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Proteolytic specificity of elastase on bovine β-casein

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Abstract

The lysosomes of the principal somatic cell type recruited on mastitic infection, polymorphonuclear leucocytes (PMN), contain a wide range of hydrolytic enzymes which aid in the destruction of ingested bacteria. These enzymes are of increasing interest in terms of milk quality, but little is known about their activity on the caseins. The objective of this study was to determine the cleavage specificity of elastase, one of the principal PMN proteinases, on β-casein. β-Casein (5 mg ml^{-1}) was dissolved in 0.1 M phosphate buffer, pH 7.5 and 1.76×10^{-3} U ml^{-1} of elastase were added. Samples were taken over a 24 h period and analysed by urea polyacrylamide gel electrophoresis and high performance liquid chromatography. Peptides were identified by N-terminal sequencing and mass spectrometry. Elastase cleaved β-casein at several sites including Ile_{26}-Asn_{27}, Gln_{40}-Thr_{41}, Ile_{49}-His_{50}, Phe_{52}-Ala_{53}, Gln_{56}-Ser_{57}, Leu_{58}-Val_{59}, Asn_{68}-Ser_{69}, Val_{82}-Val_{83}, Val_{95}-Ser_{96}, Ser_{96}-Lys_{97}, Lys_{97}-Val_{98}, Ala_{101}-Met_{102}, Glu_{108}-Met_{109}, Phe_{119}-Thr_{120}, Glu_{131}-Asn_{132}, Leu_{163}-Ser_{164}, Ala_{189}-Phe_{190}, Phe_{190}-Leu_{191} and Pro_{204}-Phe_{205}. Some of these sites are also cleaved by chymosin, plasmin or the cell envelope-associated proteinase of *Lactococcus*. The results show that elastase has a broad specificity on β-casein and it is therefore possible that indigenous elastase in milk may be of significance to the proteolysis of milk proteins.

Keywords: Elastase; β-Casein; Specificity; Somatic cells

1. Introduction

Milk from a healthy cow contains three main types of somatic cells: lymphocytes, polymorphonuclear granulocytes (PMN cells or neutrophils) and macrophages (O'Sullivan, Joyce, Sloan & Shattock, 1992; Sordillo, Shaefer-Weaver & Derosa, 1997). Upon mastitic infection, large numbers of PMN are recruited to engulf and destroy invasive bacteria, hence causing a rise in somatic cell count (SCC) (Saad & Stensson, 1990; Wever & Emanuelson, 1989). Mastitis causes an increase in the ratio of soluble to micellar casein (Ali, Andrews & Cheeseman 1980; Downey & Murphy, 1970; Sharma & Randolph, 1974) as a result of which more casein is available for proteolysis in milk during storage and less is available for incorporation into the reticulum of the curd during cheesemaking. Therefore, the use of mastitic milk for cheesemaking results in a reduction in cheese yield and quality (Grandison & Ford, 1986). The main proteolytic enzyme in milk is the alkaline protease, plasmin, which originates from the cow's blood (Bastian & Brown, 1996; Grufferty & Fox, 1988; Kaminogawa, Mizobuchi & Yamauchi, 1972). Anderson and Andrews (1977) and Ali et al. (1980) found a reduction in the concentration of β-casein in mastitic milk which was accelerated on incubation at 37°C. This suggests the occurrence of quite high levels of proteolytic activity in mastitic milk, much of which is probably due to plasmin.

As an acid phosphatase of leucocyte origin has been detected in mastitic milk or milk obtained from quarters after endotoxin infusion (Andrews & Alichanidis, 1975), it is expected that such milks should also contain elevated levels of the various proteolytic enzymes present in leucocytes together with this acid phosphatase. The lysosomes of somatic cells contain a wide range of hydrolytic enzymes (Baggiolini, Brets & Dewald, 1978; Olsson, Odeberg, Weiss & Elbach, 1978), which aid in the destruction of ingested bacteria. It has been suggested that these proteinases may contribute to the degradation of β-casein and the cleavage of κ-casein to para-κ-casein (Barry & Donnelly, 1981).

A cysteine proteinase, possibly originating from PMN leukocytes, has also been isolated from milk (Suzuki &

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Katoh, 1990). One of the principal enzymes found in PMN cells is the serine proteinase, elastase (Verdi & Barbano, 1991), which has a molecular weight of 24–30 kDa. The primary physiological function of elastase is the degradation of elastin (DelMar, Largman, Brodrick, Fassett & Geokas, 1980).

