Proteolysis of Goat β-Casein by Calf Rennet under Various Factors Affecting the Cheese Ripening Process

Antonio-José Trujillo,*,† Buenaventura Guamis,† and Carmen Carretero‡

Tecnologia dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain, and Tecnologia dels Aliments, Escola Politècnica Superior, Universitat de Girona, E-17071 Girona, Spain

The proteolytic activity of calf rennet on goat β-casein was studied under various technological parameters which affect cheese ripening (temperature, pH, salt and calf rennet concentrations). Electrophoretic studies showed that this protein hydrolyzes to give five products; β-I–β-V, in order of appearance and increasing electrophoretic mobility under alkaline conditions. In an aqueous solution, β-casein was optimally hydrolyzed to β-I at pH 6.2, β-II at pH 3.8, and β-III at pH ≥5.4. β-IV products were formed at all pH values, and β-V was optimally formed at pH ≤5.0. Both β-IV and β-V were formed in very small quantities. Proteolysis of β-casein by calf rennet is reduced by the addition of 5% NaCl, while the addition of 15% NaCl leaves only traces of β-I. The polypeptides β-I, β-II, and β-III produced from caprine and bovine β-caseins gave identical results with PAGE, which suggests that the calf rennet attacks the same regions described as susceptible to bovine β-casein cleavage by chymosin.

Keywords: *Proteolysis; calf rennet; β-casein; cheese ripening*

INTRODUCTION

Only a few studies have been dedicated to goat β-casein, although this protein represents the most abundant fraction in goat's milk (Remeuf and Lenoir, 1985; Grosclaude et al., 1987; Carretero et al., 1994). The primary structure of β-casein has recently been identified from the nucleotidic sequence of the gene (Roberts et al., 1992). This protein contains 207 amino acid residues instead of the 209 that bovine β-casein contains, which is due to the deletion of the Pro_{179}–Tyr_{180} dipeptide.

The proteolysis of casein by rennet is very important in cheese ripening. Chymosin hydrolyzes isolated bovine β-casein to yield β-I, β-II, β-III, and β-IIIb (Creamer, 1976; Pelissier et al., 1974; Visser and Slangen, 1977). β-IIIb is only formed under extreme incubation conditions, such as low pH values after prolonged incubation (Visser and Slangen, 1977).

Mulvihill and Fox (1979) studied the proteolytic activity and specificity of bovine, ovine, caprine, and porcine chymosins and pepsins on the β-caseins of these four species, and they concluded that these proteins probably have very similar amino acid sequences because they showed almost identical proteolytic patterns.

In cheese, β-I and the other characteristic bands resulting from rennet action have not been clearly identified. Ledford et al. (1966), in Cheddar cheese, and Trieu-Cuot and Gripon (1982), in Camembert cheese, concluded that rennet does not act on β-casein during the ripening of these cheeses because they failed to detect the degradation products β-I, β-II, and β-III. This may have been due to the presence of salt or low water activity or both during the ripening process. However, Marcos et al. (1979), in different varieties of cheeses, and Carretero et al. (1994), in Montsec cheese, mentioned the presence of β-I and possibly β-II.

This paper reports a study of the proteolytic activity and specificity of calf rennet on goat β-casein under different proteolytic conditions with observations on cheese technology and ripening.

MATERIALS AND METHODS

Whole goat and cow caseins were prepared by isoelectric precipitation (acetate buffer, pH 4.6) respectively from a sample of bulk goat and cow skim milks obtained from the Experimental Farm, Universitat Autònoma de Barcelona.

Crude goat β-casein was prepared from an acid-precipitated whole casein using the method described by Hipp et al. (1952) and further purified according to the batch fractionation method of Wei and Whitney (1985) with DEAE-cellulose fast flow (Sigma, St. Louis, MO). The purified fractions were dialyzed with distilled water and lyophilized, and the purity of each β-casein fraction was checked by urea–PAGE.

Enzymes. Commercial calf rennet (Renifor 15/E) was obtained from Lamirsa (Laboratorios Miret S.A., Barcelona, Spain) and contained 780 mg of chymosin (EC 3.4.23.4)/L and 565 mg of pepsin (EC 3.4.23.1)/L and had a bovine pepsin milk clotting activity of about 25% in relation to the total (International Dairy Federation, 1987).

Pure chymosin (Aniren 880) was obtained from Sanofi Bio-Ind (Beaune Cedex, France) with a declared activity of 880 mg of chymosin/L.

Pure bovine pepsin (Bovipep 1700) was also obtained from Sanofi Bio-Ind with a declared activity of 1700 mg of pepsin/L.

