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Proteolytic specificity of cathepsin G on bovine α_{s1}- and β-caseins

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Abstract

Mastitis is an inflammation of the mammary gland which results in an increase in numbers of somatic cells, particularly polymorphonuclear leucocytes (PMN), which contain very active proteinases. The objective of this study was to determine the cleavage specificity of cathepsin G, one of the principal PMN proteinases, on α_{s1}- and β-casein. α_{s1}- or β-casein (5 mg ml^{-1}) were dissolved in 0.1 M HEPES buffer, pH 7.5, containing 0.05% NaN_3. Cathepsin G was dissolved in 0.1% Brij 35 and 0.5 M NaCl and 0.25 units ml^{-1} of this stock solution was added to α_{s1}- or β-casein in buffer. Samples were taken over a 24 h incubation at 37 °C and analysed by urea polyacrylamide gel electrophoresis and high performance liquid chromatography. Isolated peptides were identified by N-terminal sequencing and mass spectrometry. Cathepsin G cleaved α_{s1}-casein at at least 16 sites and β-casein at at least 21 sites, some of which were also cleavage sites of chymosin, plasmin, elastase, cathepsin B or the cell envelope-associated proteinase of *Lactococcus*. Thus, cathepsin G had a broad specificity on α_{s1}- and β-casein and it is therefore possible that indigenous cathepsin G in milk may be of significance for the proteolysis of milk proteins. Of particular interest was the production of the small peptide α_{s1}-casein (f1-23), which also results from cleavage of the Phe_{23}-Phe_{24} bond by chymosin in cheese, and is hydrolysed rapidly during cheese ripening by the cell envelope-associated proteinase of *Lactococcus*.

Keywords: Cathepsin G; α_{s1}-Casein; β-Casein; Specificity; Somatic cells

1. Introduction

Mastitis can be defined as an inflammation of the mammary gland, resulting in an increase in somatic cell count (SCC), which is related to the presence of microbes in the mammary gland. This rise in SCC is a result of leukocytes being transferred into the milk from the blood (Kehrli & Shuster, 1994). Polymorphonuclear leukocytes (PMN) are the dominant white blood cell in milk during mastitis (Azzara & Dimick, 1985) and contain very active proteinases (Baggiolini, Bretz, & Dewald, 1978). Proteinases in somatic cells are of increasing interest since the role of such proteinases is in degrading normal connective tissue components (Roughley, 1977). Lysosomes of somatic cells contain elastase and cathepsin G, which are the predominant enzymes associated with PMN in mastitic milk (Azzara & Dimick, 1985; Newbould, 1974). Other proteinases found in PMN include the thiol protease, cathepsin B, and the acid protease, cathepsin D (Baggiolini et al., 1978). Thus, based on the presence of these proteases in lysosomes of PMN, it may be expected that proteinases from PMN could contribute to hydrolysis of casein in milk at both neutral and acid pH, particularly milk with high SCC (Verdi & Barbano, 1991). Considine, Healy, Kelly, and McSweeney (1999, 2000) and Considine (2000) reported the cleavage specificity of elastase and cathepsin B on α_{s1}- and β-caseins and showed that caseins are very suitable substrates for proteolysis by such enzymes.

Cathepsin G is a neutral serine proteinase (Starkey & Barrett, 1976), with a molecular weight of 24–26 kDa, which can occur as three isoforms and contains three disulphide bonds (Watorek, Farley, Salvesen, & Travis, 1988). Maison, Villiers, and Colomb (1991) described the purification of cathepsin G. The proteolytic specificity of cathepsin G on the oxidised B-chain of insulin was reported by Blow and Barrett (1977), who showed that peptide bonds containing the amino acids, Leu and Phe, were cleaved preferentially in this substrate.

The objective of this study was to investigate the potential significance of cathepsin G for proteolysis and

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quality of milk and dairy products by determining its cleavage specificity towards bovine α_{S1}- and β-caseins.

2. Materials and methods

2.1. Materials

Whole casein was prepared from bovine skim milk and was fractionated by ion-exchange chromatography on diethylaminoethyl cellulose (DE-52, Sigma Chemical Co., St. Louis, MO, USA; Creamer, 1974; Mulvihill & Fox, 1974). Fractions containing α_{S1}- and β-casein were pooled, dialysed against water and freeze-dried. Cathepsin G (from human leukocytes) was obtained from the Sigma Chemical Co., St. Louis, MO, USA (1 unit of cathepsin G will release one nmol *p*-nitroaniline s^{-1} from *N*-succinyl-Ala-Ala-Pro-Phe- *p*-nitroanilide at pH 7.5 and 37 °C).

2.2. Hydrolysis of the caseins

α_{S1}- and β-caseins (5 mg ml^{-1}) were dissolved separately in 0.1 M *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid]) (HEPES) buffer, pH 7.5, containing 0.05% NaN_3. Cathepsin G (0.25 units ml^{-1}) was dissolved in 0.1% polyoxyethylene 23 lauryl ether (Brij 35), with 0.5 M NaCl. Cathepsin G (0.025 U ml^{-1}) was added to α_{s1}- or β-casein and the mixture incubated at 37 °C for up to 24 h. Aliquots were taken periodically for analysis by urea polyacrylamide gel electrophoresis (urea-PAGE) and reverse-phase (RP)-HPLC. Cathepsin G was inactivated in each sample before analysis by heating at 100 °C for 5 min.