An inhibitor of bovine elastase (EI) has been identified in bovine colostrum and milk (Christensen, Wiegers, Hermansen, & Sottrup-Jensen, 1995). Different levels of this inhibitor were found in colostrum and in early, mid or late lactation milks. It has been suggested that EI might function as an inhibitor of polymorphonuclear elastase present in milk (Christensen et al., 1995).

The objective of this study was to determine the cleavage specificity of elastase towards β-casein and hence investigate the potential significance of this enzyme for milk and dairy product quality.

2. Materials and methods

Whole casein was prepared from a sample of bovine skim milk by the method of Mulvihill and Fox (1974). Casein was fractionated by ion-exchange chromatography on diethylaminoethyl cellulose (DE-52, Sigma, St. Louis, MO, USA) using 10 mM imidazole buffer, pH 7, containing 4.5 M urea and 0.15% (v/v) 2-mercaptoethanol (Creamer, 1974); proteins were eluted using a linear NaCl gradient (0–0.5 M). Fractions containing β-casein were pooled, dialysed against water and freeze-dried.

Elastase (E.C. 3.4.21.36; from porcine spleen), containing ~5 U mg^{-1} protein, was obtained from the Sigma Chemical Co., St. Louis, MO, USA (1 unit of elastase activity will solubilise 1 mg elastin in 2 min at pH 8.8 and 37°C).

β-Casein (5 mg ml^{-1}) was dissolved in 0.1 M phosphate buffer, pH 7.5, containing 0.05% w/v NaN_3. Elastase (1.76×10^{-3} U ml^{-1}) was added 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) or reverse-phase (RP)-HPLC. Elastase was inactivated in each aliquot by heating at 100°C for 5 min before analysis.

2.1. Urea-PAGE

Samples for urea-PAGE were prepared by the addition of an equal volume of double strength sample buffer (McSweeney, Olson, Fox, Healy, & Hørup, 1993). 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 (0.22 μm pore size; ProBlott™, Applied Biosystems Inc., Foster City, CA, USA) using a mini Trans-Blott™ electrophoretic transfer cell (Bio-Rad, Richmond, CA, USA). Electroblotting was performed at 90 V (300 mA) for 26 min in transfer buffer (10 mM 3-[cyclohexylamino]-1-propanesulphonic acid, pH 11, in 10% methanol). Membranes were stained for 5 min using Coomassie Brilliant Blue R250 in 50% methanol and 11% acetic acid and were destained subsequently in 50% methanol. The electroblotts were stored at −18°C until N-terminal sequencing was performed.

2.2. RP-HPLC

RP-HPLC was performed on the 1% trichloroacetic acid (TCA)-soluble fraction of the hydrolyzates. Samples were centrifuged at maximum speed (11,600 *g*) for 30 min in a Sanyo MSE microfuge (Sanyo Gallonkamp PLC, Leicester, UK) and the supernatant was retained for analysis. The supernatant was filtered through a 0.45 μm cellulose acetate filter (SRP15, Sartorius, Goettingen, Germany) prior to injection (40 μl). RP-HPLC was performed using a Shimadzu liquid chromatograph (Shimadzu Corp., Kyoto, Japan) consisting of a model LC-9A pump, SIL-9A autosampler and a SPD–6A UV spectrophotometric detector. A Nucleosil C_8 column (300 Å, 5 μm, 250×4.6 mm) was used and elution was by means of a gradient formed from solvent A (0.1% trifluoroacetic acid, TFA, in H_2O) and solvent B (0.1% TFA in acetonitrile, Aldrich Chemical Co., Milwaukee,

