A Method for Isolating β-Casein[1]

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ABSTRACT

A new method was developed for obtaining pure β-CN. Calcium caseinate (3%) was reconstituted, renneted to form a gel, cooled (4°C) to allow β-CN dissociation from the caseinate gel, and centrifuged. The supernatant was warmed to 30°C, precipitating pure β-CN from solution. Large quantities of β-CN were recovered by scaling-up this procedure, but these β-CN preparations were less pure than the β-CN that was prepared on a smaller scale. Chromatography (FPLC®) and urea-PAGE showed β-CN to be the main component in the precipitate. Chymosin, used to form the caseinate gel, did not extensively hydrolyze β-CN under the conditions of these experiments. Calcium concentration, cooling time, and caseinate concentration influenced the recovery of β-CN. Maximum recovery of β-CN, under the experimental conditions used, occurred at 10 m*M* calcium, 48 h of cooling, and 3% caseinate concentration. (**Key words**: β-casein, casein, isolation)

Abbreviation key: **βI** = β-CN fragment 1 to 192 and 1 to 189, **βII** = β-CN fragment 1 to 163, **βIII** = β-CN fragment 1 to 139, **RU** = rennin units.

INTRODUCTION

β-Casein is a milk protein that constitutes approximately 25% of the total milk protein (30) and combines with α_{s1}-, α_{s2}-, and κ-CN to form casein micelles. Isolating large quantities of β-CN may be useful in developing new products, such as surface-active agents in emulsified or aerated products, or in adjusting rennet curd strength (11). β-Casein also could be used in infant formula (23) or as the starting ingredient for obtaining bioactive peptides (21).

The method presented in this paper for isolating β-CN is based upon conditions under which β-CN is soluble and the other caseins are insoluble, which allowed the β-CN to be collected as part of the supernatant and subsequently precipitated. By changing pH, salt concentration, or temperature, proteins can be isolated (33). For example, caseins precipitate when the pH of milk is lowered to 4.6 and can be separated from the other milk components. In 1955, Sullivan et al. (29) noted that the solubility of β-CN in a buffer solution increased as the temperature decreased. They suggested that the solubility of β-CN in milk also increased as it was cooled, which partially accounted for the increase in serum casein. In 1968, Rose (26) confirmed this theory and quantified the amount of β-CN (up to 30%) that dissociated from the casein micelle at 4°C. Since 1968, others (8, 12) have studied the solubility of β-CN at low temperatures. Some (1, 3, 7) have examined parameters, such as pH, calcium, and phosphate, that influence the cold solubility of β-CN.

The use of rennet to produce an insoluble casein complex, followed by cooling to release β-CN, has been attempted before. McGann and Pyne (22) renneted colloidal phosphate-free milk and skim milk at 36°C and then cooled them to 0 to 2°C. After 12 h, they filtered the solutions and found 0 and 0.34% β-CN, respectively. Another approach was cold renneting of calcium caseinate in 50 m*M* calcium chloride to produce a coagulum depleted of β-CN (2). However, other researchers (23) found that the product was not β-CN but **βI** (β-CN fragments 1 to 192 and 1 to 189). Chymosin is the main enzyme in rennet (28) and can hydrolyze β-CN (5, 6, 17). Although β-CN is not extensively hydrolyzed by chymosin in most cheeses, β-CN in aqueous solution is rapidly hydrolyzed by chymosin to fragments that have been designated βI′ (β-CN fragment 1 to 192), βI″ (β-CN fragment 1 to 189), **βII** (β-CN fragment 1 to 163), and **βIII** (β-CN fragment 1 to 139) (16). Because the two fragments designated as βI′ and βI″ are indistinguishable by urea-PAGE (4, 17), we use the term βI (β-CN fragments 1 to 192 and 1 to 189) to include both βI′ and βI″. For this reason, there is an added emphasis in this paper on comparing the isolated product to known β-CN standards and to a preparation of βI.

Calcium changes the solubility of β-CN. In solution, isolated β-CN is sensitive to calcium and precipitates in the presence of 10 m*M* calcium chloride at

Received August 10, 1995.
Accepted March 14, 1996.
[1]Published as Paper Number 21,985 of the Contribution Series of the Minnesota Agricultural Experiment Station and based on research conducted under Project 18-036.

37°C (10, 20). The amount of β-CN that precipitates depends on pH, temperature, calcium concentration, and genetic variant of β-CN (19, 20, 25). As temperature is lowered from room temperature to near freezing, β-CN becomes more soluble and will not precipitate at 4°C in the presence of up to 400 m*M* calcium (31). κ-Casein also has a stabilizing influence on isolated β-CN. If κ-CN is added to a solution of isolated β-CN, the amount of β-CN that precipitates decreases (20, 34).