2.3. Analysis by urea-PAGE

Samples for analysis by urea-PAGE were prepared as described by Considine et al. (1999). Urea-PAGE was performed according to the method of Andrews (1983), with direct staining using Coomassie Brilliant Blue G250 by the method of Blakesley and Boezi (1977). Peptides which stained on urea-PAGE gels were electroblotted onto polyvinylidenedifluoride membranes (Considine et al., 1999) and stored at −18 °C until N-terminal sequencing was performed.

2.4. RP-HPLC analysis

RP-HPLC analysis was performed on the 2% trichloroacetic acid (TCA)-soluble fraction of the hydrolyzates. Samples were prepared as described by Considine et al. (1999) and RP-HPLC was performed using a Varian liquid chromatograph consisting of model LC-9A pump, a Rainin Dynamax® A1-200 autosampler and a Rainin Dynamax® spectrophotometric detector (Varian Inc., Walnut Creek, California). A Nucleosil C_8 column (300 Å, 5 μm, 250×4.6 mm) was used and elution was by means of a gradient described by Considine et al. (1999). Flow rate was 0.75 ml min^{-1} and detection was at 214 nm. Peptides were collected manually, freeze-dried and identified by N-terminal sequencing and mass spectrometry.

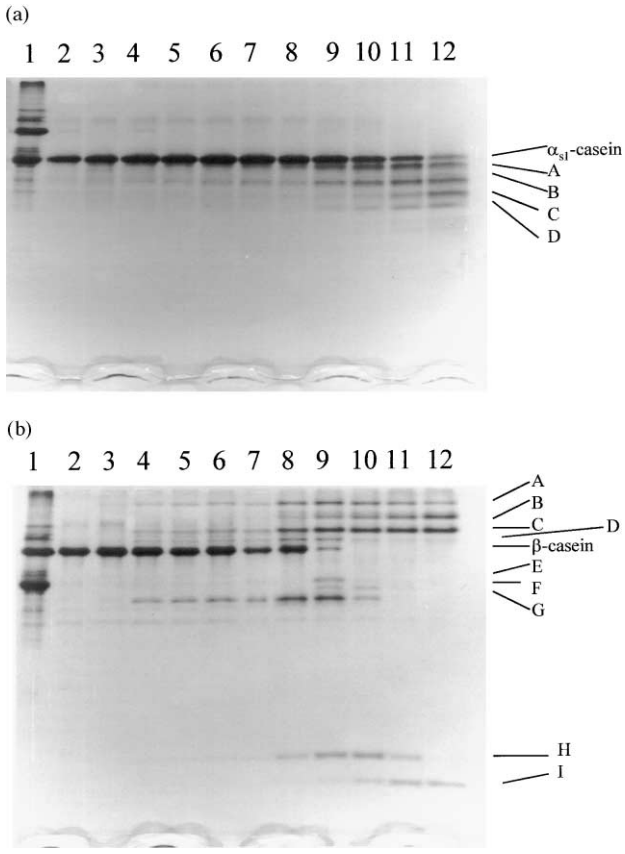

Fig. 1. Urea-polyacrylamide gel electrophoretograms of Na-caseinate (lane 1) and (a) α_{s1}- or (b) β-casein incubated in 0.1 M N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid]) (Hepes) buffer, pH 7.5, containing 0.05% NaN_3 for 0 or 24 h (lanes 2 and 3) at 37 °C or (a) α_{s1}- or (b) β-casein hydrolysed by cathepsin G (0.025 U ml^{-1}) for 5, 10, 15, 30 min, 1, 3, 6, 12 or 24 h (lanes 4–12).

2.5. Identification of peptides

Peptides were sequenced at the National Food Biotechnology Centre, University College, Cork, Ireland, by Edman degradation, as described by Considine et al. (1999). Mass spectrometric analyses were performed at the Department of Biochemistry, Faculty of Medicine and Health Sciences, Queens Medical Centre, University of Nottingham, UK, also as described by Considine et al. (1999).

3. Results and discussion

Urea-PAGE electrophoretograms of α_{s1}-casein hydrolysed by cathepsin G are shown in Fig. 1a.