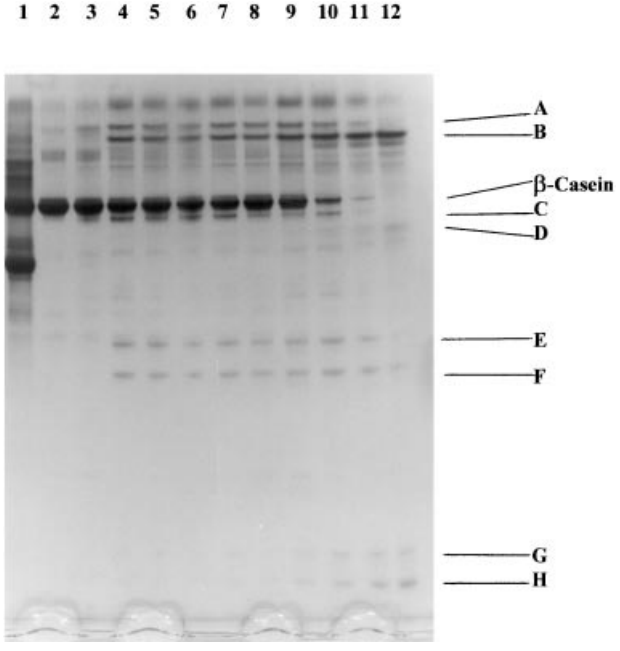

Fig. 1. Urea-polyacrylamide gel electrophoretograms of Na-caseinate (lane 1), and β-casein incubated at 37°C in 0.1 M phosphate buffer, pH 7.5, for 0 or 24 h (lanes 2, 3) or β-casein hydrolysed by elastase (1.76×10^{-3} U ml^{-1}) under the same conditions for 5, 10, 15, 30 min, and 1, 3, 6, 12 or 24 h (lanes 4–12).

WI, USA) as follows: % B increased from 0 to 30% B at a rate of 1.2% B min^{-1}; from 30 to 50% B at a rate of 0.44% B min^{-1}; 50% B was maintained for 6 min and the % B was increased to 95% at a rate of 22.5% B min^{-1}, held at 95% B for 10 min before reduction to 0% B at a rate of 47.5% B min^{-1}. Flow rate was 0.75 ml min^{-1}, detection was at 214 nm and data was recorded for 70 min. Peptides were collected manually, freeze-dried and identified by N-terminal sequencing and mass spectrometry.

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

Peptide	N-terminal sequence	Identity	N-terminal cleavage sites
A (i)	Ser-Leu-Pro-GIn-Asn	β-CN f69-[a]	Asn_{68}-Ser_{69}
A (ii)	Ser-Lys-Val-Lys-Glu	β-CN f96-[a]	Val_{95}-Ser_{96}
A (iii)	Ser-Leu-Val-Tyr-Pro	β-CN f57-[a]	Gln_{56}-Ser_{57}
A (iv)	Met-Ala-Pro-Lys-His	β-CN f102-[a]	Ala_{101}-Met_{102}
B	Met-Ala-Pro-Lys-His	β-CN f102-[a]	Ala_{101}-Met_{102}
C	Arg-Glu-Leu-Glu-Glu	β-CN fl-[a]	
D	Arg-Glu-Leu-Glu-Glu	β-CN fl-[a]	
E	Arg-Glu-Leu-Glu-Glu	β-CN fl-[a]	
F	Arg-Glu-Leu-Glu-Glu	β-CN fl	

[a] Incomplete sequence.

2.3. Identification of peptides

Peptides were sequenced at the National Food Biotechnology Centre, University College, Cork, Ireland by Edman degradation on an automated pulsed liquid-phase Procise™ 494 protein sequencer, with a 785A programmable absorbance detector (Applied Biosystems Inc., Foster City, CA, USA). Mass spectrometric analyses were performed at the Department of Biochemistry, Faculty of Medicine and Health Sciences, Queens Medical Centre, University of Nottingham, UK. Mass spectrometry was performed on the 1% TCA-soluble peptides isolated by RP-HPLC using a plasma desorption, time-of-flight mass spectrometer. Identification of peptides by mass was based on particular sequence data combined with mass searches using the GPMAW program (Lighthouse Data, Odense, Denmark). In certain cases, it was found that single