Enzyme solutions were standardized to equal milk clotting activity by a modification of Berridge's method (Collin et al., 1977). Enzyme solution (1 mL) was added to glass tubes containing 10 mL of low-heat skim milk powder (INRA, Poligny, France) dissolved in 10 mM $CaCl_2$ at 30 °C. The observation of graininess or the appearance of flecks on the tube wall gave the clotting time.

Hydrolysis Conditions. Solutions of β-casein (2.5% w/v) in 50 mM sodium acetate buffer at pH 6.6 containing 0.02% thimerosal to prevent microbial activity were heated (80 °C, 30 min) to prevent milk protease activity during incubation and were treated with calf rennet at a level of 0.1 rennet unit (RU)/mL. One RU is the amount of enzyme required to coagulate 10 mL of low-heat skim milk powder dissolved in 10 mM $CaCl_2$ in 100 s at 30 °C under the conditions specified

* Author to whom correspondence should be addressed.
† Universitat Autònoma de Barcelona.
‡ Universitat de Girona.

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by the International Dairy Federation (1987). Calf rennet used in the experiments contained 100 RU/mL. The solutions were then individually adjusted to a range of pH values (3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2 ,and 6.6) and rotated (13 rpm) at 30 °C during different incubation times (1, 2, 4, 6, 15, 30, 48, and 72 h). At the end of each period, rennet was inactivated by heating (100 °C, 5 min) and the pH was lowered to 4.6. After centrifugation (12 000 rpm, 10 min), the supernatants were filtered through 0.45 μm filters and analyzed by SDS–PAGE (polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate). The pellets were redissolved in 7.0 M urea, and samples were taken for electrophoretic separation on urea–PAGE and SDS–PAGE.

Hydrolysis conditions, as well as incubation times and rennet concentrations, were chosen according to the results of preliminary experiments to give a satisfactory hydrolysis. The incubation temperature chosen was 30 °C. Experiments were carried out at 7, 15, and 30 °C for different hydrolysis conditions and showed that these incubation temperatures do not influence the nature of proteolysis products. Other special hydrolysis conditions used in the experiment are explained under Results and Discussion.

Electrophoresis. Alkaline urea–PAGE with 0.7 mm spacers was performed according to the method described by Akroyd (1968) with a 8.8%T [grams of acrylamide plus grams of bis(acrylamide)/100 mL], 2.3%C [grams of bis(acrylamide)/%T], and 5 M urea at pH 8.9 as described by Carretero et al. (1994).

SDS–PAGE (0.7 mm thick gel) was performed following the Laemmli (1970) technique with a resolving gel of 15%T, 2.7%C, and a stacking gel of 4%T, 2.7%C. A low molecular weight calibration kit (Sigma) was used for molecular weight measurements. The molecular weight markers used were bovine serum albumin (66 000), chicken egg ovalbumin (45 000), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 000), bovine erythrocytes carbonic anhydrase (29 000), bovine pancreas trypsinogen (24 000), soybean trypsin inhibitor (20 100), bovine milk α-lactalbumin (14 200), and bovine lung aprotinin (6500).

Both gels were stained with Coomassie Blue R-250 (Uriel, 1966) and were destained by repeated washing in an ethanol/acetic acid/glycerol/water (200/50/25/725 v/v/v/v) solution.

Band scanning was carried out with a laser densitometer (LKB 2202 Ultroscan) at $\lambda = 633$ nm, connected to a Hewlett-Packard 3390A integrator used for densitometric readings.

RESULTS AND DISCUSSION

Proteolysis of Goat β-Casein by Calf Rennet. The electrophoretograms in Figure 1 show that β-casein is hydrolyzed to give five products, which we have designated β-I–β-V, according to the classification given by Creamer et al. (1971) in order of appearance and increasing electrophoretic mobility under alkaline conditions. The following special characteristics were observed: (1) β-Casein, in alkaline PAGE, migrates as two bands, β_1 and β_2, which differ in their level of phosphorylation (6/5) (Richardson and Creamer, 1974), and the products formed during the action of calf rennet also appeared as two bands, because the β-casein phosphorylation zone was included in these breakdown products. (2) β-I and β-IV showed mobilities very similar to those of β-casein and β-III, respectively. (3) β-IV and β-V bands were only slightly formed under these proteolytic conditions, and they showed poor staining capacities. (4) β-Casein was optimally hydrolyzed to β-I at pH 6.2 and to β-II at pH 3.8. Although β-III was produced at all pH values, it was optimally formed at pH ≥5.4. β-IV was formed at all pH values. β-V was optimally formed at pH ≤5.0. The last two products were formed in very small quantities. At isoelectric pH (pH 4.6), the hydrolysis of β-casein was minimal, as was the formation of its degradation products.