Table 1
Identity of peptides detected by urea-polyacrylamide gel electrophoresis after hydrolysis of α_{s1}-casein by cathepsin G (see Fig. 1a for location of peptides)

Peptide	N-terminal sequence	Identity	N-terminal cleavage sites
A (i)	Arg-Pro-Lys-His-Pro	–	–
A (ii)	Glu-Gln-Lys-His-Ile	H_2N–Glu_{77}-[a]	Val_{76}-Glu_{77}
A (iii)	Ala-Gln-Gln-Lys-Glu	H_2N–Ala_{129}-[a]	His_{128}–Ala_{129}
A (iv)	His-Ile-Gln-Lys-Glu	H_2N–Glu_{55}-[a]	Lys_{79}-His_{80}
B (i)	Glu-Asp-Ile-Lys-Gln	H_2N–Glu_{55}-[a]	Met_{54}-Glu_{55}
B (ii)	Arg-Pro-Lys-His-Pro	–	–
B (iii)	Arg-Gln-Phe-Tyr-Gln	H_2N–Arg_{151}-[a]	Phe_{150}-Arg_{151}[b,c]
B (iv)	Glu-Gln-Lys-His-Ile	H_2N–Arg_{151}-[a]	Val_{76}-Glu_{77}
C (i)	Ile-Val-Pro-Asn-SerP	H_2N–Ile_{71}-[a]	Glu_{70}-Ile_{71}
C (ii)	Ile-Val-Pro-Asn-SerP	H_2N–Ile_{111}-[a]	Glu_{110}-Ile_{111}
C (iii)	Ala-Tyr-Phe-Tyr-Pro	H_2N–Ala143-[a]	$Leu1_{42}$-Ala_{143}
C (iv)	Arg-Gln-Phe-Tyr-Gln	H_2N–Arg_{151}-[a]	Phe_{150}-Arg_{151}[d]
C (v)	Glu-Asp-Ile-Lys-Gln	H_2N–Glu_{55}-[a]	Met_{54}-Glu_{55}
D (i)	Arg-Gln-Phe-Tyr-Gln	H_2N–Arg_{151}-[a]	Phe_{150}-Arg_{151}[d]
D (ii)	Ala-Tyr-Phe-Tyr-Pro	H_2N–Ala_{143}-[a]	Leu_{142}-Ala_{143}
D (iii)	Arg-Pro-Lys-His-Pro	H_2N–Arg_1-[a]	–
D (iv)	Leu-Asp-Ala-Tyr-Pro	H_2N–Leu_{186}-[a]	Gln_{155}-Leu_{156}

[a] Incomplete sequence.
[b] Chymosin cleavage site.
[c] Lactococcal cleavage site.
[d] Plasmin cleavage site.

α_{s1}-Casein was readily degraded by cathepsin G, with the formation of peptides of higher electrophoretic mobilities. Electrophoretograms of the control, containing no enzyme, showed no α_{s1}-casein degradation after 24 h incubation, indicating the absence of indigenous proteinase activity in the α_{s1}-casein preparation. Hydrolysis of α_{s1}-casein by cathepsin G was rapid, with the first degradation products being evident after 15 min incubation. The intensity of all the peptide bands increased from 5 min to 24 h and five bands were present in the 24 h sample. Four bands in the 24 h hydrolysate (A–D, Fig. 1a) were isolated by electroblotting and their N-terminal sequence determined, thus allowing identification of some of the primary sites of cathepsin G action on the protein (Table 1). Many bands contained more than one peptide. Large peptides, produced by cathepsin G from α_{s1}-casein, originated from cleavage at Met_{54}-Glu_{55}, Glu_{70}-Ile_{71}, Val_{76}-Glu_{77}, Lys_{79}-His_{80}, Glu_{110}-Ile_{111}, His_{128}-Ala_{129}, Leu_{142}-Ala_{143} and Phe_{150}-Arg_{151} and Gln_{155}-Leu_{156}. The C-termini of these large peptides were not determined.

Urea-PAGE electrophoretograms of β-casein hydrolysed by cathepsin G are shown in Fig. 1b. β-Casein was readily degraded by cathepsin G with the formation of peptides of both higher and lower electrophoretic mobilities. Again, no indigenous proteinase activity was found in the β-casein preparation and hydrolysis of β-casein by cathepsin G was also rapid. Some bands were less intense in the electrophoretogram of the 24 h sample than that of the 6 h sample, indicating further hydrolysis of primary proteolysis products by the enzyme. Four bands with very slow electrophoretic mobility (A–D, Fig. 1b) accumulated in the hydrolysate and increased in intensity by 24 h, with the exception of band D, which was hydrolysed subsequently within 3 h. Five bands of very high mobility were also observed (bands E–I) and their intensity decreased on incubation for 24 h, with the exception of band I, the intensity of which increased. Five peptides (A–D and H, Fig. 1b) were isolated by electroblotting and their N-terminal sequences determined (Table 2). As with α_{s1}-casein, there were more than one peptide in each band. Large peptides produced from β-casein, by cathepsin G, originated from cleavage at Gln_{56}-Ser_{57}, Asn_{68}-Ser_{69}, Phe_{119}-Thr_{120}, Pro_{153}-Thr_{154}, Leu_{163}-Ser_{164} and Lys_{176}-Ala_{177}.