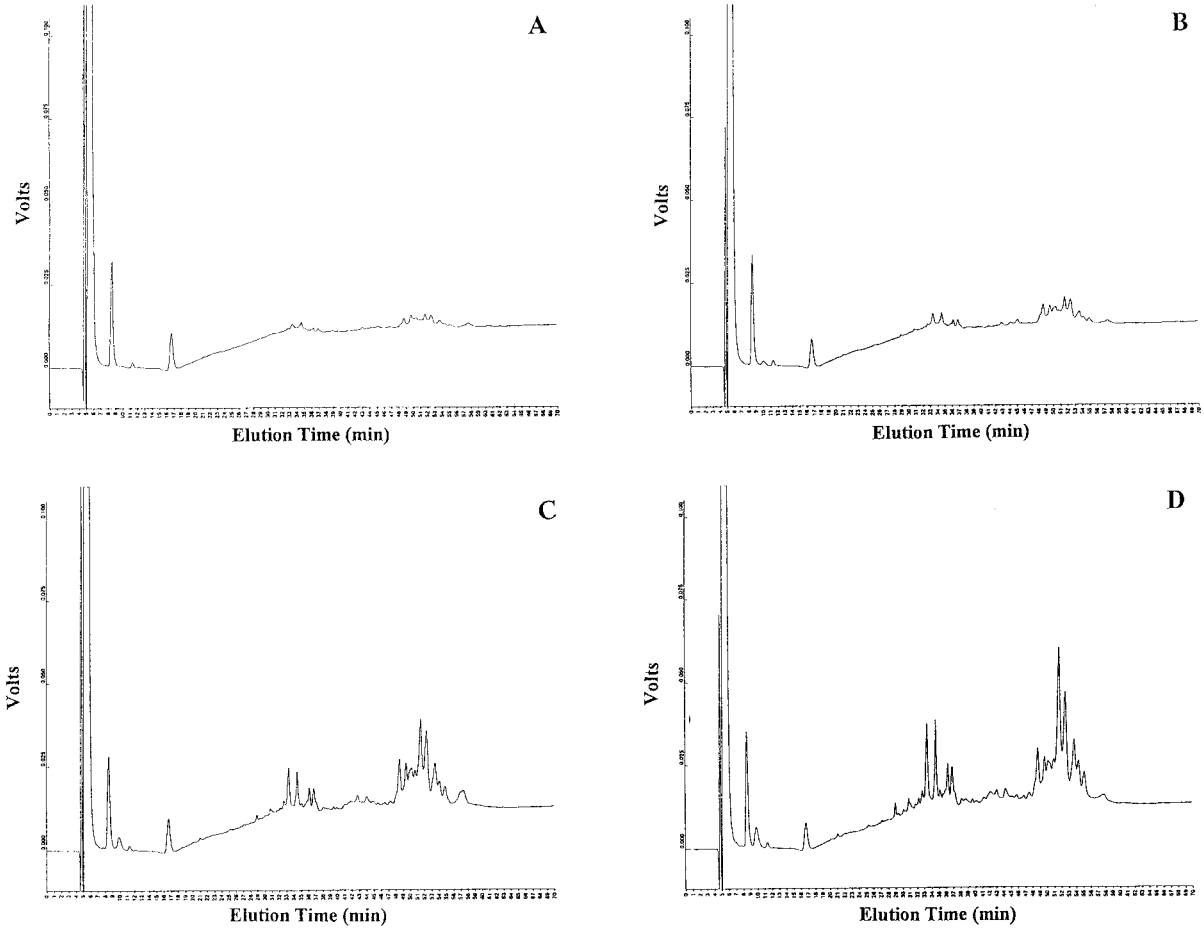

Fig. 2. Reverse-phase HPLC chromatograms of 1% TCA-soluble peptides produced from β-casein (5 mg ml^{-1}) by elastase (1.76×10^{-3} U ml^{-1}) incubated in 0.1 M phosphate buffer, pH 7.5 for 3 h (A), 6 h (B), 12 h (C) or 24 h (D).

electroblott bands or HPLC peaks contained more than one peptide. These peptides were sequenced in parallel.

3. Results and discussion

Urea-PAGE electrophoretograms of β-casein hydrolysed by elastase are shown in Fig. 1. β-Casein was readily degraded by elastase with the formation of peptides of both higher and lower electrophoretic mobilities. Electrophoretograms of the control, containing no enzyme, showed no β-casein degradation after 24 h incubation, indicating the absence of indigenous proteinase activity in the β-casein preparation. Hydrolysis of β-casein by elastase was rapid, with the first degradation products being evident after 5 min incubation. The intensity of the peptide bands increased from 5 min to 12 h and 15 bands were present in the 3 h sample. Most bands were less intense in the electrophoretogram of the 24 h sample than that of the 12 h sample, indicating their subsequent degradation by elastase. Two bands with high electrophoretic mobility (G and H, Fig. 1) accumulated in the hydrolysate. Six peptides (A–F, Fig. 1) were isolated by electroblotting and their N-terminal sequence determined, thus allowing identification of some of the primary sites of elastase action on the protein (Table 1). Large peptides produced by elastase originated from cleavage sites at Gln_{56}-Ser_{57}, Asn_{68}-Ser_{69}, Val_{95}-Ser_{96} and Ala_{101}-Met_{102} while 4 peptides (C–F) had the same N-terminal sequence as β-casein. The C-termini of the large peptides were not determined.

