Buffalo α S_1-casein; its terminal residues and amino acid composition

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Summary. Buffalo αS_1-casein was treated with carboxypeptidase-A to determine the C-terminal amino acid residue, which was - leucine-tryptophan. The Sakaguchi reagent was used to confirm arginine as the N-terminal amino acid. The colored p-phenylazophenyl thiohydantions of tryptophan and arginine released from C- and N-terminals respectively were quantitatively estimated and from this molecular weights of buffalo αS_1 casein were calculated and found to be 26,300 and 26,100 respectively.

Casein is a major component of milk and is heterogeneous in nature. It is precipitated at pH 4.8. Different constituents of buffalo milk casein have already been identified[3] and a standard procedure to isolate pure buffalo αS_1-casein has been reported[4]. A modified and convenient technique[5] to determine the sequence of the first 15 N-terminal amino acids of buffalo αS_1-casein has also been developed[6]. In this communication the C-terminal amino acid and the amino acid composition of buffalo αS_1-casein are described. The Sakaguchi reagent[7] was used to confirm the N-terminal amino acid.

Materials and methods. Buffalo milk (Murrah variety) was collected from Haringhata Dairy Farm, West Bengal. Casein was precipitated from skimmed milk at pH 4.8. Homogeneous αS_1-casein was prepared according to Sengupta et al.[4]. Homogeneity was checked by polyacrylamide gel electrophoresis[8] using Tris-glycine buffer at pH 8.3. αS_1-casein was suspended in water, and carboxypeptidase-A solution in 0.2 M N-ethylmorpholine acetate buffer (pH 8.5) was added in the ratio of enzyme:casein/1:100 (w/w) in presence of 0.001 M diisopropylflurophosphate. The mixture was incubated at 37 °C. Aliquots of 1 ml reaction mixture were pipetted out at definite intervals of time and HCl was added to bring down the pH to 2.5 when the excess enzyme is destroyed. The released amino acids present in the solution were converted into p-phenylazophenyl thiohydantoins (PAPTH) and identified by TLC on silica gel plates[10,11] to determine the C-terminal end.

Buffalo αS_1-casein was treated with p-phenylazophenyl isothiocyanate (PAPITC)[5] to determine the N-terminal amino acid. The result was confirmed by reacting the colored end product with Sakaguchi reagent[7]. The PAPTH-amino acids released from C- and N-terminal ends were scraped carefully from their respective thin layer chromatograms and transferred to stoppered centrifuge tubes with 4 ml ethyl acetate. The mixture was centrifuged. The absorbances of the colored supernatants were read at 590 nm and the amino acid concentrations were calculated from the standard curve.

Standard amino acids the same as those identified as C- and N-terminal residues were added to the reaction mixture prior to enzymatic degradation and PAPITC addition to determine the recovery percentage. Buffalo αS_1-casein (Murrah variety) and electrophoretically pure bovine αS_1-casein (prepared according to Thompson et al.[12]) from Red Sindhi cow milk were hydrolyzed separately with 6 N HCl at 110 °C for 24 h to determine the amino acid composition. Hydrolysates were analyzed on a Beckman Multichrom Liquid Column Chromatograph 4255 automatic amino acid analyser. Cystine and cysteine were estimated according to Schram et al.[13] and Bailey et al.[14] respectively. Tryptophan was determined spectrophotometrically[15].

Results. Kinetic analysis of amino acids liberated from the C-terminal end was conducted. Tryptophan was released first, followed by leucine (fig.). Under the experimental conditions used tryptophan was completely released at a much higher rate than that of leucine. Released amino acids were also treated with 1-dimethyl aminonapthalene 5-sulfonyl (DNS) chloride and identified as DNS derivatives[16] to confirm our results.

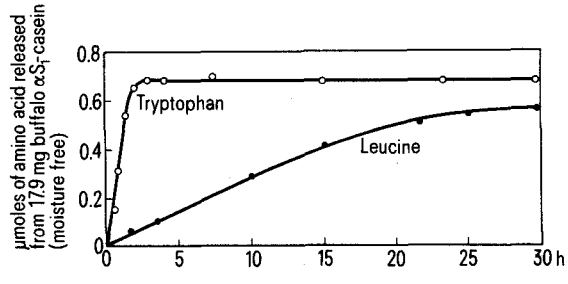
It has already been reported[6] that arginine is the N-terminal amino acid of buffalo αS_1-casein. The modified Edman degradation technique[17,18] of Fraenkel-Conrat[19] was adopted to confirm this result. Tryptophan and arginine were further identified by specific color reactions[20,21]. Moreover, the color intensity of red PAPTH-arginine was increased when Sakaguchi reagent[7] was added to it.

The amino acid compositions of bovine and buffalo αS_1-caseins are tabulated in the table. Cystine and cysteine were absent.