Figure 1 also shows that the pH level did not influence the proteolytic specificity of rennet on β-casein, but the rennet activity was highly dependent on the pH value.

After only 1 h of incubation, the polypeptides β-I and β-II appeared at all pH values. β-Casein was optimally degraded at pH 5.4, although at pH 4.2 it also showed strong degradation. Minimum hydrolysis on β-casein at pH 4.6 (isoelectric pH) was observed. At pH values of 3.8, 5.0, and 6.6 little casein was degraded. Polypeptide β-II was optimally formed at extreme acid values studied (Figure 1A).

After 2 h of incubation, the electrophoretic pattern did not change but an intensification of the bands was evident.

After 4 h of incubation and at pH 6.2, maximum β-casein degradation and β-I formation were observed. At isoelectric pH, minimal β-casein degradation and β-I formation continued to be observed. At pH 6.6, nearly 50% of the initial β-casein was degraded, 50% of β-I was formed, and very little β-II was produced (Figure 1B).

After 6 h of incubation and at a pH range of 5.8–6.2, β-casein disappeared and at the same time polypeptide β-I was formed; at pH 6.2, β-I represented 90% of the total of polypeptides formed (Figure 1C).

After 15 h of incubation, β-casein disappeared for pH values ≥5.4. At pH 6.6 there was maximum β-I formation but minimal or no β-II production. It would seem that β-I is highly resistant to enzyme action, and thus it accumulated without being hydrolyzed. β-II polypeptide was produced at all pH ranges, with a minimum at pH 6.2–6.6 and a maximum at pH 3.8 (Figure 1D). At this latter pH little β-I was formed but high quantities of β-II were produced. This could be because the β-I formed is quickly hydrolyzed to β-II or because different conformational changes might occur in β-casein due to the state of aggregation (protein precipitation occurred in pH range 3.8–5.0), and thus the susceptible cleavage sites for the production of β-I might not be completely available.

After 30 h of incubation, β-III, β-IV, and β-V were formed. β-III and β-IV were optimally produced at pH ≥5.4 and β-V at pH ≤5.0 (Figure 1E). The polypeptides appeared sequentially as there was no evidence in the experiments of the formation of any β-casein degradation product if its predecessor product was not formed. The rates of formation of β-II and β-III were only affected when the rate of formation of β-I was affected too. Thus, the first attack of rennet on β-casein produces the polypeptide β-I, which is further degraded to β-II, and then β-II may be attacked to give β-III. Finally, β-III might give β-IV and β-V. This fact may be explained by the conformation adopted by β-casein, which makes other sites of attack unavailable.

After 48 h of incubation, electrophoretic bands corresponding to β-III, β-IV, and β-V increased. The electrophoretic pattern at 72 h, the longest incubation period, was similar except for β-IV and β-V, which appeared slightly reduced (Figure 1F).

SDS–PAGE showed that the goat β-casein breakdown products, pH 4.6 insoluble fraction, ranged in relative molecular mass (M_r) from 22 300 to 12 800. The incubation conditions (pH and incubation time) used in these experiments were chosen to obtain all of the breakdown products sequentially (Figure 2). The enzyme level used was 0.1 RU/mL.

The estimated M_r for β-casein was 27 500; this result is not wholly consistent with the molecular weight calculated from its primary structure (M_r = 23 800). Data from different laboratories show that the molecular weights for bovine α_{s1}- and β-caseins derived from

