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SHORT COMMUNICATION

α_{s1} -CASEIN A (*BOS TAURUS*): A PROBABLE SEQUENTIAL DELETION OF EIGHT AMINO ACID RESIDUES AND ITS EFFECT ON PHYSICAL PROPERTIES

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Abstract—1. The major casein of cow's milk (α_{s1} -) occurs in four known polymorphic forms, A, B, C and D.

2. α_{s1} -Casein A is devoid of eight amino acid residues (apparently in sequence) which are present in the more common variants, B and C.

3. α_{s1} -A is soluble in calcium chloride solutions above 0.10 M at 1°C while the B and C variants are insoluble.

4. The increased solubility of the A variant appears to have arisen as a result of either less extensive hydrophobic bonding and/or a conformational change of the protein brought about by the deletion of the nonpolar amino acids alanine, valine, two phenylalanines and three leucines and one polar amino acid, arginine.

INTRODUCTION

PRACTICALLY all of the caseins and whey proteins of different species of lactating mammals exhibit genetically variable forms. Detailed genetic information is more abundant, however, on the milks of *Bos taurus* and *Bos indicus* cattle than on any other species studied to date. Aschaffenburg (1961) has shown the caseins to be complex. β -Caseins, for example, exist in at least six electrophoretically distinguishable forms, A¹, A², A³ (Peterson & Kopfler, 1966), B, C and D, the latter of which has been found only in *Bos indicus* (Indian Deshi breed and African Boran Zebu cattle) (Aschaffenburg *et al.*, 1968).

κ -Casein exists in two forms, A and B (Neelin, 1964), while the major caseins, α_{s1} -, which comprise 55 per cent of the total casein, exist in two principal forms, B and C, in Eastern and Western cattle with A occurring in Holstein (Thompson *et al.*, 1962) and perhaps Danish RDM (Thymann & Larsen, 1965) and D in French Flamande (de Koning & van Rooijen, 1967).

The A variant of the α_{s1} -series has been of particular interest not only because of its apparent limited occurrence in certain breeds of cattle, but because of its unusual physical properties as compared to the common B and C (and presumably

less common D) variants. It is the purpose of this communication to report some of the differences in chemical-physical properties of α_{s1} -casein A as compared to α_{s1} -B.

MATERIALS AND METHODS

Preparation of α_{s1} -caseins

The α_{s1} -caseins were prepared by the method of Thompson & Kiddy (1964) and purified by column chromatography on DEAE-cellulose, pH 7.0, imidazole-HCl buffer, 3.3 M urea in the presence of 2-mercaptoethanol (Thompson, 1966).

Amino acid composition and peptide fingerprinting

The amino acid composition of the α_{s1} -caseins was determined as described in detail by Gordon *et al.* (1965). Chymotryptic digestion of α_{s1} -caseins was carried out at pH 8.0 at a protein-enzyme ratio of 100 : 1. Digests were lyophilized and fingerprinting was accomplished by high-voltage electrophoresis followed by ascending chromatography of the peptides as described by Kalan *et al.* (1966).

Calcium solubility of α_{s1} -casein A

Calcium solubility of α_{s1} -casein A was carried out at temperatures of 0, 5, 15, 25 and 30°C at CaCl₂ concentrations of 0, 0.05, 0.10, 0.20, 0.30 and 0.40 M. To 20 mg of α_{s1} -casein A, dissolved in 2 ml of deionized water at pH 7.0, was added 2 ml of CaCl₂ solution at twice the desired CaCl₂ concentration at the appropriate temperature. After equilibration for 30 min the solutions were centrifuged at 43,800 g for 15 min in a swinging bucket rotor. Supernatant protein, representing the soluble casein, was determined by absorption at 2800 Å.

Gel electrophoresis of caseins

Polyacrylamide-gel electrophoresis of caseins, carried out at pH 9.1, Tris-citrate-borate buffer, 4.5 M urea, was used for phenotyping of most caseins (Aschaffenburg *et al.*, 1968).

Ultracentrifugation

Sedimentation velocity experiments were carried out in a Beckman Model E ultracentrifuge at 59,780 rev/min and 3°C in a Kel-F, 4° sector cell.

RESULTS AND DISCUSSION

Figure 1 shows polyacrylamide-gel electrophoresis of α_{s1} -caseins A, B and C and a mixture of A and B at pH 9.1, 4.5 M urea. The A variant also migrates faster than the B and C variants (which are not resolved) in acid buffer at pH 3.0, 4.4 M urea; an observation which was unexpected. Semi-quantitative densitometry of polyacrylamide-gel electrophoresis strips indicates that homozygous α_{s1} -casein A constitutes only 40–45 per cent of the total casein in the milk as compared to about 55 per cent for the B and C variants. In the heterozygotes A/B and A/C the ratio of A to B or C is about 40 : 60.