Methods that have been developed for isolating β-CN on a large scale include microfiltration (15) and ultrafiltration (23) of sodium caseinate at 4°C to obtain a fraction rich in β-CN. Other methods for isolating β-CN are ion-exchange chromatography (21, 24) and continuous electrophoresis (21), although these methods are less practical for large-scale production of β-CN.

The main purpose of this research was to develop an alternative method for isolating large quantities of β-CN. We optimized the recovery conditions by changing the calcium concentration, cooling time, and caseinate concentration. We compared the isolated β-CN to β-CN standards and to hydrolyzed β-CN using FPLC®, amino acid analysis, and PAGE. These comparisons were made to identify the isolated protein as β-CN and to determine its purity.

MATERIALS AND METHODS

Materials

Calcium caseinate was purchased from New Zealand Milk Products Inc. (Santa Rosa, CA). Calcium chloride, urea, NaCl, acetate, and Whatman number 4 filter paper were obtained from Fisher Scientific (Fair Lawn, NJ). Spectra/Por®6 dialysis tubing (1000 molecular weight cutoff) also was obtained from Fisher Scientific and used for dialysis of samples before amino acid analysis. Chymosin (Chymax™) was donated by Pfizer Inc. (Milwaukee, WI). Bis-Tris-propane, Tris·HCl, κ-CN, and β-CN were obtained from Sigma Chemical Company (St. Louis, MO); this β-CN was used as a standard for comparison and is referred to as standard β-CN.

Filters (0.8 and 0.2 μm) used to filter buffer solutions were obtained from Millipore (Bedford, MA). Filters (0.2 μm) from Chrom Tech, Inc. (Apple Valley, MN) were used to filter samples before injection into the FPLC®. A mono Q HR 5/5 column and 8 to 25% gradient gels were purchased from Pharmacia Biotechnology (Uppsala, Sweden). The FPLC® system and PhastSystem® (Pharmacia Biotechnology) were used for fast protein liquid chromatography and PAGE. Becton Dickinson number 6901 sedimentation tubes were purchased from Curtin Matheson Scientific Inc. (Eden Prairie, MN). Sodium acetate trihydrate and Bacto® agar 0140 were from VWR Scientific (Chicago, IL).

Optimization of Isolation Procedure

To determine the amount of calcium chloride, calcium caseinate, and the cooling time needed for optimal recovery of β-CN, we designed an experiment using three different values for each variable. Caseinate concentrations used were 1, 2, and 3%. Calcium chloride concentrations were 10, 15, and 20 m*M*. Cooling times were 24, 48, and 72 h. All 27 possible combinations were made in duplicate for a total of 54 samples. Samples of 45 ml were prepared according to the method described in this paper for small-scale isolation. Least squares means and standard errors were calculated using SAS (27) software.

Small-Scale Isolation of β-CN

Calcium caseinate (3%) was dissolved in a 10-m*M* solution of calcium chloride and stirred for 1 h as the temperature was increased to 31°C. The pH of the solution was adjusted to 6.8, and 23.8 rennin units (**RU**) of chymosin/L of caseinate solution was added to form a gel. Chymosin activity was determined according to the description by Ernstrom (14), except that coagulation times were detected using a Formagraph (Foss Electric, Hillerød, Denmark). Thirty minutes after rennet addition, the gel that formed was disrupted by stirring for 2 to 3 min. The aggregated caseinate particles settled to the bottom of the beaker. The resulting solution was cooled to 4°C and left undisturbed for 48 h. After 48 h, samples were stirred again to resuspend the caseinate particles and the dissociated β-CN. Samples were centrifuged (5520 × *g* at 4°C for 15 min); then the supernatant was collected and filtered at 4°C through Whatman number 4 filter paper. Warming the supernatant in a 45°C water bath caused the β-CN to precipitate. The precipitate was removed with a spatula, dried in a desiccator, and weighed.

The percentage of β-CN recovered was calculated by dividing the grams of β-CN recovered by the amount of β-CN in the original calcium caseinate powder. The amount of β-CN in the calcium caseinate was determined by FPLC® according to the procedure of Davies and Law (9). The β-CN peak was collected, and the concentration was calculated by reading the absorbance at 280 nm and using an extinction coeffi-

cient of 4.6. The calcium caseinate contained 91.8% protein.