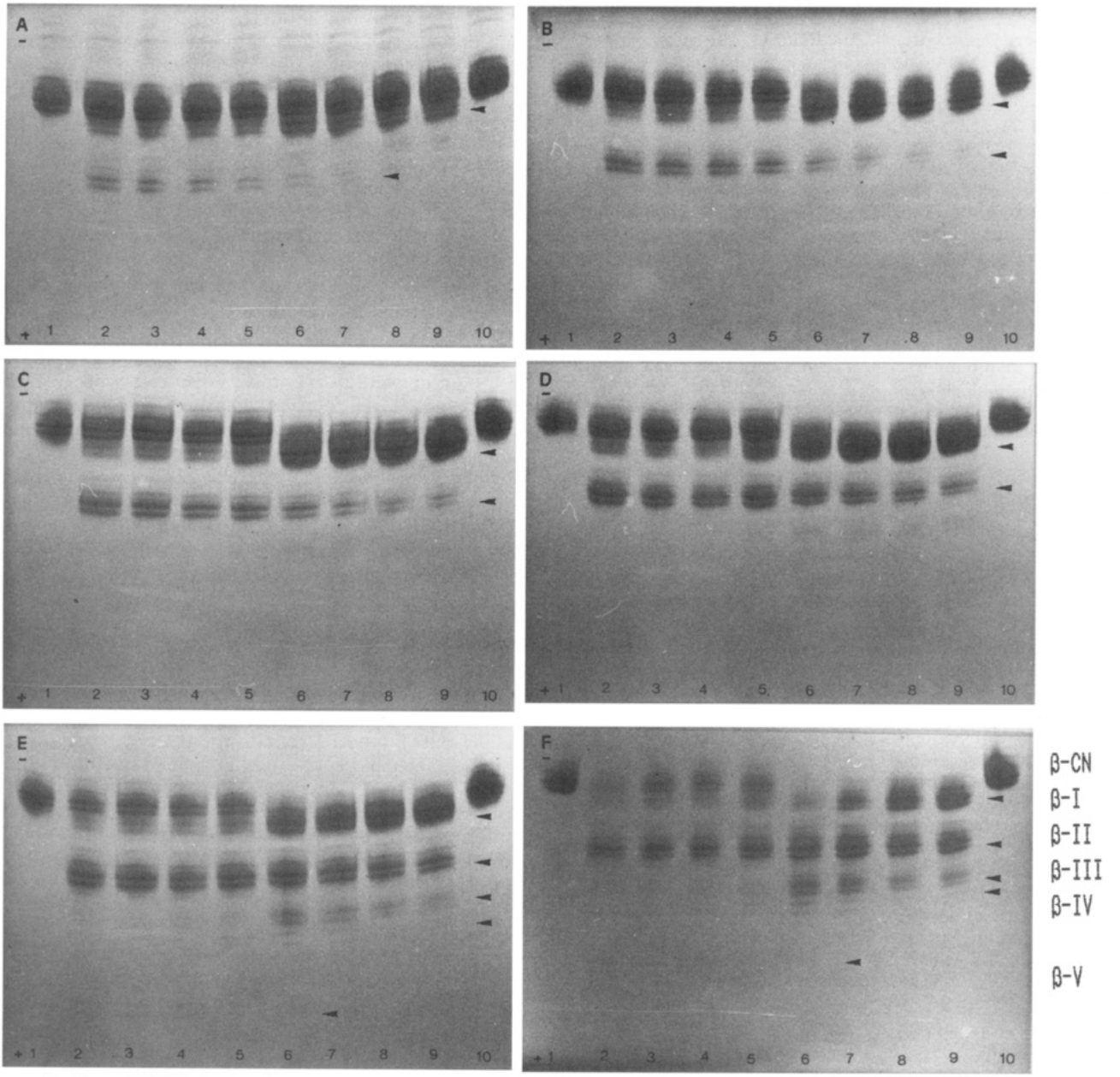

Figure 1. Urea–PAGE electrophoretograms of the effect of pH on the proteolysis of β-casein by calf rennet at 30 °C for (A) 1, (B) 4, (C) 6, (D) 15, (E) 30 and (F) 72 h. Unrenneted controls (lanes 1 and 10) and samples hydrolyzed at pH 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, and 6.6 (lanes 2–9), respectively, are shown.

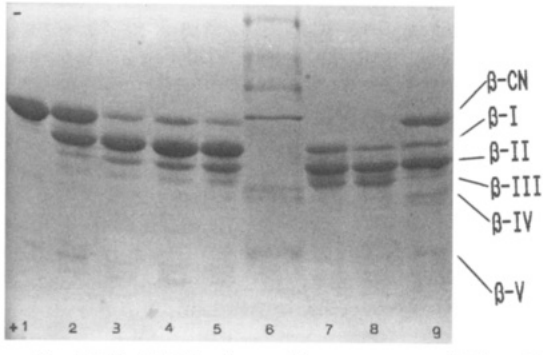

Figure 2. SDS–PAGE electrophoretograms of the pH 4.6 insoluble breakdown products of goat β-casein produced by calf rennet. Unrenneted control (lane 1) and samples hydrolyzed at pH 5.4 for 1, 6, 15, 30, 48, and 72 h (lanes 2–7, and 8), respectively, are shown. Molecular weight calibration kit (slot 6) and sample hydrolyzed at pH 3.8 for 30 h (lane 9) are also shown.

migration in SDS gels are higher than those calculated from amino acid sequence studies, yet they are well within the range of values given by other physical measurements. This fact could be explained because caseins behave abnormally in the Laemmli system, migrating to a position which is near or above that of carbonic anhydrase (M_r 29 000). It appears that individual caseins bind different amounts of SDS. This may lead to anomalous results, which may be due to competing equilibria as the caseins either bind SDS or interact with other casein molecules (Basch et al., 1985). Our calculated apparent molecular weight agrees with the works of Groves et al. (1972) and Green and Pastewka (1976).