RP-HPLC elution profiles of the 1% TCA-soluble fractions of β-casein hydrolysed by elastase for time periods up to 24 h are shown in Fig 2. The RP-HPLC analysis indicated that 1% TCA-soluble peptides were produced gradually over time during incubation. Eight peaks were isolated from the 24 h hydrolyzate and identified by sequencing and by mass spectrometry (Fig. 3; Table 2). Cleavage sites were identified at Ile_{26}-Asn_{27}, Gln_{40}-Thr_{41}, Ile_{49}-His_{50}, Phe_{52}-Ala_{53}, Gln_{56}-Ser_{57}, Leu_{58}-Val_{59}, Val_{82}-Val_{83}, Val_{95}-Ser_{96}, Ser_{96}-Lys_{97}, Lys_{97}-Val_{98}, Ala_{101}-Met_{102}, Glu_{108}-Met_{109}, Phe_{119}-Thr_{120}, Glu_{131}-Asn_{132}, Leu_{163}-Ser_{164}, Ala_{189}-Phe_{190}, Phe_{190}-Leu_{191} and Pro_{204}-Phe_{205}. The location of the peptides and elastase cleavage sites on β-casein are summarised in Fig. 4. The appearance of a number of peptides may be as a result of the initial cleavage of β-casein at several sites throughout the molecule, producing a number of large 1% TCA-insoluble polypeptides which were subsequently degraded to smaller peptides.

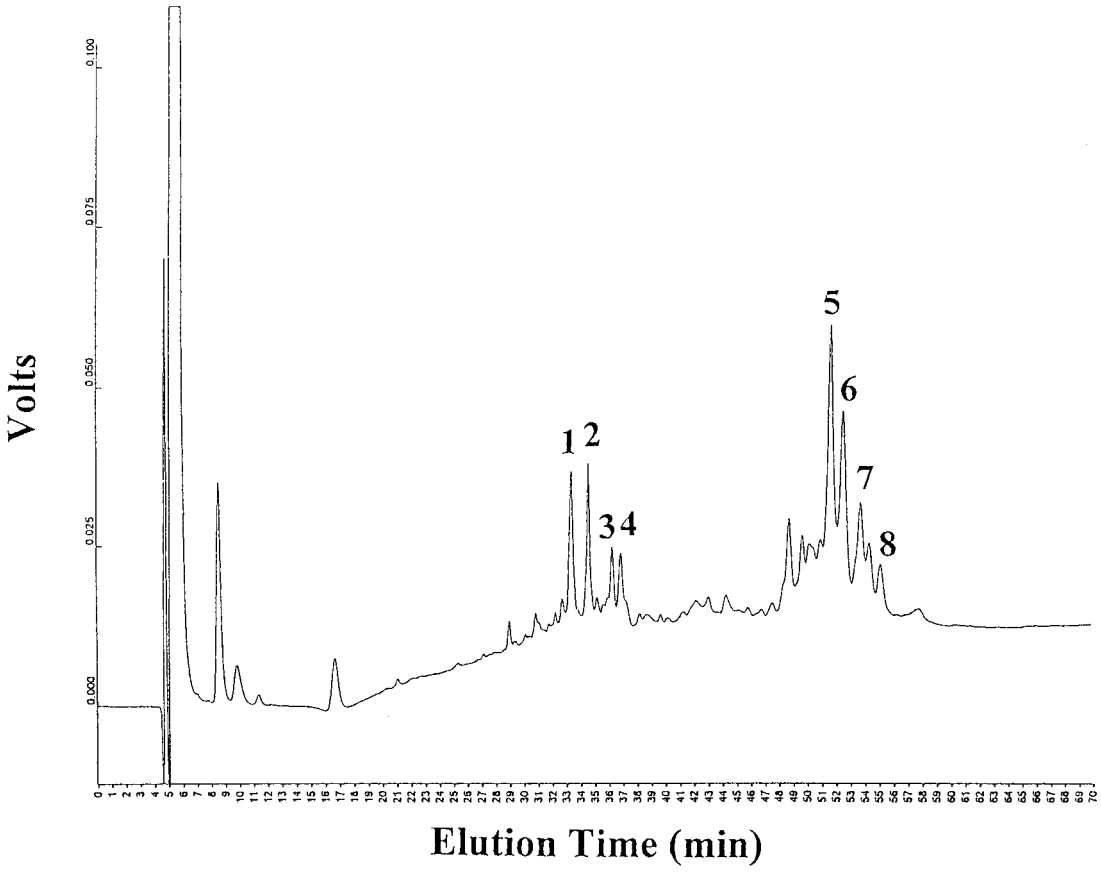

Fig. 3. Reverse-phase HPLC profile of 1% TCA-soluble peptides from bovine β-casein (5 mg ml^{-1}) hydrolysed by elastase (1.76×10^{-3} U ml^{-1}) incubated in 0.1 M phosphate buffer, pH 7.5 at 37°C for 24 h. Peptides that were isolated and identified are indicated (1–8).