Scale-up of the Isolation Procedure

The procedure used for laboratory-scale isolation of β-CN was modified for scale-up. A 160-L solution of 3% calcium caseinate and 10 m*M* calcium chloride solution was prepared in a 190-L jacketed tank. The temperature was maintained at 31°C during the 1 h of stirring. The pH of the large-scale isolation was not adjusted. This solution had an unadjusted pH range of 6.6 to 6.7. The renneting procedure was the same as that used for small-scale isolation. The gel was disrupted by stirring for 2 to 3 min. The caseinate solution was cooled by circulating 9°C water through the jacket and then storing the tank at 4°C. Cooling time was 48 h. Supernatant was collected by two methods: the liquid fraction was siphoned after the caseinate particles resettled to the bottom of the tank, or the supernatant was obtained by clarification using a separator (model MP 1254 Westfalia separator; Centrico Corporation, Englewood, NJ). The fractions collected using these two techniques were warmed to precipitate β-CN. The precipitate was resuspended in water and spray-dried (Niro Atomizer P-6.3 spray-dryer; Niro Atomizer, Columbia, MD). The inlet temperature was 200°C, and the exit temperature was 100°C. The percentage of yield was calculated using the same formula as previously described for small-scale isolation.

FPLC®

Isolated β-CN was compared with standard β-CN (from Sigma Chemical Co.) and hydrolyzed samples of β-CN using FPLC® (9). Buffer 1, consisted of 0.005 *M* bis-Tris-propane and 3.3 *M* urea, adjusted to pH 7.0 with HCl. Buffer 2 was identical to buffer 1, except that 1 *M* NaCl was added. Both buffers were filtered through 0.8- and 0.2-μm filters before use. Samples of isolated β-CN and β-CN standards were dissolved in buffer 1 and filtered through a 0.2-μm filter; 500 μl were injected onto a mono Q HR 5/5 column (Pharmacia Biotechnology). The flow rate used was 1 ml/min. The absorbances were measured at 280 nm.

PAGE

Samples were dissolved in 5 ml of urea-Tris-acetate buffer at pH 6.4. The buffer contained 6.6 *M* urea, 0.112 *M* Tris, and 0.112 *M* acetate. Two drops of β-mercaptoethanol and 1 drop of bromophenol blue (4.5%) were added, and the samples were boiled for 5 min. Samples were applied to an 8 to 25% gradient gel that had been modified according to Van Hekken and Thompson (32) for urea-PAGE on a Phast-System®. Running conditions, staining, and destaining also were as described by Van Hekken and Thompson (32).

Hydrolyzed Samples of β-CN

Chymosin hydrolyzes β-CN to form βI, βII, and βIII, listed here in the order of increasing electrophoretic mobility (5, 6, 17). Samples of 1% (wt/vol) standard β-CN were hydrolyzed at 30°C for 12 h with chymosin. Chymosin concentration was 0.0238 RU/ml. Three samples were prepared, and the pH of the samples was adjusted (2.2, 5.5, and 7.0) to give different concentrations of βI and βII.

In a separate experiment, β-CN (1%) was hydrolyzed by chymosin under conditions that were similar

TABLE 1. The ANOVA of the effect of caseinate concentration, calcium concentration and cooling time on the recovery of β-CN.[1]

Source	df	MS	*P*
Replication (Rep)	1	23.09	0.26
Time	2	48.77	0.08
Rep × time	2	2.55	0.87
Calcium	2	208.01	0.00
Rep × calcium	2	8.40	0.62
Time × calcium	4	44.12	0.07
Caseinate	2	777.11	0.00
Rep × caseinate	2	4.47	0.78
Time × caseinate	4	23.19	0.29
Calcium × caseinate	4	12.82	0.58
Model	25	94.26	0.00
Error	27	17.55	

[1]R^2 = 0.838.

TABLE 2. Least squares means for time, caseinate concentration, and calcium concentration on the recovery of β-CN.

	LSM[1]
	(%)
Time, h	
24	55.38[a]
48	58.46[b]
72	58.18[ab]
Caseinate, %	
1	49.84[a]
2	59.53[b]
3	62.64[c]
Calcium, m*M*	
10	60.81[a]
15	53.84[b]
20	57.37[c]

[a,b,c]Means within categories with no common superscripts differ ($P < 0.05$).

[1]SE = 1.00. Recovery is expressed as the percentage recovered.

to the isolation conditions (pH 6.8; 4°C). Hydrolysis was monitored using PAGE. Samples were taken every 2 h and monitored for formation of βI using PAGE. The sample at 12 h was analyzed using FPLC®, and the major peaks were collected and analyzed for amino acid composition.

Residual Chymosin Activity

Residual chymosin activity in β-CN preparations was determined by using a κ-CN agar diffusion assay (18). Becton-Dickinson sedimentation tubes were filled with hot (70°C) κ-CN agar, leaving enough space for sample addition. The tubes were sealed with parafilm (Fisher Scientific, Fair Lawn, NJ) and stored at 4°C. After the tubes were warmed to room temperature and the parafilm was removed, a 10-μL sample was applied. The diffusion of chymosin was followed by monitoring the opacity of the agar. As κ-CN was hydrolyzed, the agar turned from transparent to white. Tubes were held at 37°C, and the distance of diffusion was measured after 48 h. A standard curve was prepared simultaneously with unknown samples. Sample preparation for the unknowns consisted of preparing a solution of isolated β-CN such that the residual activity was within the range of the standard curve. Residual chymosin activity was reported as rennin units per gram of isolated β-CN.