Recovery percentages of tryptophan and arginine added to buffalo αS_1-casein were 83.6 and 88.2 respectively. Release of amino acid residues from the protein chain was assumed to be 100%.

Amino acid compositions of bovine and buffalo αS_1-casein

Amino acid	Observed values of αS_1-casein	
	Bovine (residues, mol. wt 28,000)	Buffalo (residues, mol. wt 26,300)
Lysine	19.2	18.6
Histidine	6.1	6.8
Arginine	5.4	4.3
Aspartic acid	16.1	16.4
Threonine	6.9	7.8
Serine	18.5	16.9
Glutamic acid	44.3	45.1
Proline	19.9	20.6
Glycine	10.2	9.3
Alanine	9.3	7.2
Valine	12.3	11.5
Methionine	5.4	5.8
Isoleucine	12.8	11.4
Leucine	16.3	15.1
Tyrosine	13.8	14.2
Phenylalanine	7.9	7.2
Tryptophan	2.2	2.5



Rates of amino acid release from buffalo αS_1-casein by the action of carboxypeptidase-A. Ratio of enzyme to casein is 1:100 (by weight); pH 8.5 and temperature = 37 °C.

Molecular weights of buffalo αS_1-casein calculated separately on the basis of tryptophan and arginine contents were found to be 26,300 and 26,100 respectively. Here buffalo αS_1-casein was assumed to be a single polypeptide chain[22].

Discussion. Different techniques showed that the carboxyl terminal amino acid residue of buffalo αS_1-casein is leucine-tryptophan. It was also observed that tryptophan was completely released from the C-terminal end under present experimental conditions. It is assumed that 1 mole of amino acid is released per mole of protein and thus the molecular weight of buffalo αS_1-casein was calculated from the data on terminal amino acids. Molecular weights determined on the basis of tryptophan and arginine contents were in reasonable agreement. The molecular weight of buffalo αS_1-casein by ultracentrifugal analysis[23] is reported to be 25,900 which agrees quite well with our value.

Primarily we identified tryptophan, leucine and arginine as their PAPTH derivatives. As amino acid PAPTHs are colored[5], visual identification of these compounds on thin layer chromatograms were convenient. Moreover, the percentage recoveries of these 3 amino acid derivatives were higher than those of others, particularly DNP (2,4 dinitrophenyl)[24] or DNS derivatives.

Although the C-terminal amino acid residues of both buffalo αS_1 and bovine αS_1-caseins are - leucine-tryptophan, the rates of release of these 2 amino acids from the carboxypeptidase-A treated casein differed. It is reported[25] that the concentration of leucine released from bovine αS_1-casein was higher than that of released tryptophan after 5 h, and traces of tyrosine, phenylalanine and valine were also released within 22 h. On the other hand, the concentration of leucine released from buffalo αS_1-casein did not overlap the tryptophan concentration within 30 h, and also no other amino acid was released during this period. The amino acid composition of buffalo αS_1-casein was in reasonable agreement with that of bovine αS_1-casein. Amino acid residues per mole of bovine αS_1-casein was calculated on the basis of a mol. wt value of 28,000[26]. The red color of PAPTH-arginine[5] was intensified by reaction with Sakaguchi reagent[7]. The procedure was also useful to distinguish PAPTH-arginine from PAPTH-lysine, which has the same mobility in some solvent systems.

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Detection of gamma-carboxy glutamic acid residues in an extract of cock spermatozoa

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Summary. In a dialyzed extract of cock spermatozoa - containing proacrosin, a precursor of a trypsin-like endopeptidase - gamma-carboxy glutamic acid residues (Gla) were detected. Such residues are known to exist in several zymogens of vitamin K-dependent serine proteases.

Although the biological activity of vitamin K in the blood clotting system has been known for more than 40 years[3], its molecular function in this process was elucidated only recently.

With prothrombin as an example it was shown that in a post-translational step, which is dependent on the presence of vitamin K and bicarbonate ions, specific glutamic acid residues positioned in the N-terminal part of this protein are converted to gamma-carboxy glutamic acid residues (Gla residues) (Suttie and Jackson[4]). Later on, Gla was detected in factors VII, IX and X[5,6] as well as in other proteins not related to blood coagulation[6,7].

All vitamin K-dependent blood clotting factors are zymogens of proteolytic enzymes belonging to the class of serine-proteases. In addition a vitamin K dependence could be demonstrated for the activities of other serine-proteases such as trypsin, chymotrypsin[8] and collagenase and elastase[9].

Acrosin, a trypsin-like enzyme occuring in sperm cells, where it is stored as an inactive precursor called proacrosin, belongs to the same class of endopeptidases. Its activity in cock sperms was shown to be reduced after chronic marcoumar treatment and subsequently to be restored after application of vitamin K_1[10].