Calf rennet degraded goat β-casein to give five pH 4.6 insoluble polypeptides (β-I–β-V) of M_r = 22 300, 21 400, 20 700, 18 900, and 12 800, respectively.

SDS–PAGE analysis of the pH 4.6 soluble fraction showed that these degradation products had M_r values in the range 23 600–8500. The predominant bands varied with the pH and ionic conditions used in the experiments. The electrophoretic patterns at the pH values studied were very similar, but little differences

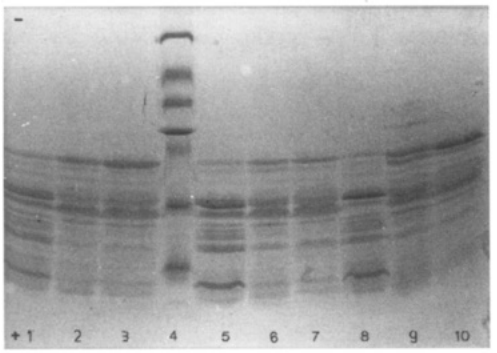

Figure 3. SDS–PAGE electrophoretograms of the pH 4.6 soluble breakdown products of goat β-casein produced by calf rennet at pH 3.8 (lanes 1–3), pH 5.4 (lanes 5–7), and pH 6.6 (lanes 8–10) for 1, 6, and 15 h, respectively. Molecular weight calibration kit (lane 4) is also shown.

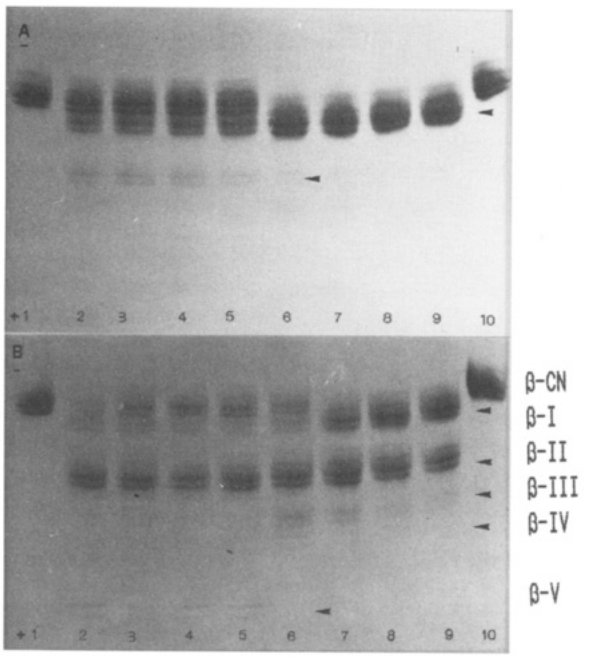

Figure 4. Urea–PAGE electrophoretograms of goat β-casein treated with (A) chymosin and (B) pepsin at 30 °C for 15 h. Unrenneted controls (lanes 1 and 10) and samples hydrolyzed at pH 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, and 6.6 (lanes 2–9), respectively, are shown.

were observed at pH 3.8. The degradation products formed at pH 5.4 and 6.6 included peptides in the range M_r = 23 600–14 700, but other peptides with M_r values lower than 14 500, formed at pH 3.8, did not appear clearly. After 15 h incubation and at pH 6.6, new peptides with M_r = of 19 900 and 19 400 were formed (Figure 3).

Proteolysis of Goat β-Casein by Pure Bovine Chymosin and Pepsin. Gastric proteinases are traditionally used for the manufacture of most cheese varieties, with calf rennet and bovine rennet being the most common types. Both contain chymosin and pepsin at different levels depending on the type, individual, and age of calves, cows, etc. (Andren and Collin, 1986, 1988). Due to the shortage of abomasa from very young suckling calves, calf rennets nowadays contain more bovine pepsin than previously. As the proteolytic activity of bovine pepsin has been shown to be greater and it seems to be more pH sensitive to clotted milk than to chymosin (Fox, 1969), it is of interest to establish the differences, if any, in its ability to hydrolyze goat β-casein.