RESULTS AND DISCUSSION

Optimizing the Isolation Procedure

Analysis of variance and the results for calcium concentration, caseinate concentration, and cooling

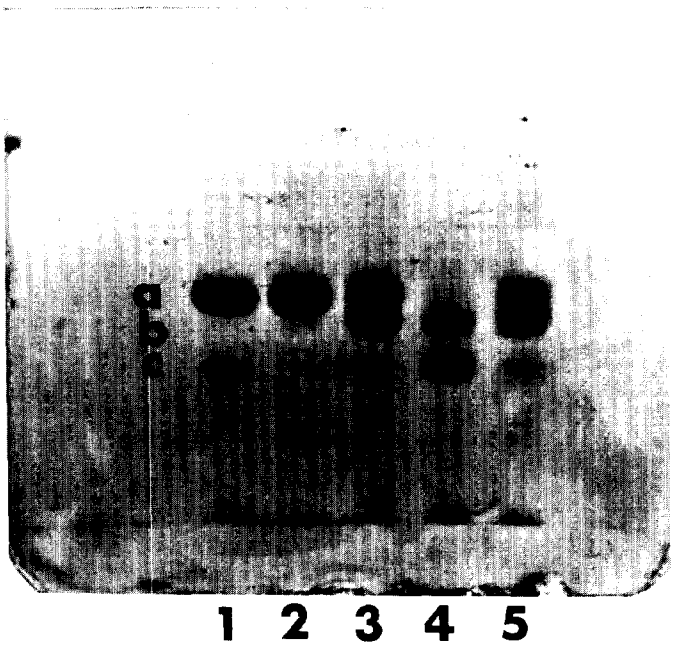

Figure 1. Urea-PAGE of standard β-CN (lane 1), isolated β-CN (lane 2), and β-CN hydrolyzed by chymosin (lanes 3 to 5). β-Casein was hydrolyzed at pH 2.2 (lane 4), pH 5.5 (lane 5), and pH 7 (lane 3) at 37°C for 12 h. Chymosin concentration in all of the samples was 0.0238 RU/ml. Hydrolysis under these conditions produced varying amounts of βI and βII. a = β-CN, b = βI, and c = βII. FPLC® analyses of the different lanes are shown in Figure 5.

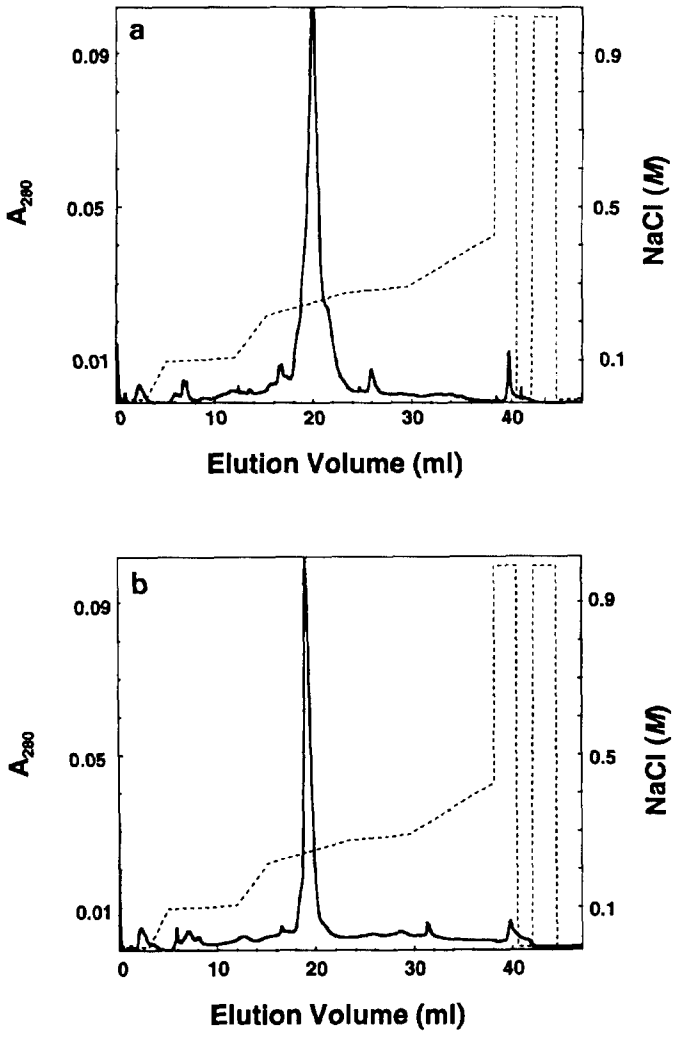

Figure 2. The FPLC® chromatograms of standard β-CN (a) and isolated β-CN (b). Absorbance at 280 nm (—) and salt gradient (- - -) are shown.