The B variant of the α_{s1} -series clearly differs from the C variant by a glu/gly substitution involving the probable coding triplets, GA^{A/G}/GG^{A/G}, while the D variant (seen only in Western Europe to date) differs from B by a pro/ser substitution (coding triplets, CCA/UCA) (de Koning & van Rooijen, 1967). A difference in no less than ten amino acid residues exists between the A and B variants: A

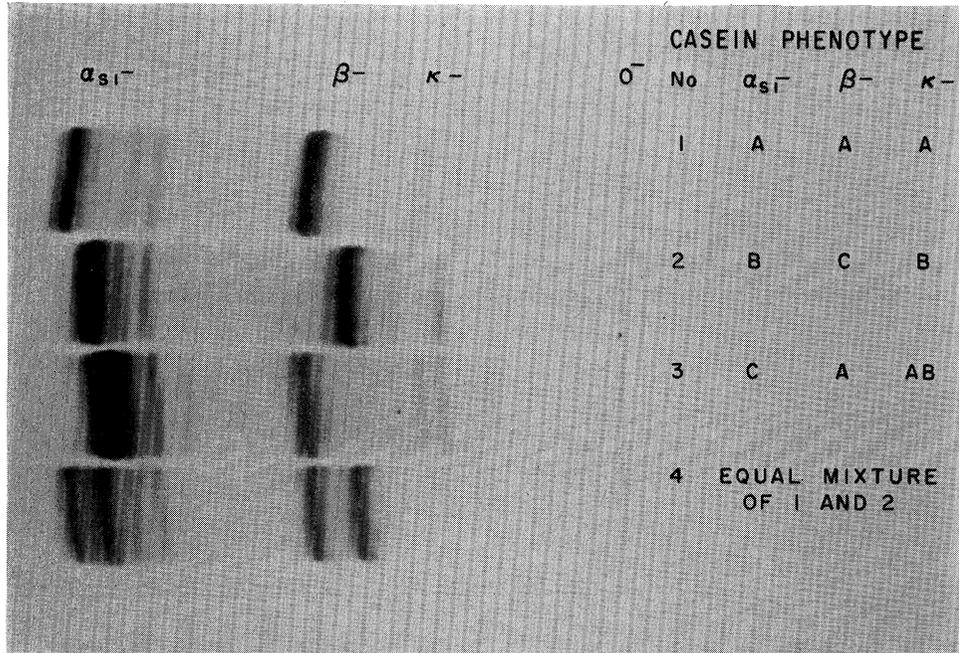


FIG. 1. Polyacrylamide-gel electrophoresis of whole caseins at pH 9.1, 4.5 M urea.