Samples of β-casein (2.5% w/v) treated with pure

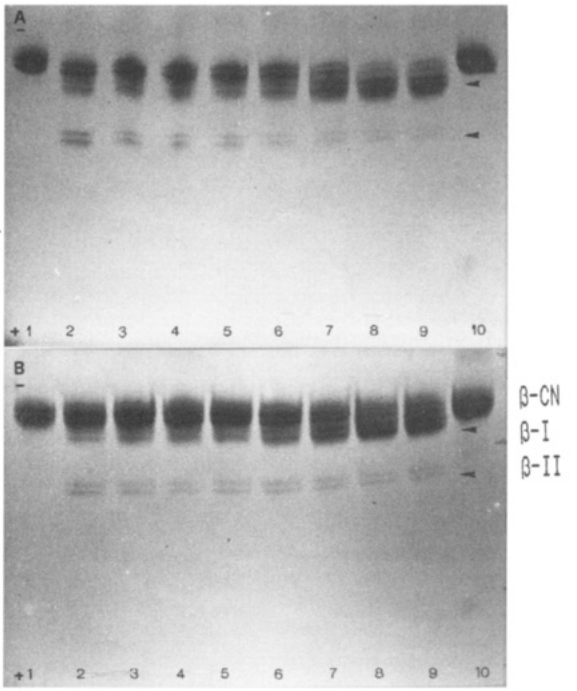

Figure 5. Urea–PAGE electrophoretograms of goat β-casein treated with calf rennet at rates of (A) 4×10^{-3} and (B) 1.2×10^{-3} RU/mL for 15 h at 30 °C. Unrenneted controls (lanes 1 and 10) and samples hydrolyzed at pH 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, and 6.6 (lanes 2–9), respectively, are shown.

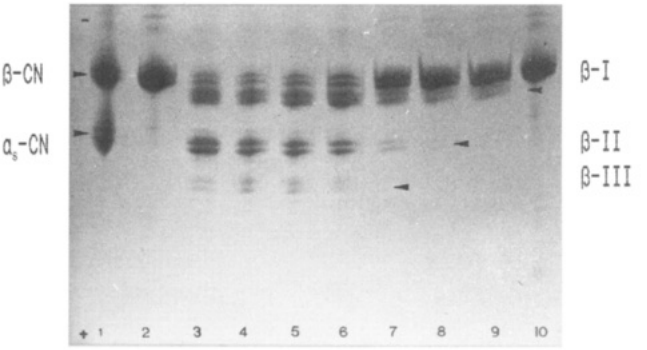

Figure 6. Urea–PAGE electrophoretograms of the effect of NaCl on the proteolysis of goat β-casein by calf rennet at pH 5.4, 30 °C, for 15 h. Whole casein (lane 1), unrenneted controls (lanes 2, and 10), and samples containing 0, 0.5, 1, 2.5, 5, 10, and 15% (w/v) NaCl (lanes 3–9), respectively, are shown.

chymosin and bovine pepsin at 0.1 RU/mL (enzyme solutions standardized to equal milk clotting activity) were adjusted to the different pH values and were incubated at 30 °C for 15 h.

The results from the electrophoretograms (Figure 4) showed that, as a group and for equal milk clotting activities, bovine pepsin was more proteolytic than chymosin. Goat β-casein was hydrolyzed to its β-I polypeptide by both bovine chymosin and pepsin, chymosin being the more active. β-I polypeptide was quite resistant to further hydrolysis by bovine chymosin, especially at pH ≥5.4. β-II polypeptide was only formed at pH ≤5.0 under the experimental conditions described above, whereas it was hydrolyzed to β-II, β-III, β-IV, and β-V by bovine pepsin.

Effect of Rennet Concentration on the Proteolysis of β-Casein. Most of the rennet added to cheese milk is lost in the whey and only small quantities remain enclosed in the curd after cheese manufacture, depending on the conditions during manufacture [see Guinee and Wilkinson (1992)]. Residual rennet in cheese curd appears to be highly stable during cheese

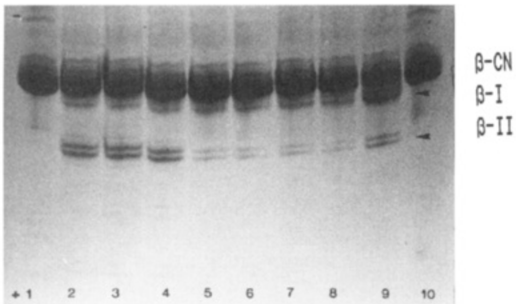

Figure 7. Urea–PAGE electrophoretograms of the effect of pH and NaCl (5%, w/v) on the proteolysis of goat β-casein by calf rennet at 30 °C for 15 h. Unrenneted controls (lanes 1 and 10) and samples hydrolyzed at pH 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, and 6.6 (lanes 2–9), respectively, are shown.

ripening (Matheson, 1981; Boudjellab et al., 1994), and it makes a major, perhaps essential, contribution to proteolysis during ripening and consequently to flavor and texture development (Fox, 1989).