time are shown in Tables 1 and 2. A cooling time of 24 h was necessary to recover 55% of the β-CN. Increasing the cooling time to 48 h increased the amount of β-CN that was recovered to 58.5%. Additional cooling after 48 h did not increase the amount of β-CN recovered. Caseinate concentration had the largest influence on the amount of β-CN recovered. Increasing the caseinate concentration from 1 to 3% greatly increased the amount of β-CN that was recovered (50 to 63%). The trend was opposite for calcium concentration. As the calcium concentration was increased from 10 to 20 m*M*, the amount of recovered β-CN decreased. Addition of 10 m*M* calcium resulted in recovery of 61% of the β-CN compared with 57% from solutions of 20 m*M* Ca.

Purity of β-CN Isolated on a Laboratory Scale

Standard β-CN was compared with isolated β-CN to identify the isolated protein as β-CN and to determine its purity. Gel electrophoresis (Figure 1) showed that standard β-CN had one major β-casein band and a slight βII band. Gel electrophoresis of isolated β-CN showed only one band that directly corresponded with the standard β-CN band. Hydrolysis of β-CN by chymosin produced peptides (βI and βII) that had increased mobility (Figure 1). We did not observe formation of βIII under the hydrolysis conditions used in these experiments.

The FPLC® results for standard β-CN (Figure 2a) showed one major peak at 20 min that corresponded to β-CN. A shoulder before and after the main peak on the standard β-CN indicated that the β-CN contained a small amount of another protein or peptide. This peptide might have been βII, which was the only other peptide detected using gel electrophoresis. The isolated β-CN eluted at 20 min (Figure 2b) as one major peak. A small shoulder before the main peak was observed. The shoulder observed after the main peak for standard β-CN (Figure 2a) was not observed for the isolated β-CN, indicating the absence of βII. Gel electrophoresis also failed to show the presence of any βI or βII in the isolated β-CN (Figure 1). Amino acid analysis of the isolated β-CN was done, and the calculated amino acid composition was compared with the predicted composition (Table 3). The comparisons were made using β-CN A^2-5P (13).

TABLE 3. Amino acid analysis of the isolated β-CN and β-CN A^2-5P.

Amino acid	Predicted	Calculated
Aspartic acid	9.00	9.17
Threonine	9.00	8.23
Serine	16.00	11.93
Glutamic acid	39.00	38.61
Proline	35.00	34.04
Glycine	5.00	5.21
Alanine	5.00	6.12
Half-cystine	0.00	0.13
Valine	19.00	18.23
Methionine	6.00	6.02
Isoleucine	10.00	8.98
Leucine	22.00	22.56
Tyrosine	4.00	4.20
Phenylalanine	9.00	9.07
Histidine	5.00	5.56
Lysine	11.00	10.84
Arginine	4.00	4.06

