

PLASMIN CLEAVES HUMAN β -CASEIN

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SUMMARY: Plasmin cleaves isolated human β -casein to form specific fragments in a manner similar to the generation of γ_1 -, γ_2 -, and γ_3 -caseins from the bovine homologue. Identification of a protein previously isolated from human milk as a specific plasmin cleaved portion of β -casein indicates that endogenous plasmin is active in whole milk. These findings suggest that protease activity should be considered in casein quantitation or isolation of components from human milk. © 1984 Academic Press, Inc.

Values for the protein content and distribution in human milk, the recommended nutritional regimen for pre-term and term infants, remain largely unsettled. Total protein concentrations of 0.8 to 1.7% have been reported with the casein fraction estimated between 30 and 50% of the total protein. Working values of 0.9% protein (1), one third as caseins (2), are currently used by the pediatric nutritional community pending definitive resolution of the question.

Comparison of the primary structure of the major component of the casein fraction in mature human milk, β -casein, with the bovine homologue yields a value of 50% sequence identity (3). Both milk systems contain small amounts of proteinases possibly associated with casein micelles and fat globule membranes (4,5). Studies on the bovine γ -casein family which includes several temperature sensitive (higher solubility with decrease in temperature) minor casein proteins indicated a close relationship of these materials to β -casein. Comparison of the amino terminal sequences of these molecules with the primary structure of β -casein revealed that these γ_1 -

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γ_2^- , and γ_3^- -caseins represent successively smaller carboxyl terminal fragments of β -casein (6). Bovine γ_1^- , γ_2^- , and γ_3^- -caseins consist of residues 29-209, 106-209, and 108-209 of β -casein (6). Under a proposed new nomenclature system (7), all casein fragments would be named as portions of the parent proteins; the aforementioned γ -caseins would be referred to as β -CN B (f29-209), β -CN B (f106-209), and β -CN B (f108-209). Eigel (8) demonstrated production of these components by incubation of bovine β -casein with plasmin, which together with its zymogen has been identified in bovine and human milk (9-11). In 1970, before the aforementioned bovine β -casein plasmin cleavage was described, Schade and Reinhart (12) reported the isolation and characterization of a temperature sensitive protein from human milk whey having a molecular weight of 14,000 which they called galactothermin. In the present communication we report that plasmin cleaves human β -casein highly specifically in analogous fashion to the bovine protein, that galactothermin is a product of this proteolysis, and that this phenomena may contribute to the uncertainty in protein and casein quantitation.

MATERIALS AND METHODS

The whole β -casein fraction from mature human milk and its six components having identical amino acid compositions but differing in phosphate content from zero to five phosphate groups per molecule were isolated as described by Groves and Gordon (13). Sequence studies were carried out on the intact protein and on peptides produced by enzymatic and chemical cleavages. The complete primary structure including location of the phosphate groups is reported elsewhere (3).

Plasmin mediated proteolysis was performed by a modification of the procedure described by Eigel (8) for the bovine system. To the 37°C incubation mixture containing human β -casein-1-P (5 mg/ml) in 0.05 M tris-HCl, pH 7.4, was added 0.025 mg/ml human plasmin (Sigma¹). Aliquots (50 μ l) were removed after 0.5, 1, 4, and 8 min and mixed with equal volumes of 8 M urea containing 0.2% 2-mercaptoethanol.

The extent of digestion was monitored by disc gel electrophoresis at pH 9.6, 4 M urea (14), and by sodium dodecyl sulfate gel electrophoresis using 10% gel (15).

RESULTS AND DISCUSSION

Plasmin mediated proteolysis of human β -casein proceeds rapidly and appears to effect a highly specific cleavage. At a protein to enzyme ratio

