985) and Dalgleish (1989) have reviewed other evidence that suggests that possible changes to the structure of native CCP may occur on heating, particularly at temperatures above 80 °C. Calcium and phosphate are clearly implicated in the dissociation behavior of casein micelles at temperatures ≥90 °C as an increase in soluble calcium or a decrease in soluble phosphate reduces micellar dissociation (Visser et al., 1986; Singh and Fox, 1987).

There was a temperature-dependent change in dissociation behavior at about 70 °C, in which a reduced level of α_s-casein and β-casein was dissociated from the micelles as the temperature was raised above this level. As this temperature approximately corresponds to the onset of whey protein denaturation (Dannenberg and Kessler, 1988), this phenomenon may be a consequence of the interaction of the denatured whey proteins with κ-casein. It is known that the complex formed between κ-casein and denatured β-lg is less capable at stabilizing α_s-casein and β-casein to calcium ions than uncomplexed κ-casein (Zittle et al., 1962; S. G. Anema, unpublished observation). At temperatures below 70 °C, the dissociated α_s-casein and β-casein are able to remain in the serum as small soluble aggregates stabilized by the dissociated κ-casein. At higher temperatures, the κ-casein−β-lg complex can no longer effectively stabilize these proteins; therefore, either they do not dissociate from the micelles or the dissociated casein precipitates in the presence of calcium and deposits with the casein micelles on ultracentrifugation. As the calcium and phosphate concentrations are decreased on heat treatment and increase again on cooling (Pouliot et al., 1989a,b), the precipitation of α_s-casein and β-casein may be more pronounced when the samples are returned to ambient temperatures.

The results of this study have demonstrated that there is a considerable pH- and temperature-dependent dissociation of casein protein from the micelles when reconstituted skim milk is heated at temperatures below 100 °C. This type of dissociation behavior, particularly at temperatures below 80 °C, has not been reported previously. Further research on this dissociation behavior is currently in progress, particularly the effect of milk concentration and a comparison of the present results with those from fresh skim milk counterparts.

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