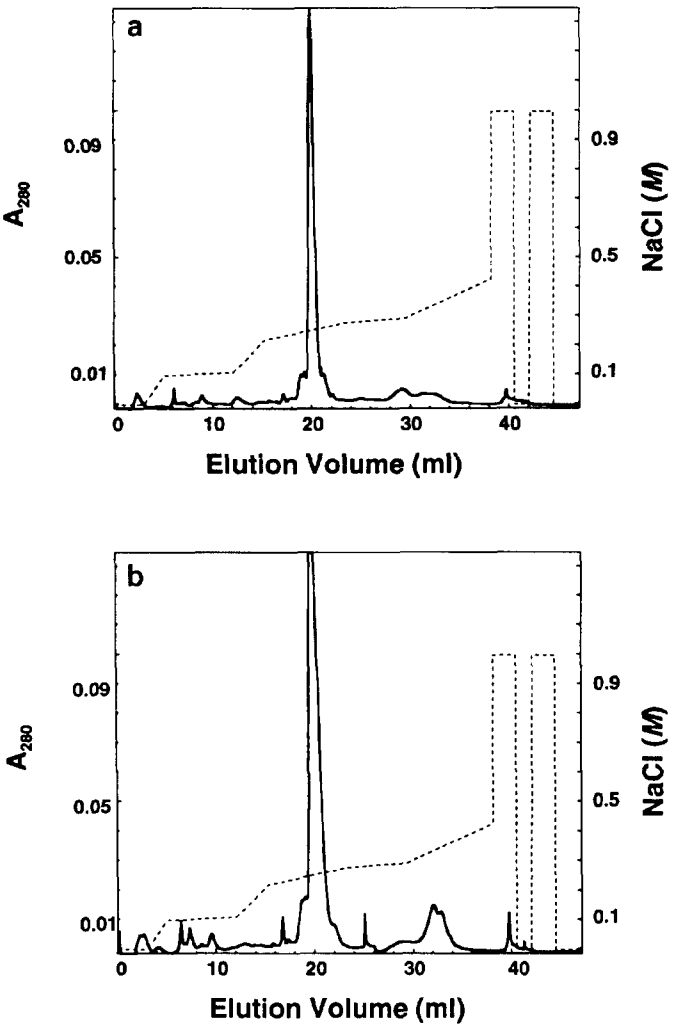

Figure 3. The FPLC® chromatograms of β-CN isolated from large samples and collected by clarifier (a) and by siphoning (b). Absorbance at 280 nm (—) and salt concentration (- - -) are shown.

TABLE 4. Amino acid analysis data from protein fractions separated by FPLC®.[1]

	Peak 1 (peptide 193 to 209)		Peak 2 (peptide 1 to 189)		Peak 3 (peptide 1 to 192)		Peak 4 (peptide 1 to 192)	
Amino acid	Predicted	Calculated	Predicted	Calculated	Predicted	Calculated	Predicted	Calculated
Aspartic acid	0.00	0.09	9.00	9.56	9.00	9.04	9.00	9.33
Threonine	0.00	0.00	9.00	8.99	9.00	8.48	9.00	8.46
Serine	0.00	0.08	16.00	12.60	16.00	12.98	16.00	12.20
Glutamic acid	2.00	2.17	37.00	36.31	37.00	37.26	37.00	36.91
Proline	4.00	3.82	31.00	27.55	31.00	30.19	31.00	28.52
Glycine	2.00	1.85	3.00	4.33	3.00	3.40	3.00	3.60
Alanine	0.00	0.09	5.00	5.55	5.00	5.02	5.00	4.97
Half-cystine	0.00	0.02	0.00	0.18	0.00	0.12	0.00	0.17
Valine	3.00	2.72	16.00	14.31	16.00	14.66	16.00	14.32
Methionine	0.00	0.03	6.00	4.56	6.00	5.30	6.00	5 14
Isoleucine	2.00	1.26	8.00	8.13	8.00	7.44	8.00	7.77
Leucine	1.00	1.16	19.00	18.56	21.00	20.30	21.00	19.80
Tyrosine	1.00	0.92	3.00	3.00	3.00	3.06	3.00	2.96
Phenylalanine	1.00	1.00	7.00	7.29	8.00	7.81	8.00	7.71
Histidine	0.00	0.00	5.00	4.57	5.00	5.16	5.00	4.94
Lysine	0.00	0.08	11.00	10.53	11.00	10.66	11.00	10.80
Arginine	1.00	0.93	3.00	3.04	3.00	3.14	3.00	3.11

[1]See text and Figure 4 for assignment of peaks. Peaks are labeled 1 to 4 as on the chromatogram. The amino acid composition for each peak was calculated and then compared to peptides from β-CN. Peptides that had similar compositions are indicated in the figure with their corresponding data. All predictions were based on the composition of β-CN A^2-5P.

Purity of β-CN from Isolation on a Pilot Plant Scale

The purity and yield of β-CN decreased as the process was scaled up, because separating the fines from the supernatant was more difficult, which resulted in some α_s-CN contamination. Two methods were employed for recovering the supernatant from large samples. First, the pilot plant separator was used to remove precipitate depleted of β-CN from the mixture. Figure 3a is a chromatogram of β-CN isolated on a pilot plant scale using the pilot plant separator to clarify the supernatant. The major peak at 20 min corresponded to β-CN. A small peak at 2 min and shoulders on the β-CN peak indicate that the product was slightly hydrolyzed. For the second method, caseinate particles were allowed to settle to the bottom of the tank, and supernatant was siphoned off the top. The chromatogram of β-CN that was isolated this way also showed several other peaks (Figure 3b).