RP-HPLC elution profiles of the 2% TCA-soluble fractions of β- and α_{s1}-casein hydrolysed by cathepsin G for time periods up to 24 h, are shown in Fig. 2. Several cleavage sites of cathepsin G on α_{s1}- and β-casein were very close or identical to chymosin cleavage sites (Phe_{23}-Phe_{24}, Phe_{32}-Gly_{33}, Leu_{149}-Phe_{150}, Phe_{179}-Ser_{180}, McSweeney, Olson, Fox, & Højrup, 1993; and Leu_{163}-Ser_{164}, Creamer, 1976; Visser & Slangen, 1977), suggesting that cathepsin G may contribute to chymosin-like activity in dairy products, which may influence cheese texture (Creamer & Olson, 1982; Tables 3 and 4).

The small peptide which results from cleavage of Phe_{23}-Phe_{24}, α_{s1}-CN (f1-23), is hydrolysed rapidly by the cell envelope-associated proteinase (CEP) of *Lactococcus* (Fox & McSweeney, 1997).

α_{s1}- And β- caseins were cleaved, also, by cathepsin G close to many plasmin cleavage sites (Arg_{151}-Gln_{152} of α_{s1}-casein, McSweeney et al., 1993 and Lys_{97}-Val_{98}, Lys_{105}-His_{106}, Lys_{107}-Glu_{108}, Lys_{169}-Val_{170}, Lys_{176}-

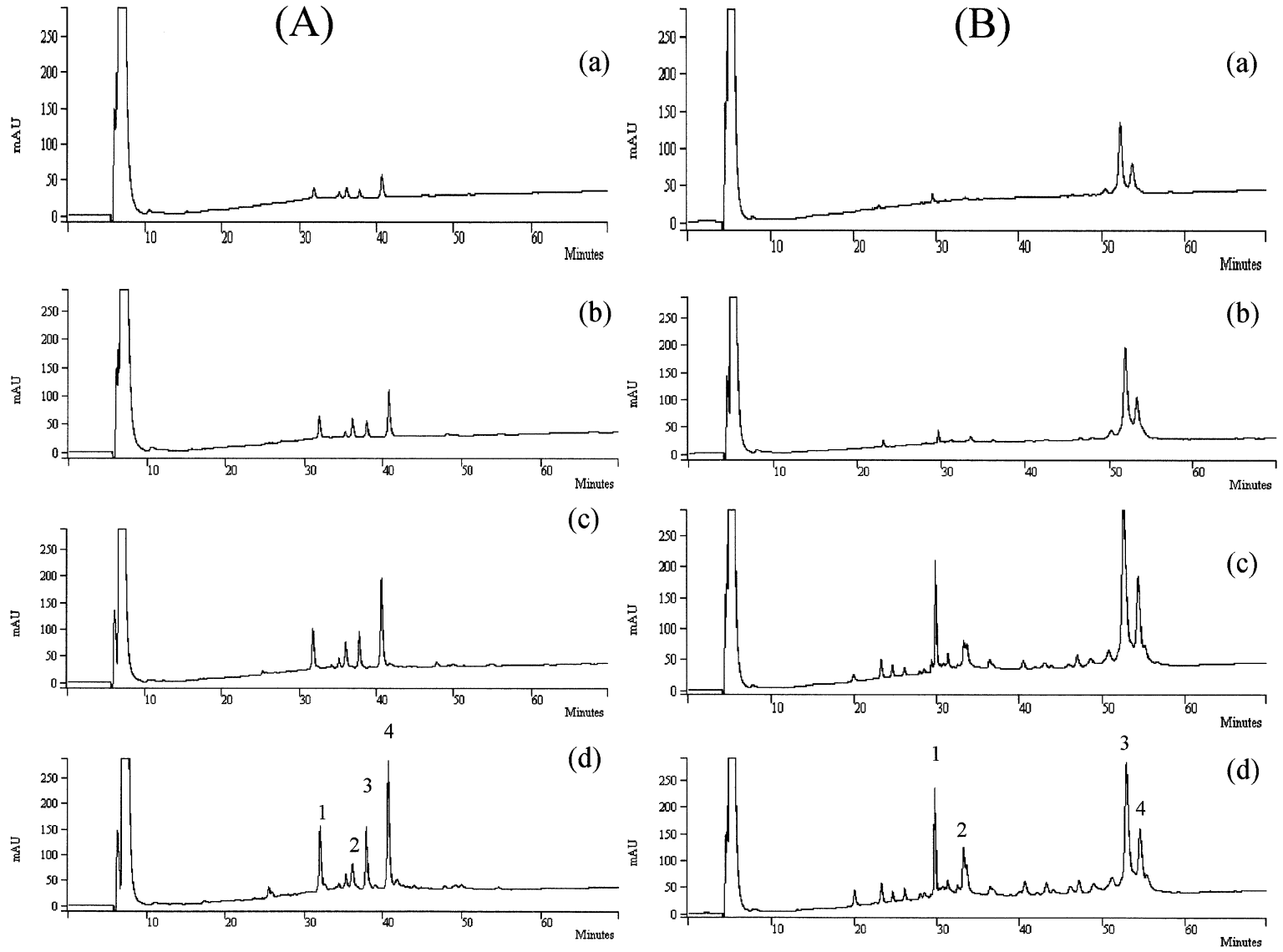

Fig. 2. Reversed-phase HPLC chromatograms of 2% TCA-soluble peptides produced from (A) α_{s1}- and (B) β-casein (5 mg ml^{-1}) by cathepsin G (0.025 U ml^{-1}) incubated in 0.1 M N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid]) (Hepes) buffer, pH 7.5, containing 0.05% NaN_3, for (a) 1, (b) 6, (c) 12 or (d) 24 h.