Table 2
Identity of 1% TCA-soluble peptides produced from bovine β-casein (5 mg ml^{-1}) by elastase (1.76×10^{-3} U ml^{-1})

HPLC peak no.	Sequence identity	Experimental mass (Da)	Theoretical mass (Da)	Probable peptide identity	N-terminal cleavage site	C-terminal cleavage site
1(i)	H_2N-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Gln-Ser-Glu-Lys-Gln-Gln-Gln-Thr-Glu-Asp-Leu-Gln-Asp-Lys-Ile-COOH	2877.7	2835.11	β-CN f27-49	Ile_{26}-Asn_{27}	Ile_{49}-His_{50}
1(ii)	H_2N-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Val-Pro-Gln-Lys-Ala-Val-Pro-Tyr-Pro-Gln-Arg-Asp-Met-Pro-Ile-Gln-Ala-COOH	2877.7	2878.37	β-CN f164-189	Leu_{163}-Ser_{164}	Ala_{189}-Phe_{190}
2	H_2N-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Gln-Ser-Glu-Lys-Gln-Gln-Gln-COOH	1773.8	1762.98	β-CN f27-40	Ile_{26}-Asn_{27}	Gln_{40}-Thr_{41}
3 (i)	H_2N-Leu-Leu-Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-COOH	1511.1	1537.82	β-CN fl91-204	Phe_{190}-Leu_{191}	Pro_{204}-Phe_{205}
3 (ii)	H_2N-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Val-Pro-Gln-Lys-Ala-Val-Pro-Tyr-Pro-Gln-Arg-Asp-Met-Pro-Ile-Gln-Ala-Phe-COOH	3024.2	3025.5	β-CN fl64-190	Leu_{163}-Ser_{164}	Phe_{190}-Leu_{191}
4 (i)	H_2N-Ser-Lys-Val-Lys-Glu-Ala-Met-Ala-Pro-Lys-His-Lys-Glu-COOH	1485.1	1482.76	β-CN f96-108	Val_{95}-Ser_{96}	Glu_{108}-Met_{109}
4 (ii)	H_2N-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-COOH	2180.4	2173.6	β-CN f102-119	Ala_{101}-Met_{102}	Phe_{119}-Thr_{120}
5 (i)	H_2N-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-lle-Pro-Asn-Ser-Leu-Pro-Gln-Asn-lle-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-COOH	3203.3	3215.69	β-CN f53-82	Phe_{52}-Ala_{53}	Val_{82}-Val_{83}
5 (ii)	H_2N-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro- Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-Glu-Ser-Gln-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-COOH	3449.1	3477.98	β-CN f102-131	Ala_{101}-Met_{102}	Glu_{131}-Asn_{132}
6 (i)	H_2N-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-COOH	4630.8	4609.39	β-CN f53-95	Phe_{52}-Ala_{53}	Val_{95}-Ser_{96}
6 (ii)	H_2N-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu-Ala-COOH	4630.8	4623.46	β-CN f59-101	Leu_{58}-VaI_{59}	Ala_{101}-Met_{102}
7 (i)	H_2N-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-COOH	4228.8	4195.96	β-CN f59-97	Leu_{58}-Val_{59}	Lys_{97}-Val_{98}
7 (ii)	H_2N-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-COOH	4228.8	4268.02	β-CN f57-96	Gln_{56}-Ser_{57}	Ser_{96}-Lys_{97}
8	H_2N-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Gln-Pro-Glu-Val-Met-Gly-Val-COOH	4184.7	4180.95	β-CN f57-95	Gln_{56}-Ser_{57}	Val_{95}-Ser_{96}

