

Note

Plasmin Cleavage of Human β -CaseinNorihiko AZUMA^{*,†} and Kunio YAMAUCHI^{**}^{*} Department of Applied Biochemistry, Utsunomiya University, Mine-machi 350, Utsunomiya 321, Japan^{**} Department of Food Technology, Nihon University, 34-1, Shimouma-3, Tokyo 154, Japan

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Human casein is considered to consist mainly of β - and κ -caseins. In addition to these, we have found a 20,000-dalton casein component in a human casein fraction prepared by either acid precipitation or ultracentrifugation, and demonstrated that this fragment was derived from β -casein, being cleaved by the action of plasmin.¹⁾ The formation of the γ -casein group (β -CN(f29—209), β -CN(f106—209), and β -CN(f108—209)) from bovine β -casein has been investigated and ascribed to the action of plasmin.^{2–4)} However, the human 20,000-dalton casein fragment described above contained the N-terminal phosphoserine region, and is one of the major components of human casein. These findings led us to presume that plasmin primarily attacks the C-terminal portion of human β -casein, unlike its attack on bovine β -casein,²⁾ and releases the C-terminal fragment from human β -casein instead of converting it to component(s) like bovine γ -caseins.³⁾ In this study, we isolated the C-terminal fragment of human β -casein that was released by plasmin action, and identified the cleavage point.

Human β -casein was prepared from acid casein by gel filtration on Sephacryl S-300 (Pharmacia, Uppsala, Sweden) in a 50 mM imidazole-HCl buffer at pH 7, containing 4 M urea and 0.1% 2-mercaptoethanol as described elsewhere.¹⁾ The β -casein was further fractionated on DEAE-Sephacel (Pharmacia) in accordance with its phosphorus content as described previously,⁵⁾ and the major β -casein component of 4-phosphorus form was used in this study.

Hydrolysis of human β -casein by plasmin from human plasma (EC 3.4.21.7; Sigma, St. Louis, MO) was done in a 0.2 M phosphate buffer at pH 7.4 with a substrate to enzyme ratio of 50 to 1 for 1.5 hr at 37°C. The reaction was stopped by heating in a boiling water bath for 10 min. A sample of the hydrolysate was put directly onto reverse-phase high-performance liquid chromatography (HPLC). A Tri Rotor SR-2 liquid chromatograph (Jasco, Tokyo, Japan) with a C4 hi-pore column (SSC-SC4, 250 × 4.6 mm, Senshu Kagaku, Tokyo, Japan) was used. Elution was done with a linear gradient of acetonitrile (1% per min) in 0.1% trifluoroacetate at 40°C and a flow rate of 1 ml per min. The peptide concentration was monitored at 230 nm, and each peptide fraction was manually collected and lyophilized.

The amino acid composition of each fraction was analyzed in a Hitachi 835 amino acid analyzer after it was hydrolyzed in 6 M HCl at

110°C for 24 hr. The peptide that possibly was the C-terminal fragment was analyzed by automated Edman degradation on a 470A protein sequencer (Applied Biosystems, California, U.S.A.).

The C-terminal peptide corresponding to the part of human β -casein other than the 20,000-dalton fragment was isolated by reverse-phase HPLC from the plasmin hydrolysate of human β -casein (Fig. 1). The

Table I. Amino Acid Composition and Sequence of the C-Terminal Peptide Released by the Action of Plasmin from Human β -Casein

Amino acid	mol%	Nearest integer	β -CN (f160—210) ^a
Asx	6.1	3	3
Thr	4.4	2	2
Ser	2.2	1	1
Glx	17.1	8	8
Pro	18.0	9	10
Gly	nd	0	0
Ala	7.0	3	3
Cys	nd	0	0
Val	15.7	8	7
Ile	6.1	3	3
Leu	15.6	8	8
Tyr	3.1	2	2
Phe	nd	0	0
Lys	nd	0	0
His	3.2	2	2
Arg	1.9	1	1
Trp	nd	0	0

Sequence (up to 40th residue):

VLPQPQVVPYPQRAVPVQALLNQELLNPTHQIYPVTQ

^a From the sequence data of Lönnerdal *et al.*⁶⁾
nd, not detected.