Table 2
Identity of peptides detected by urea-polyacrylamide gel electrophoresis after hydrolysis of β-casein by cathepsin G (see Fig. 1b for location of peptides)

Peptide	N-terminal sequence	Identity	N-terminal cleavage sites
A (i)	Ser-Leu-Pro-Gln-Asn	H_2N–Ser_{69}-[a]	Asn_{68}-Ser_{69}
A (ii)	Ser-Leu-Ser-Gln-Ser	H_2N–Ser_{164}-[a]	Leu_{163}-Ser_{164}[b,c]
A (iii)	Ser-Leu-Val-Tyr-Pro	H_2N–Ser_{57}-[a]	Gln_{56}-Ser_{57}
A (iv)	Thr-Val-Met-Phe-Pro	H_2N–Thr_{154}-[a]	Pro_{153}-Thr_{154}
A (v)	Ala-Val-Pro-Tyr-Pro	H_2N–Ser_{177}-[a]	Lys_{176}-Ala_{177}[d]
B (i)	Thr-Glu-Ser-Gln-Ser	H_2N–Thr_{120}-[a]	Phe_{119}-Thr_{120}
B (ii)	Ser-Leu-Ser-Gln-Ser	H_2N–Ser_{164}-[a]	Leu_{163}-Ser_{164}
C (i)	Thr-Val-Met-Phe-Pro	H_2N–Thr_{154}-[a]	Pro_{153}-Thr_{154}
C (ii)	Thr-Glu-Ser-Gln-Ser	H_2N–Thr_{120}-[a]	Phe_{119}-Thr_{120}
D (i)	Thr-Glu-Ser-Gln-Ser	H_2N–Thr_{120}-[a]	Phe_{119}-Thr_{120}
D (ii)	Ser-Leu-Pro-Gln-Asn	H_2N–Ser_{69}-[a]	Asn_{68}-Ser_{69}
D (iii)	Ser-Leu-Ser-Gln-Ser	H_2N–Ser_{164}-[a]	Leu_{163}-Ser_{164}
H (i)	Arg-Glu-Leu-Glu-Glu	H_2N–Arg_1-[a]	–

[a] Incomplete sequence.
[b] Chymosin cleavage site.
[c] Lactococcal cleavage site.
[d] Plasmin cleavage site.

Table 3
Identity of 2% TCA-soluble peptides produced from bovine α_{s1}-casein (5 mg ml^{-1}) by cathepsin G (0.025 U ml^{-1})

HPLC peak no.	Sequence identity	Experimental mass (Da)	Theoretical mass (Da)	Peptide identity	N-terminal cleavage site	C-terminal cleavage site
1 (i)	H_2N-Arg-Gln-Phe-Tyr-Gln-Leu-Asp-Ala-Tyr-Pro-Ser-Gly-Ala-Trp-Tyr-COOH	1876.5	1865.03	αs_1-CN (f151-165)	Phe_{150}-Arg_{151}	Tyr_{165}-Tyr_{166}
1 (ii)	H_2N-Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln-Glu-Val-Leu-COOH	1876.5	1877.22	α_{s1}-CN (f1-16)	–	Leu_{16}-Asn_{17}
2 (i)	H_2N-Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln-Glu-Val-Leu-Asn-Glu-Asn-Leu-Leu-COOH	2458.7	2460.86	α_{s1}-CN (f1-21)	–	Leu_{21}-Arg_{22}
2 (ii)	H_2N-Lys-Glu-Lys-Val-Asn-	2458.7	–	α_{S1}-CN (f34-[a])	Gly_{33}-Lys_{34}[b,c]	–
3	H_2N-Ser-Asp-Ile-Pro-Asn-Pro-Ile-Gly-Ser-Glu-Asn-Ser-Glu-Lys-Thr-Thr-Met-Pro-Leu-Trp-COOH	2216	2214.1	α_{s1}-CN (f180-199)	Phe_{179}-Ser_{180}	–
4 (i)	H_2N-Thr-Asp-Ala-Pro-Ser-Phe-Ser-Asp-Ile-Pro-Asn-Pro-Ile-Gly-Ser-Glu-Asn-Ser-Glu-Lys-Thr-Thr-Met-Pro-Leu-Trp-COOH	2825.9	2835.08	α_{s1}-CN (f174-199)	Tyr_{173}-Thr_{174}[b]	–
4 (ii)	H_2N-Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln-Glu-Val-Leu-Asn-Glu-Asn-Leu-Leu-Arg-Phe-COOH	2764.2	2764.22	α_{s1}-CN (f1-23)	–	Phe_{23}-Phe_{24}[b,c]

[a] Incomplete sequence.
[b] Chymosin cleavage site.
[c] Lactococcal cleavage site.