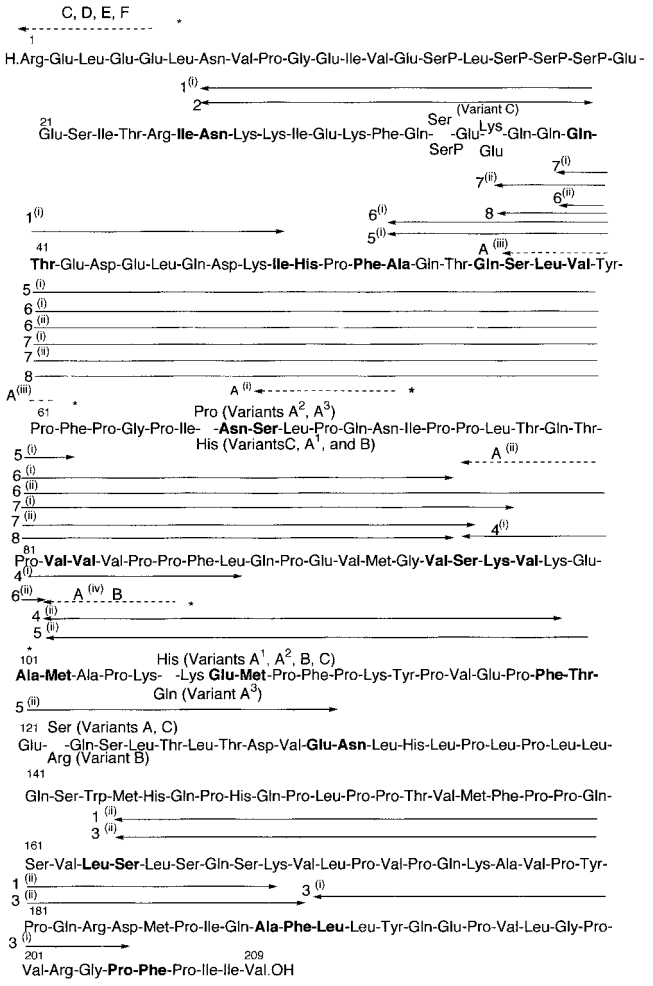

Fig. 4. Primary structure of bovine β-casein (Swaisgood, 1992) showing the position of the peptides produced on hydrolysis with elastase at pH 7.5. Cleavage sites are indicated in bold. Peptides identified by urea-polyacrylamide gel electrophoresis (Table 1) are indicated as ..... and peptides isolated by reverse-phase-HPLC (Table 2) as —. (*Incomplete sequence).

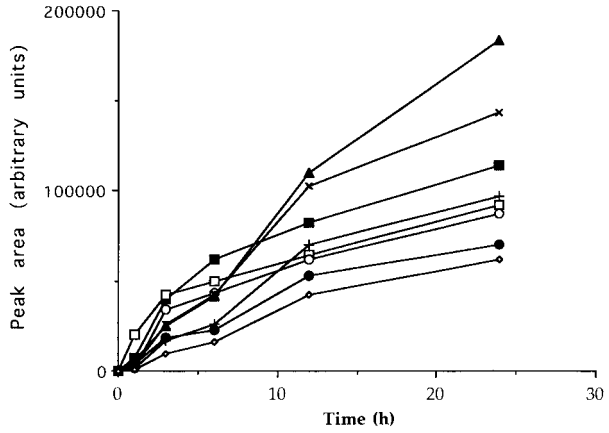

Fig. 5. Time course of changes in the area of the HPLC peaks representing the major peptides produced on hydrolysis of bovine β-casein by elastase at 37°C in 0.1 M phosphate buffer, pH 7.5: ■, f27-49, f164-189; □, f27-40; ●, f191-204, f164-190; ○, f96-108, f102-119; ▲, f53-82, f102-131; X, f53-95, f59-101; +, f59-97, f57-96; ◆, f57-95. Experimental details are given in the text.

The order in which the 1% TCA-soluble peptides were produced from β-casein is shown in Fig 5. Areas of all peptide peaks increased with incubation time, suggesting that they were not degraded further by elastase. The peptides β-CN (f27-40), (f27-49) and (f164-189) were produced most rapidly, suggesting that Ile_{26}-Asn_{27} is one of the principal cleavage sites of elastase on β-casein. There was a gradual increase in peak areas of peptides β-CN (f191-204), (f164-190), (f96-108), (f102-119), (f59-97), (f57-96) and (f57-95). There was a marked increase in the areas of the peaks corresponding to β-CN (f53-82), (f102-131), (f53-95) and (f59-101) after 14 h. The concentrations of all 1% TCA-soluble peptides increased throughout incubation suggesting that they were not degraded further by elastase.