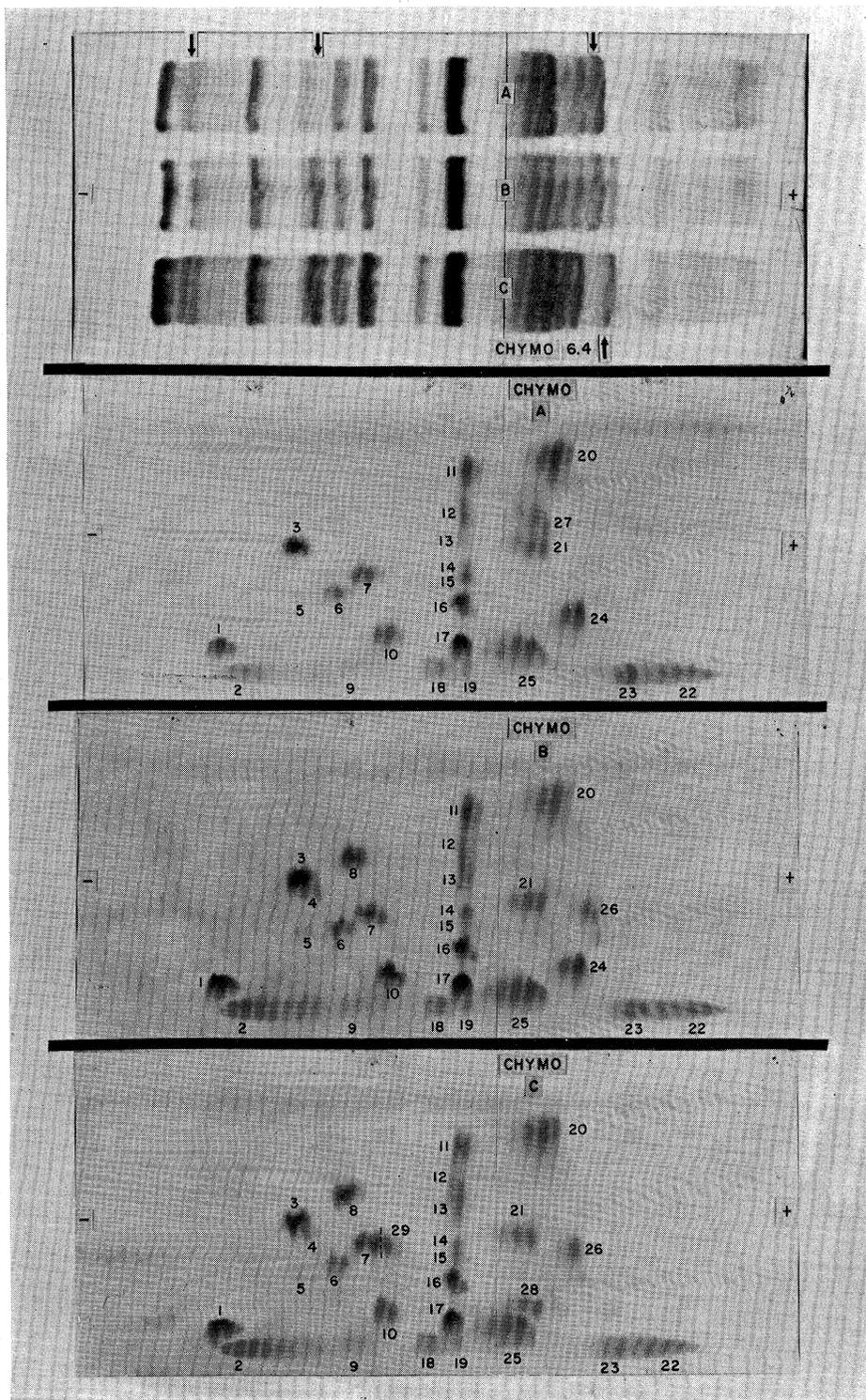


FIG. 2. Two-dimensional high-voltage electrophoresis (pH 6.4) and ascending chromatography of chymotryptic digests of α_{s1} -caseins A, B and C (Kalan *et al.*, 1966).

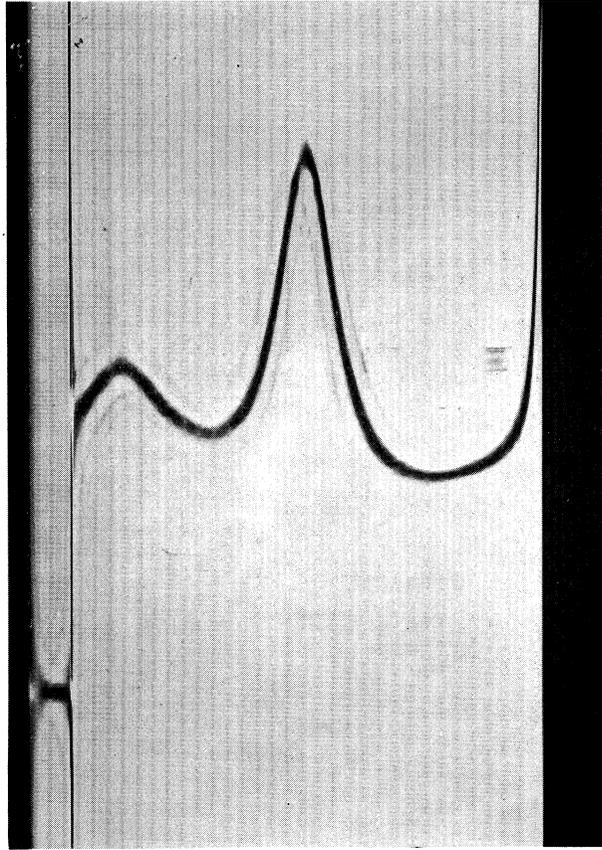


FIG. 3. Sedimentation diagram of α_{s1} -casein A in 0.15 M CaCl_2 , 0.01 M imidazole-HCl buffer, pH 7.0, 1.5% protein. Runs were made at 3°C, 59,780 rev/min. Photograph taken 80 min after up-to-speed.

contains one more lysine, but one less each of arginine, aspartic acid, alanine and valine, two less phenylalanines and three less leucines, or a net deletion of nine amino acid residues. That the deletion has not occurred in the terminal positions of the molecule is certain; each variant has N-terminal arginine and a leu-leu-try C-terminal sequence. Additionally, no apparent alterations in the phosphopeptide region of the caseins has occurred as evidenced by identical phosphorus contents (Thompson & Kiddy, 1964).