Ala_{177}, Arg_{183}-Asp_{184} of β-casein, Singh, Fox, Højrup, & Healey, 1994; Visser, Nooman, Slangen, & Rollema, 1989).

Cathepsin G cleaved α_{s1}- and β-casein at many sites which are close or identical to cleavage sites of the CEP of *Lactococcus lactis* subsp. *lactis* NCDO 763 (Glu_{148}-Leu_{149}; Monnet, Ley, & Gonzàlez, 1992) *Lactococcus lactis* subsp. *cremoris* SK11 (Phe_{23}-Phe_{24} and Gly_{33}-Lys_{34}, Reid, Ng, Moore, Coolbear, & Pritchard, 1991), *Lactococcus lactis* subsp. *cremoris* SK11 (Phe_{52}-Ala_{53}, Leu_{163}-Ser_{164}, Reid, et al., 1991) and *Lactococcus lactis* subsp. *cremoris* HP (Leu_{163}-Ser_{164}, Visser, Slanger, Exterkate, & de Veer, 1988; Fig. 3).

Several cleavage sites of cathepsin G on α_{s1}-casein, such as Phe_{150}-Arg_{151}, Tyr_{165}-Tyr_{166}, Tyr_{173}-Thr_{174} and Phe_{179}-Ser_{180} and Phe_{52}-Ala_{53}, Gln_{56}-Ser_{57}, Leu_{58}-Val_{59}, Asn_{68}-Ser_{69}, Ser_{96}-Lys_{97}, Ala_{101}-Met_{102}, Glu_{108}-Met_{109}, Phe_{119}-Thr_{120}, Leu_{163}-Ser_{164}, Phe_{190}-Leu_{191} were identical to elastase cleavage sites (Considine et al., 1999, 2000), while sites, such as Phe_{23}-Phe_{24}, Gly_{33}-Lys_{34}, Glu_{70}-Ile_{71}, Val_{76}-Glu_{77}, Glu_{110}-Ile_{111}, Leu_{142}-Ala_{143}, Gln_{155}-Leu_{156} and Tyr_{165}-Tyr_{166} and Glu_{108}-Met_{109}, Lys_{169}-Val_{170}, Lys_{176}-Ala_{177}, Asp_{184}-Met_{185} and Phe_{190}-Leu_{191}, were also cleaved by cathepsin B (Considine et al., 2000).

The results of this study indicate that cathepsin G has a very broad cleavage specificity on both α_{s1}- and β-caseins, having a preference for bonds incorporating Glu, Ala, Phe, Arg and Leu in α_{s1}-casein and Ser, Thr, Leu and Phe in β-casein. This is in partial agreement with Blow and Barrett (1977), who reported that peptide bonds containing Leu and Phe were cleaved preferentially in the β-chain of insulin. Some cathepsin G cleavage sites are identical or near to those cleaved by the action of the lactococcal CEP, plasmin or chymosin.