In this study the quantities used in the experiments are equivalent to those retained in cheese curd according to different authors (Fox, 1988; Boudjellab et al., 1994). The values indicated below as rennet units per milliliter correspond to 20 and 6% of rennet retention in curd when milk cheese is treated at a rate of 20 mL/100 l.

Samples of β-casein (2.5% w/v) were treated with calf rennet at 4×10^{-3} and 1.2×10^{-3} RU/mL, adjusted to the different pH values and then incubated at 30 °C for 15 h.

The electrophoretograms in Figure 5 show that calf rennet, in both concentrations studied, produced the polypeptides β-I and β-II along the whole range of pH values. Other β-casein degradation products were not formed due to the low enzyme concentrations used for this incubation time. When the incubation time was prolonged, all of the β-casein degradation products were formed.

Effect of NaCl on the Proteolysis of β-Casein by Rennet. NaCl principally influences cheese ripening

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                                        10                                      20
     Leu                    Pro        Ile
Arg-Glu-Gln-Glu-Glu-Leu-Asn-Val-Val-Gly-Glu-Thr-Val-Glu-Serp-Leu-Serp-Serp-Serp-Glu-

                                        30                                      40
             Arg
Glu-Ser-Ile-Thr-His-Ile-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Gln-Serp-Glu-Glu-Gln-Gln-Gln-

                                        50                                      60
                                                    Thr
Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Ala-Gln-Ser-Leu-Val-Tyr-

                                        70                                      80
    Pro                                             Pro
Pro-Phe-Thr-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Leu-Pro-Leu-Thr-Gln-Thr-

                                        90                                      100
                                            Val         Ser
Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Ile-Met-Gly-Val-Pro-Lys-Val-Lys-Glu-

                                        110                                     120
Ala     Ala
Thr-Met-Val-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-

                                        130                                     140
                            ↓               Asn             Pro Leu    ↓Leu
Glu-Ser-Gln-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-Lys-Leu-His-Leu-Pro-Leu-Pro-Leu-Val-

                                        150                                     160
                            His             Pro
Gln-Ser-Trp-Met-His-Gln-Pro-Pro-Gln-Pro-Leu-Ser-Pro-Thr-Val-Met-Phe-Pro-Pro-Gln-

                                        170                                     180
            ↓       ↓       ↓Ser                                    Pro Tyr
Ser-Val-Leu-Ser-Leu-Ser-Gln-Pro-Lys-Val-Leu-Pro-Val-Pro-Gln-Lys-Ala-Val-*-*-Pro-Gln-

                                        190                                     200
                            ↓       ↓
Arg-Asp-Met-Pro-Ile-Gln-Ala-Phe-Leu-Leu-Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-Val-Arg-

                        207
                        Ile
Gly-Pro-Phe-Pro-Ile-Leu-Val
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Figure 8. Primary structure of goat β-casein (Roberts et al., 1992) compared with bovine β-casein A^2 (Ribadeau Dumas et al., 1972; Grosclaude et al., 1973; Carles et al., 1988). In the bovine sequence, only the different residues are plotted in italics, above the caprine sequence. The cleavage sites susceptible to hydrolysis by chymosin in bovine β-casein are indicated by arrows (Creamer, 1976; Pelissier et al., 1974; Visser and Slangen, 1977). Asterisks indicate a dipeptide deleted in goat β-casein.

through its effect on water activity, but it probably has some more specific effects, partly due to water activity [see Guinee and Fox (1987)]. In cheese, characteristic bands resulting from rennet action have not been clearly identified. During the ripening of Cheddar cheese, β-casein undergoes limited hydrolysis and it does not appear to be hydrolyzed by coagulants (Ledford et al., 1966; Phelan et al., 1973). However, other authors (Marcos et al., 1979; Carretero et al., 1994) mentioned the presence of the breakdown products from β-casein by rennet in cheese, perhaps due to the cheese technology used and the ripening conditions.

Samples of β-casein (2.5% w/v) made at 0, 0.5, 1, 2.5, 5, 10, and 15% w/v with NaCl were treated with rennet at 0.1 RU/mL, adjusted to pH 5.4, and incubated at 30 °C for 15 h.