Characterization of β-CN Peptides

Hydrolysis of isolated β-CN by chymosin at pH 6.8 and 4°C was monitored using PAGE until all of the β-CN was converted to βI. Twelve hours were required to complete this hydrolysis (Figure 4). Although chymosin completely hydrolyzed β-CN in 12 h under the conditions used for isolating β-CN from caseinate, we did not observe extensive hydrolysis of β-CN (see Figure 2b) in the renneted calcium caseinate solution. The FPLC® chromatograms of the hydrolyzed sample showed a cluster of peaks around 20 min and a single large peak at 2 min (Figure 4). Four major peaks were collected and analyzed for amino acid composition. Table 4 shows the amino acid composition for these peaks compared with predicted compositions for β-CN A^2-5P peptides. Peak 2 was similar to

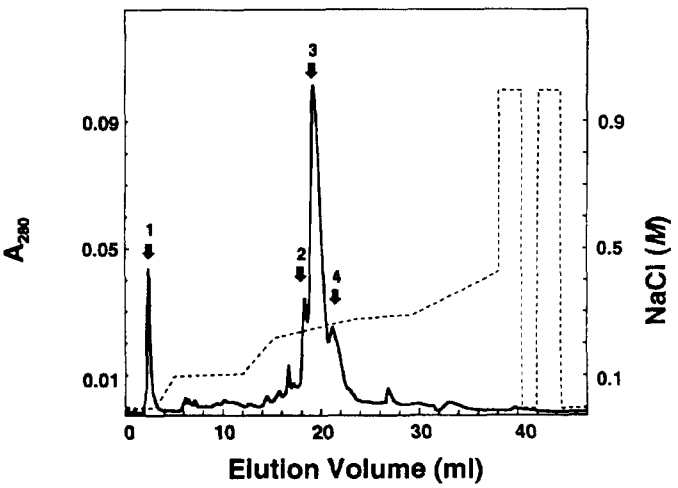

Figure 4. The FPLC® chromatogram of β-CN hydrolyzed by chymosin long enough to completely convert the protein to βI (β-CN fragment 1 to 92 and 1 to 89; pH 6.8 at 4°C for 12 h). This hydrolyzed sample showed a single band on a polyacrylamide gel corresponding to β-CN. Major peaks were collected and analyzed for amino acid composition. Amino acid results are summarized in Table 4.