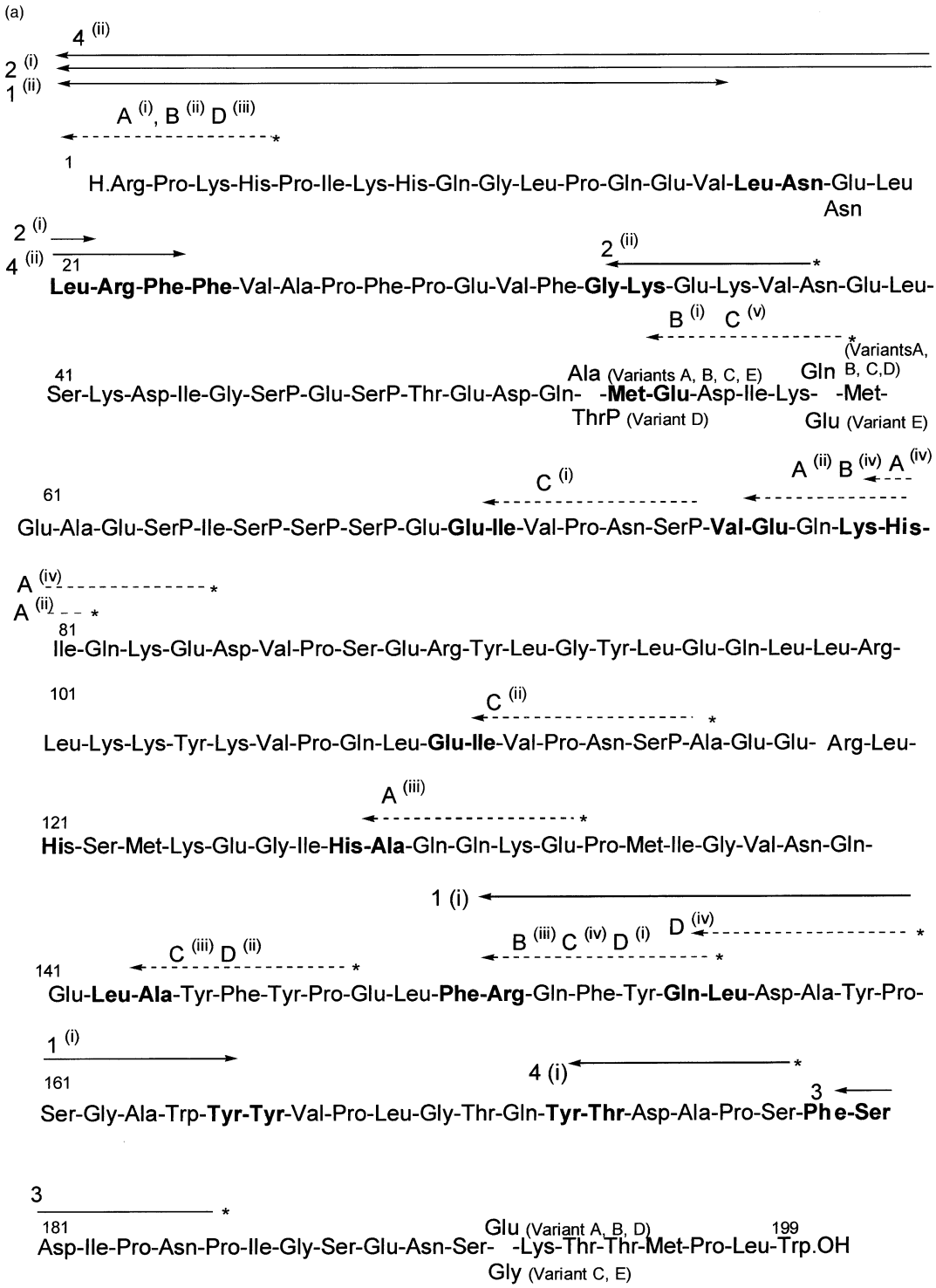

Fig. 3. Primary structure of bovine (a) α_{s1}- and (b) β-casein (Swaisgood, 1992), showing the position of the 2% TCA-soluble peptides produced by hydrolysis by cathepsin G at pH 7.5. Cleavage sites are indicated in bold. Letters represent peptides identified by urea-polyacrylamide gel electrophoresis (- - - - -), (Tables 1 and 2). Numbers correspond to peptides isolated by reverse-phase-HPLC (————), (Tables 3 and 4; [a] incomplete sequence).