One cleavage site of elastase on β-casein was found at Ala_{189}-Phe_{190}, which is one of the principal chymosin cleavage sites on β-casein (Visser & Slangen, 1977). Elastase also cleaves β-casein at Phe_{190}-Leu_{191}, which is very close to another of the principal cleavage sites of chymosin on β-casein, Leu_{192}-Tyr_{193} (Visser & Slangen, 1977). Hydrolysis of the latter bond results in the release of an extremely bitter C-terminal fragment β-CN (f193-209), which has been identified in Gouda (Visser, Slangen, Hup & Stadhouders, 1983) and Cheddar (Kelly, Fox & McSweeney, 1996) cheeses. Thus, cleavage of β-casein towards its C-terminus by elastase may also result in the production of bitter peptides.

β-Casein was also cleaved by elastase at Ile_{26}-Asn_{27} and Glu_{108}-Met_{109}. These sites are close to the three principal plasmin cleavage sites on β-casein, Lys_{28}-Lys_{29}, Lys_{105}-His_{106} and Lys_{107}-Glu_{108} (Eigel et al., 1984; Fox, Singh, & McSweeney, 1994). Peptides originating from these plasmin cleavage sites are present in Cheddar cheese (McSweeney, Pochet, Fox & Healy, 1994). β-Casein was cleaved by elastase at Lys_{97}-Val_{98}, a site also cleaved by plasmin and at Pro_{204}-Phe_{205} which is very close to the plasmin cleavage site Arg_{202}-Gly_{203} (Fox et al., 1994). Thus, elastase from somatic cells may potentially make a contribution to plasmin-like activity in high SCC milk. This is in contrast to the conclusions of Barry and Donnelly (1981) who thought that the major proteolytic enzymes of PMN leucocytes (elastase, cathepsin G and collagenase) contribute little, if any, to the overall breakdown of α_{s1}− and β-caseins in high SCC milk, although these authors did not determine the specificity of these proteinases on the caseins.

The specificities of cell envelope-associated proteinases (CEP) of a number of strains of *Lactococcus* on β-casein are known, allowing comparison with elastase action (Fox et al., 1994). Elastase cleaved β-casein at Asn_{68}-Ser_{69}, which is identical to one of the principal cleavage sites of P_1−type CEP from *Lc. lactis* subsp. *cremoris* Wg2 (Juillard et al., 1995). Elastase also cleaved β-casein

at Phe_{190}-Leu_{191}, which is very close to a principal cleavage site of the P_I-type proteinase of *Lc. lactis* subsp. *cremoris* Wg2 (Leu_{191}-Leu_{192}; Juillard et al., 1995). Also, the cleavage site Phe_{190}-Leu_{191} of elastase on β-casein is close to a site hydrolysed by the P_I-type proteinase of *Lc. lactis* susp *cremoris* H2 (Tyr_{193}-Gln_{194}) and a cleavage site of the P_{III}-type proteinase of *Lc. lactis* subsp. *cremoris* SK112 (Leu_{192}-Tyr_{193}; Reid, Ng, Moore, Coolbear, & Pritchard, 1991). Proteinases of *Lc. lactis* subsp. *lactis* NCDO 763 (Monnet, Le Bars, & Gripon, 1986) and *Lc. lactis* subsp. *lactis* HP (Visser, Slanger, Exterkate & deVeer, 1988) cleave β-casein at Tyr_{193}-Gln_{194} and Tyr_{193}-Gln_{194}, respectively, which are also close to an elastase cleavage site (Phe_{190}-Leu_{191}). Elastase cleaved β-casein at Phe_{52}-Ala_{53} which is also cleaved by the CEP of *Lc. lactis* subsp. *cremoris* AC1 and *Lc. lactis* subsp. *lactis* NCDO 763 (Monnet, Bockelmann, Gripon, & Teuber, 1989).

The peptide β-CN (f57-95) was found in a 10 kDa diafiltrate retentate of the water-soluble fraction of Cheddar cheese by Singh, Fox, and Healy (1995). This peptide is also produced by the action of elastase on β-casein, although the bond Gln_{56}-Ser_{57} is also cleaved by the CEP of *Lactococcus* (Fox & McSweeney, 1996). The proteolytic agent responsible for the cleavage of Val_{95}-Ser_{96} cannot be explained in terms of the known specificities of chymosin, plasmin and or CEP of *Lactococcus* on β-CN (Fox & McSweeney, 1996) but is consistent with the action of elastase.

The results of this study indicated that elastase has a broad cleavage specificity on β-casein and some of the elastase cleavage sites are identical to or near those cleaved by the action of CEP, plasmin or chymosin. Indigenous elastase in milk may thus be of significance to proteolysis in milk or dairy products, particularly those produced from milks of elevated SCC.

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