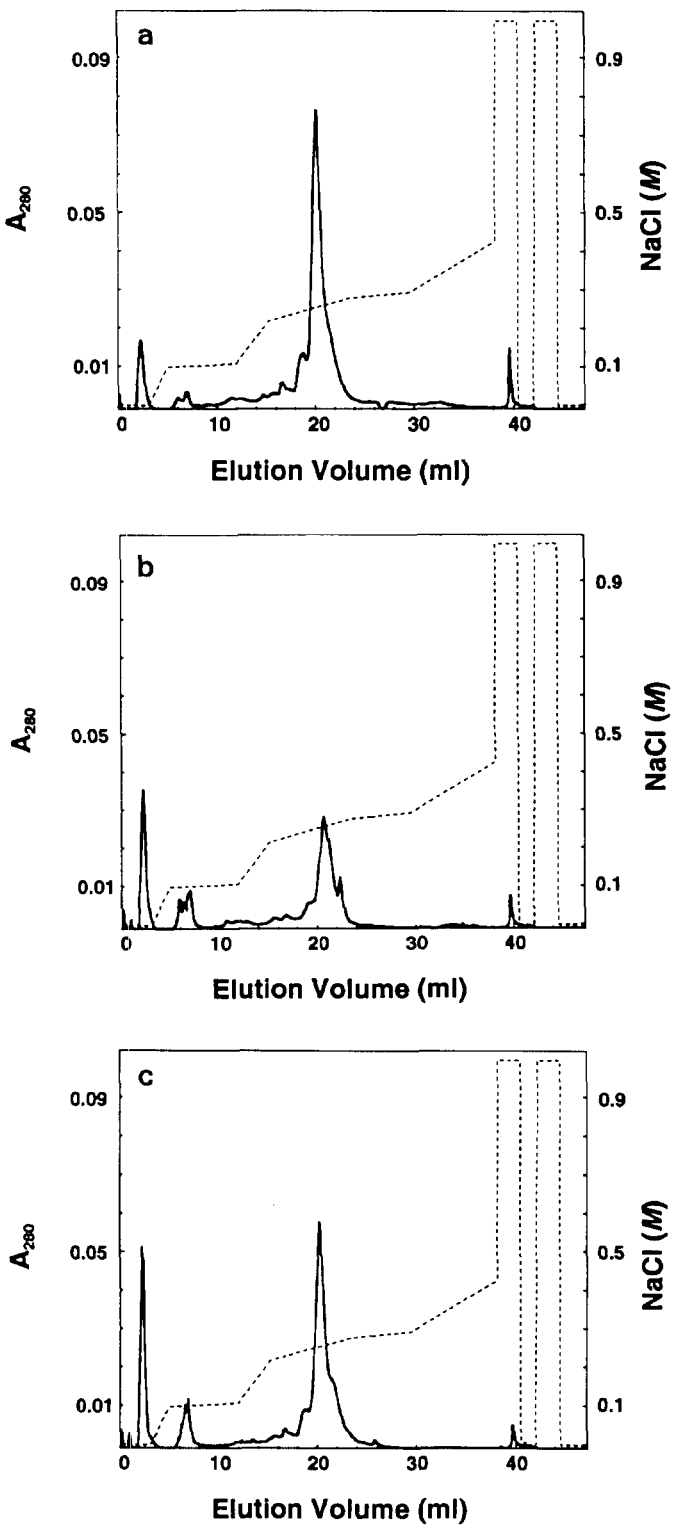

Figure 5. Influence of chymosin hydrolysis on β-CN. β-Casein was hydrolyzed at pH 7 (a), 2.2 (b), and 5.5 (c), and the products were monitored using FPLC®. Chromatograms can be compared with results in Figure 1 from PAGE. Figure 5a corresponds to lane 3, 5b corresponds to lane 4, and 5c corresponds to lane 5. Absorbance at 280 nm (—) and salt gradient (- - -) are shown.

TABLE 5. Residual chymosin was determined by using a κ-CN agar diffusion assay.[1]

Sample	Activity recovered	Maximum activity recovered
	(RU/g)	(%)
β-CN Precipitate	0.448	14.33
Spray-dried β-CN	0.139	4.45

[1]The results are expressed as rennin units (RU) per gram of β-CN recovered. The maximum possible amount of activity that could be recovered is expressed as a percentage. This calculation is based on recovering 100% of the β-CN.

β-CN fragments 1 to 189. Peaks 3 and 4 both showed similarity to β-CN fragments 1 to 192. The difference in elution time between peaks 3 and 4 might possibly have been due to different genetic variants. Peak 3 was shifted slightly to the right compared with unhydrolyzed, isolated β-CN. These results indicate that the large βI peptides (fragments 1 to 189 and 1 to 192) eluted at approximately the same time as β-CN. Peak 1, which eluted at 2 min (Figure 4), was identified as the C-terminal peptide (β-CN fragment 193 to 209) corresponding to βI (Table 4).

The results of β-CN hydrolysis by chymosin at 37°C (pH 2.0, 5.5, and 7.0) can be seen in lanes 3 to 5 of Figure 1 and in the FPLC® chromatograms shown in Figure 5. Because chymosin can hydrolyze β-CN and chymosin was used in the isolation procedures to form a gel, hydrolyzed samples were compared with the isolated protein to confirm that the sample was β-CN and not a hydrolyzed product of β-CN. The same solutions of hydrolyzed β-CN were used for PAGE and FPLC®; the chromatogram in Figure 5a corresponds to lane 3, Figure 5b corresponds to lane 4, and Figure 5c corresponds to lane 5. Initial hydrolysis of β-CN resulted in the formation of βI (lane 3). The chromatogram of lane 3 (Figure 5a) showed the C-terminal peptide at 2 min and a peak at 19 min that contained a βI fragment. Further hydrolysis increased the amount of βI, as indicated by the larger, darker βI band (lane 5) and an increased peak at 2 min (Figure 5c). Complete hydrolysis of β-CN resulted in βI and βII (lane 4). The chromatogram of lane 4 showed two major peak areas (Figure 5b), a peak at 2 min and a cluster of peaks at 21 min, showing that the β-CN had been hydrolyzed. These results indicate that the purity of β-CN can be monitored to some extent by the C-terminal peptide that elutes at 2 min and the cluster of peaks that appears at 20 min.

Residual Chymosin Activity

One of the obstacles in isolating β-CN using chymosin is that some residual chymosin remains in the

product. Residual chymosin activity was determined using the method of Holmes et al. (18). Table 5 shows residual chymosin activity in β-CN fractions obtained during our experiments. β-Casein that was precipitated out of solution contained 0.448 RU/g of β-CN. The activity decreased in the spray-dried product to 0.139 RU/g of β-CN. The spray-dried product was monitored for hydrolysis of β-CN during storage at room temperature for 8 mo, and no loss of β-CN occurred, suggesting that the residual activity measured using the agar diffusion assay had little impact on β-CN during storage.

CONCLUSIONS

Pure gram quantities of β-CN can be obtained using this method. Larger quantities of β-CN also can be obtained, but the purity decreases. Higher yields may be obtained after optimization of factors such as pH, rennet concentration, gel strength, and conditions influencing β-CN hydrolysis. Further work is underway to optimize the recovery conditions for isolating β-CN on a large scale and to increase the yield and purity.

ACKNOWLEDGMENTS

We thank the Minnesota-South Dakota Dairy Research Center for funding this project. We also thank the Micro-Chemicals facility at the University of Minnesota for performing the amino acid analysis.

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