Fingerprints of chymotryptic digests of the A, B and C variants are shown in Fig. 2. The absence of peptide 26 in A and the appearance of peptide 27 could be explained by a mutational event involving a lys/asN substitution, probable codons, AA^{A/G}/AA^{U/C}. Three points concerning the chymotryptic peptide map of α_{s1} -A are significant: (1) the absence of peptide 8; (2) the absence of peptide 4; and (3) a decrease in stain intensity in the region of peptide 13. Isolation and analysis of peptide 8 from an α_{s1} -B digest gives a tripeptide with a sequence of arg-phe-phe. The presence of N-terminal arginine was verified by the Edman procedure. The remaining dipeptide stayed in the neutral band region upon high voltage electrophoresis. Yet to be accounted for (assuming that lys/asN are involved in one mutational event) are alanine, valine and three leucine residues. Presumably these nonpolar amino acids are in either peptide 4 or the neutral band (peptide 13) region.

Thus, with α_{s1} -casein A, it is probable that a two-step mutational event (not necessarily simultaneously) has occurred, one involving a straightforward amino acid substitution, the other involving a deletion of eight amino acid residues which are likely in sequence. This suggestion is, in fact, borne out by the essential identity of the peptide maps of α_{s1} -A and -B with the exceptions previously noted. Were the amino acid deletions distributed throughout the α_{s1} -casein A molecule, a substantial number of peptides would be expected to differ. They do not. Multiple deletions, while not common, have been reported to occur—one of the most recent by Bradley *et al.* (1967) involving hemoglobin Gun Hill (HbGH) which differs from HbA (BTpX + XI) by one less each of asp, his, leu, lys, cys in sequence, the result of which is manifested by impaired heme-globin binding. The major deletion of α_{s1} -casein A may have arisen by unequal crossing over between the closely linked α_{s1} -Cn and β -Cn genetic loci (King *et al.*, 1965).

The deletion of a largely nonpolar peptide in the α_{s1} -A molecule has resulted in structural alterations which remarkably distinguish it from the more common B and C variants. The latter two are insoluble in calcium chloride solutions in excess of 0.005 M at 1°C and remain insoluble over a broad range of calcium ion concentrations and temperatures. The A variant, however, while insoluble in CaCl₂ from 0.005 to 0.08 M, becomes soluble when the calcium ion level is increased beyond 0.10 M. Table 1 shows the solubility of the protein as a function of temperature and CaCl₂ concentrations. While the protein may be precipitated at 33°C (and 0.40 M CaCl₂), it is readily resolubilized by cooling. The protein, therefore, behaves much like a β -casein which, when warmed to 18°C in the presence of calcium, readily precipitates, an effect which is reversed upon cooling.

Because of its solubility in CaCl_2 solutions (Table 1), α_{s1} -casein A may be studied by ultracentrifugation in the presence of calcium ions at low temperature (2–4°C). Figure 3 illustrates the sedimentation behavior of the protein at a concentration of 1.5% in 0.15 M CaCl_2 , pH 7.0, 3°C. The major peak ($S_{20} = \sim 6$) illustrates that the protein remains, for the most part, in a highly aggregated form; the slower moving component ($S_{20} = 1$) is presumably monomeric α_{s1} -A. The slow component persists at 0.40 M CaCl_2 . Sedimentation velocity of whole casein (α_{s1} -type A), under the above conditions, indicates a faster major peak (the α_{s1} -A, κ -casein complex which is not disrupted by Ca^{2+}) and slower moving β -casein. Ultracentrifugal examination of an equal mixture of α_{s1} -A and κ -casein, in 0.40 M CaCl_2 , shows that a portion of the α_{s1} -A and κ -casein have formed a complex (asymmetric peak with much boundary spreading) which is stable even when the temperature is elevated to 40°C. With the α_{s1} -B and -C variants to which κ -casein had been added a precipitate would form in the presence of CaCl_2 at 40°C.

TABLE 1—SOLUBILITY OF α_{s1} -CASEIN A IN CaCl_2 AT VARIOUS TEMPERATURES

Temp. (°C)	CaCl_2 (moles/l)					
	0	0.05	0.10	0.20	0.30	0.40
0	S ¹	I ²	S	S	S	S
5	S	I	I	S	S	S
15	S	I	I	S	S	S
25	S	I	I	I	S ³	S ³
30	S	I	I	I	I	S ³

1. Denotes soluble. 2. Denotes insoluble. 3. Turbid solution.

Lastly, naturally occurring casein micelles (in individual cow's milks) containing the α_{s1} -casein A variant differ in physical behavior from micelles formed by the more common B and C variants. Micelles, harvested by centrifugation of α_{s1} -A containing milks, are solvated at a level of 1.4–1.6 g water/g protein, whereas the solvation of micelles harvested from milks containing the more common variants averages 1.9 g water/g protein.

Clearly the deletion of a large segment of principally nonpolar amino acid residues has resulted in substantial differences in physical behavior of the α_{s1} -A variant. The deletion of these amino acid residues may reduce the extent of hydrophobic bonding and/or alter protein conformation in solution. Extensive details will be communicated in a subsequent publication.

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