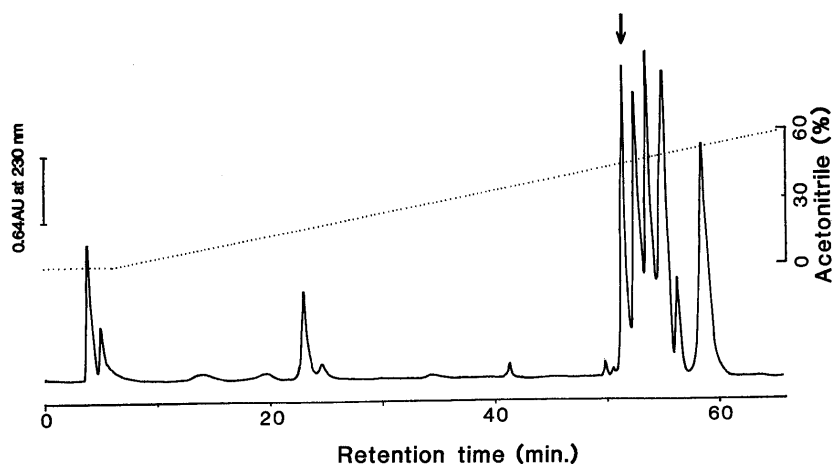


Fig. 1. Reverse-phase HPLC Pattern for Human β -Casein after Treatment with Plasmin. The desired peptide peak is indicated by the arrow.

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peptide indicated by the arrow in Fig. 1 was found to be the desired peptide from its amino acid composition and sequence.

The result of the amino acid composition of this peptide is shown in Table I, together with its N-terminal amino acid sequence up to the 40th residue. Table I also has the amino acid composition of the corresponding fragment of human β -casein (residues 160 to 210) analyzed by Lönnerdal *et al.*⁶⁾ from the nucleotide sequence. The amino acid sequences of these portions were identical. Consequently, the plasmin cleavage point was clarified to be the peptide bond between ¹⁵⁹Lys–¹⁶⁰Val. The amino acid residues at positions 166 (Q/E), 168 (V/L), 172 (Q/V), 191 (T/P), 198 (T/P), and 199 (Q/E) (residue numbers are according to Lönnerdal *et al.*⁶⁾) are in conflict with those reported by Greenberg and Groves,⁷⁾ and unlikely to be due to genetic variants.

As the amino acid composition of this peptide was closely similar to that of the C-terminal fragment from residues 160 to 210, as found by Lönnerdal *et al.*,⁶⁾ this peptide is considered to have the full amino acid sequence to the C-terminus.

In our previous paper,¹⁾ we clarified that, by plasmin action, human β -casein was converted to the 20,000-dalton fragment (termed β -CN(f)1), with the phosphoserine residues in the N-terminal region. Because β -CN(f)1 can be found in the casein fraction prepared by either acid precipitation or ultracentrifugal precipitation, and because the C-terminal peptide considered to be the other part of β -casein can be released by the action of plasmin, it is likely that the peptide bond between ¹⁵⁹Lys–¹⁶⁰Val is primarily hydrolyzed by plasmin. This cleavage point was not indicated by Greenberg and Groves⁸⁾; they showed the occurrence of γ -casein like peptides which has comparable mobilities to that of bovine γ_2 - and γ_3 -caseins on alkaline-urea polyacrylamide gel electrophoresis (PAGE). The band of their γ_{2+3} -casein like peptides may be identical with our β -CN(f)1 with one phosphorus, judging from their mobilities on

alkaline-urea PAGE. The cleavage points for the occurrence of γ -casein like peptides estimated by Greenberg and Groves⁸⁾ from the homology between human and bovine β -caseins are all included in β -CN(f)1, and the cleavage point for releasing our C-terminal peptide is included in all γ -casein homologues.

These findings suggest that the occurrence of γ -casein homologues is inconsistent with the occurrence of β -CN(f)1 accompanied by the release of the C-terminal peptide.

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