The electrophoretogram in Figure 6 shows that β-casein is more resistant to hydrolysis as NaCl concentration increases up to 15%. At 15% NaCl, only very slight proteolysis of β-casein was evident. Characteristic β-casein degradation peptides were formed until the NaCl concentration increased to 2.5%. The greatest change in the degradation peptides was between 5 and 15% NaCl, where β-II was formed only slightly at 5% NaCl but not at higher concentrations. β-III was not formed. At 10 and 15% NaCl concentration only β-I was formed in very small quantities.

Effect of pH on Proteolysis of β-Casein in 5% NaCl by Rennet. Samples of β-casein (2.5% w/v) with 5% NaCl were adjusted to different pH values, treated with rennet at 0.1 RU/mL, and incubated at 30 °C for 15 h.

The electrophoretogram in Figure 7 shows that the inhibitory effect of NaCl on goat β-casein varied with pH. The inhibition was more pronounced as pH decreased, above all at pH 5.0.

β-I was produced at all pH values, and it increased as the pH was increased, especially at pH 6.6. At low pH values (pH ≤4.6) polypeptide β-II was the main breakdown product observed. β-III was not formed for any pH values in the presence of 5% NaCl.

In the presence of 5% NaCl at pH 5.4, ionic conditions in many young cheeses, β-I and β-II were formed.

The inhibitory effect of NaCl on bovine β-casein is pH dependent too. However, at low pH levels, NaCl also alters the proteolytic specificity of chymosin and pepsin; NaCl (2.5%) inhibits the formation of β-III and promotes the formation of two peptides, which were only resolved by a modified gel electrophoresis method (Mulvihill and Fox, 1978). On goat β-casein in the proteolytic conditions and electrophoretic system described above, no other products except β-I and β-II were formed.

Comparison of the Degradation Products of Caprine and Bovine β-Caseins. Figure 8 shows the sequences of caprine and bovine β-caseins. The homology between these two proteins is very high (>90%), with similar amino acid sequences in the regions described as susceptible to cleavage by calf chymosin in bovine β-casein. Calf chymosin hydrolyzes isolated bovine β-casein at residues 189−190 and/or 192−193, 163−164 and/or 165−166 and/or 167−168, 139−140, and 127−128 to yield β-I, β-II, β-III, and β-IIIb (Creamer, 1976; Pelissier et al., 1974; Visser and Slangen, 1977). The most significant difference is due to the deletion of the Pro_{179}−Tyr_{180} dipeptide in goat β-casein.

Goat β-casein and whole bovine casein were treated with rennet at a level of 0.1 RU/mL, pH 5.4 at 30 °C, for different incubation times (1, 2, 4, 6, and 15 h). The electrophoretogram in Figure 9 shows the proteolytic action of rennet on goat β-casein and whole bovine

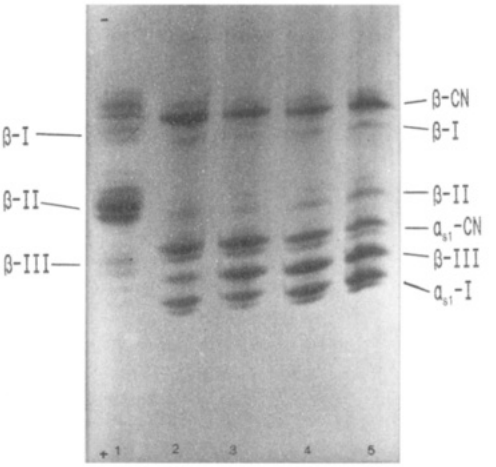

Figure 9. Urea−PAGE electrophoretograms showing the degradation products of caprine and bovine β-caseins produced at pH 5.4 by calf rennet. Goat β-casein hydrolyzed for 15 h (lane 1) and bovine whole casein hydrolyzed for 6, 15, 30, and 48 h (lanes 2−5), respectively, are shown.

casein. Under these proteolytic conditions, whole bovine casein in urea−PAGE yields four breakdown products identified as β-I, β-II, and β-III from β-casein hydrolysis and α_{s1}-I from α_{s1}-casein hydrolysis by rennet (de Jong and Groot Mostert, 1977). The degradation products β-I, β-II, and β-III produced from bovine and caprine β-caseins by rennet appeared to be identical or almost similar by PAGE. The fastest peptides of goat β-casein, β-I, β-II, and β-III, appeared to have electrophoretical mobility identical to that of bovine β-caseins β-I, β-II, and β-III, respectively. These results suggest that goat β-casein could be attacked by calf rennet in the same regions described to be susceptible to cleavage for bovine β-casein by chymosin. The presence of additional polypeptides, β-IV and β-V, may be explained by the different protein conformation adopted, with regard to bovine β-casein, which is perhaps due to the dipeptide deletion, which might make other susceptible bonds available to calf rennet action.

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