¹Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

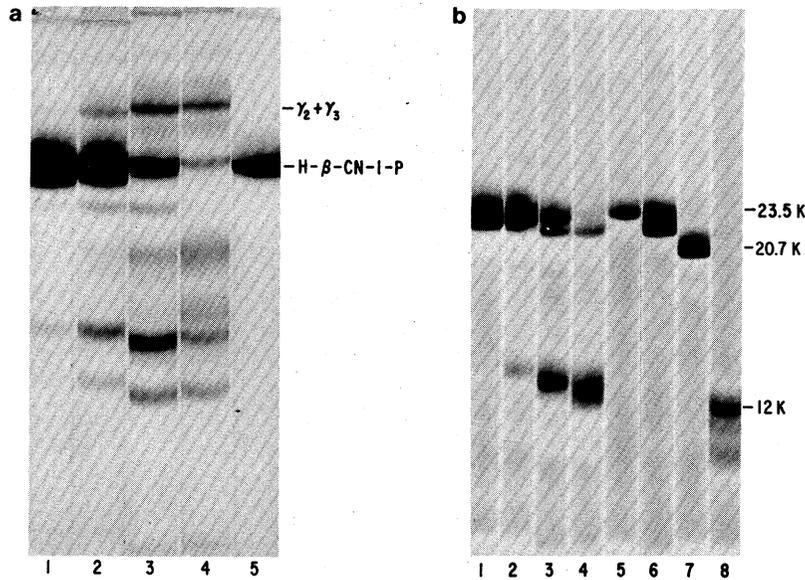


Figure 1. Disc gel electrophoresis of human β -casein-1-P incubated at 37°C with human plasmin.
 a. pH 9.6, 4 M urea
 b. SDS-10% gel
 Samples 1 through 5 on both gels are the same, (1) 0.5, (2) 1, (3) 4, (4) 8 min incubation, (5) human β -casein-1-P, (6b) bovine β -casein B, (7b) γ_1 -casein, (8b) $\gamma_2 + \gamma_3$ -caseins.

of 200:1 the alkaline gel electrophoretic patterns (Fig. 1a) clearly indicate the disappearance of the parent protein and concomitant appearance of new bands in less than 1 min. The 37°C digestion mixture became increasingly turbid in the same time period confirming the release of temperature sensitive fragments. The γ -casein-like components have comparable mobilities to the equivalents formed in the bovine system, although the latter are not shown on the pH 9.6 gel. The human $\gamma_2 + \gamma_3$ -equivalent band represents the major fragment as judged by staining intensity. The faster moving bands at pH 9.6 may comprise the amino terminal portions of β -casein released during plasmin cleavage which in the bovine system have been identified as components of the proteose-peptone fraction (16,17). The SDS-gel (Fig. 1b) which also indicates rapid formation of fragments, includes bovine β -, γ_1 -, and $\gamma_2 + \gamma_3$ -caseins for molecular weight comparisons. The human β -casein digestion products appear to be of slightly higher molecular weights than their bovine counterparts.

Table 1
Amino Acid Composition (Residues/Mole)

$T_2 + T_3^a$	Galactothermin ^b	$T_2 + T_3^a$	Galactothermin ^b		
Asp	6	6.0	Met	2	1.8
Thr	5	3.3	Ile	6	5.3
Ser	4	1.5	Leu	19	17.6
Glu	18	16.4	Tyr	2	2.5
Pro	27	23.7	Phe	2	1.9
Gly	-	0.5	Lys	3	2.8
Ala	4	4.7	His	3	2.6
Val	11	11.5	Arg	1	1.6
			Try	1	1

^aFrom sequence, Ref. (3).

^bData of Schade and Reinhart (12) recalculated based on Asp = 6.0, 16 hr hydrolyzate.

Recently Monti and Jolles (18) reported on another temperature sensitive human milk whey protein, also referred to as "galactothermin," which they concluded was intact zero-phosphate β -casein. Increased solubility of this form during casein precipitation at pH 4.6 allows its distribution between the whey and casein fractions.

In addition to an active plasmin system, an enzyme with chymotryptic-like activity has been reported in human milk (19). One study concerned with the variation of casein from individual milk samples (20) reported that on occasion no pH 4.6 precipitate was evident and gel patterns of these milks showed the absence of β -casein bands and the presence of lower molecular weight peptides. During many such studies, human milk was allowed to stand either at 37°C, room temperature, or 4°C for extended periods of time (1 to 24 hr). Now that the existence of endogenous protease activity (plasmin and others) is apparent, this may be a partial reason for the large variations reported in casein content. It is likely that this substantial protease

activity plays a beneficial role for the infant in enhancing digestibility, but it must be considered in casein quantitation or isolation of components.

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