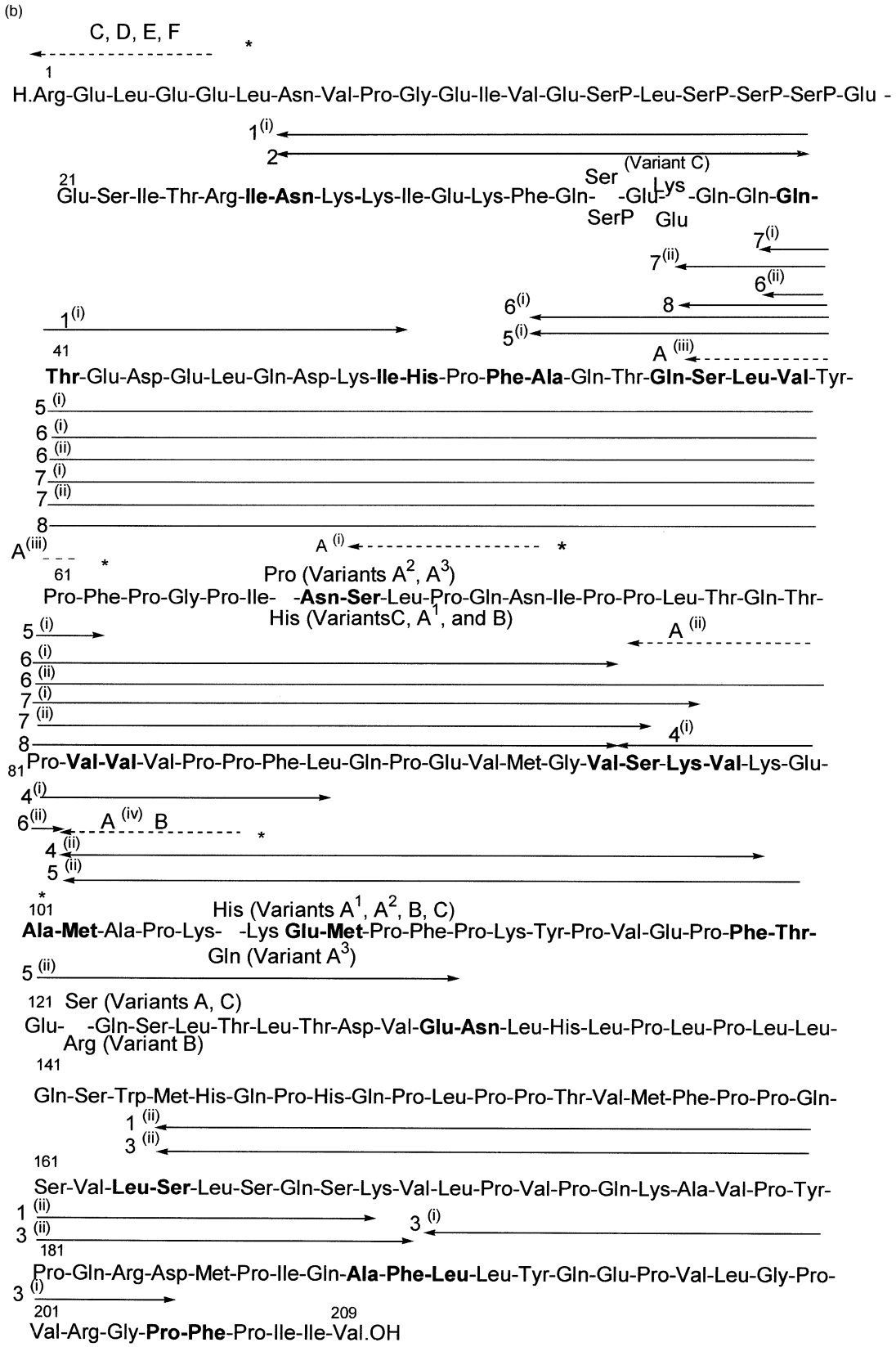

Fig. 3. continued.

Table 4
Identity of 2% TCA soluble peptides produced from bovine β-casein (5 mg ml^{-1}) by cathepsin G (0.025 U ml^{-1})

HPLC peak no.	Sequence identity	Experimental mass (Da)	Theoretical mass (Da)	Peptide identity	N-terminal cleavage site	C-terminal cleavage site
1 (i)	H_2N-Thr-Glu-Ser-Gln-Ser-	709.2	–	β-CN (f120-[a])	Phe_{119}-Thr_{120}	–
1 (ii)	H_2N-Ala-Pro-Lys-His-Lys-His-Lys-Glu-COOH	709.2	708.81	β-CN (f103-108)	Met_{102}-Ala_{103}[b]	Glu_{108}-Met_{109}[b]
1 (iii)	H_2N-Met-Pro-Ile-Gln-Ala-Phe-COOH	709.2	705.87	β-CN (f185-190)	Asp_{184}-Met_{185}[b]	Phe_{190}-Leu_{191}
1 (iv)	H_2N-Ser-Leu-Pro-Gln-Asn-	709.2	–	β-CN (f69-[a])	Asn_{68}-Ser_{69}	–
1 (v)	H_2N-Lys-Val-Lys-Glu-Ala-COOH	709.2	704.88	β-CN (f97-101)	Ser_{96}-Lys_{97}[b]	Ala_{101}-Met_{102}
1 (vi)	H_2N-Val-Leu-Pro-Val-Pro-	709.2	–	β-CN (f170-[a])	Lys_{169}-Val_{170}[b]	–
2	H_2N-Leu-Gln-Pro-Glu-Val	–	–	β-CN (f88-[a])	Phe_{87}-Leu_{88}	–
3 (i)	H_2N-Thr-Asp-Val-Glu-Asn	–	–	β-CN (f128-[a])	Leu_{127}-Thr_{128}	–
3 (ii)	H_2N- Ala-Gln-Thr-Gln-Ser	–	–	β-CN (f53-[a])	Phe_{52}-Ala_{53}[c]	–
3 (iii)	H_2N- Thr-Gln-Ser-Leu-Val	–	–	β-CN (f55-[a])	Gln_{54}-Thr_{55}	–
3 (iv)	H_2N- Val-Pro-Pro-Phe-Leu	–	–	β-CN (f84-[a])	Val_{83}-Val_{84}	–
3 (v)	H_2N-Val-Glu-Asn-Leu-His	–	–	β CN (f130-[a])	Asp_{129}-Val_{130}	
3 (vi)	H_2N-Val-Tyr-Pro-Phe-Pro	–	–	β-CN (f59-[a])	Leu_{58}-Val_{59}	Leu_{58}-Val_{59}
3 (vii)	H_2N-His-Leu-Pro-Leu-Pro	–	–	β-CN (f134-[a])	Leu_{133}-His_{134}	Leu_{58}-Val_{59}
4 (i)	H_2N-Ala-Gln-Thr-Gln-Ser	–	–	β-CN (f53-[a])-	Phe_{52}-Ala_{53}	Leu_{58}-Val_{59}
4 (ii)	H_2N-Val-Tyr-Pro-Phe-Pro	–	–	β-CN (f59-[a])	Leu_{58}-Val_{59}	Leu_{58}-Val_{59}
4 (iii)	H_2N-Ser-Leu-Val-Tyr-Pro	–	–	β-CN (f57-[a])	Gln_{56}-Ser_{57}	Leu_{58}-Val_{59}

[a] Incomplete sequence.
[b] Plasmin cleavage site.
[c] Lactococcal cleavage site.

Thus, cathepsin G from somatic cells may potentially make a significant contribution to proteolysis in high SCC milk or cheese made from such milk. Since some of the cleavage sites of cathepsin G were identical to both elastase and cathepsin B, it is possible that these lysosomal enzymes together may be involved in proteolysis in products made from high SCC milk.

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