

A genetic and biochemical analysis of a polymorphism of bovine α_{s2} -casein

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SUMMARY. Using gel electrophoresis a genetic polymorphism of α_{s2} -casein (Cn) was discovered in individual milk samples from 2 bovine breeds of the eastern part of France (Vosgienne and Montbéliarde). The 3 observed phenotypes (Plate 1) are determined by 2 co-dominant alleles at an autosomal locus. The α_{s2} -Cn A variant was the only one known up to now in European breeds (reference variant) and α_{s2} -Cn D is a new variant, whose bands overlap the β -casein A band at pH 8.6, and migrate faster than α_{s2} -Cn A at pH 3.0.

The sequence of the polypeptide chain α_{s2} -Cn D differs from that of α_{s2} -Cn A by the deletion of a very acidic nonapeptide, which includes a cluster of 3 phosphoserine residues. Due to the characteristics of the reference sequence, this deletion cannot be exactly located but it involves residues 50-58, or 51-59, or 52-60.

A genetic analysis shows that locus α_{s2} -Cn is closely linked to the cluster α_{s1} -Cn- β -Cn- κ -Cn. The 4 casein species are thus synthesized by 4 closely linked loci.

The first example of genetic polymorphism of α_{s2} -casein (Cn) was discovered and investigated recently by Grosclaude *et al.* (1976) in the milk of yaks, cattle and zebus bred in a high Nepalese valley. In addition to the usual or classical variant, α_{s2} -Cn A, 2 other variants were found: α_{s2} -Cn B in cattle (*Bos taurus*) and zebus (*Bos indicus*), and α_{s2} -Cn C in yaks (*Bos grunniens*). Although no family data were available, there were indications of a possible linkage of the α_{s2} -Cn locus to the cluster α_{s1} -Cn- β -Cn- κ -Cn. The α_{s2} -Cn C variant was later found in milk from yaks from the Republic of Mongolia (Grosclaude, unpublished), and the α_{s2} -Cn B in milk from zebus from the Republic of South Africa (Guérin, personal communication).

In this communication, the first example of genetic polymorphism of α_{s2} -Cn found in European breeds is described and investigated.

METHODS AND RESULTS

Description and genetic analysis of the polymorphism

The polymorphism was first discovered in the Vosgienne breed where 3 phenotypes were found (Plate 1). Of 227 individual milk samples, the respective numbers of each phenotype were: I, 189; II, 36; III, 2. Since type I is the homozygous type for the usual variant α_{s2} -Cn A, type III was possibly the homozygous type for a new variant α_{s2} -Cn D, and type II, the heterozygous type. In this hypothesis the frequency of allele α_{s2} -Cn^D in the Vosgienne sample is 0.088, and the observed phenotypic proportions fit exactly those expected according to the Hardy-Weinberg law. In the

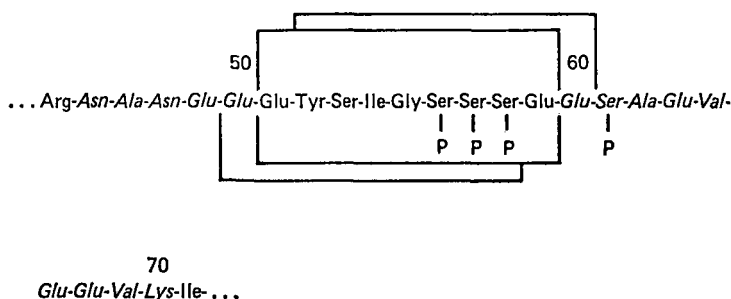


Fig. 1. Localization of the deletion found in variant α_{82} -Cn D. The reference sequence is that of α_{82} -Cn A (Brignon *et al.* 1977); only the region with the deletion is reproduced. The sequence in italics is of peptide α_{82} -Cn T4 b2 established by manual Edman degradation. The deletion is framed (3 alternatives).

Montbéliarde breed, of 798 cows only 780 type I and 18 type II were found. In this case the frequency of α_{s2} -Cn^D is 0.011; the phenotypic proportions also fit those expected according to Hardy-Weinberg, the absence of type III resulting from the low frequency of α_{s2} -Cn^D. In addition, in the progeny of 3 heterozygous bulls, alleles α_{s2} -Cn^A and α_{s2} -Cn^D were transmitted to 20 and 22 offspring respectively, i.e. not differing statistically from a 50:50 ratio. The polymorphism of α_{s2} -Cn described here thus appears to be controlled by 2 co-dominant alleles at an autosomal locus, α_{s2} -Cn.

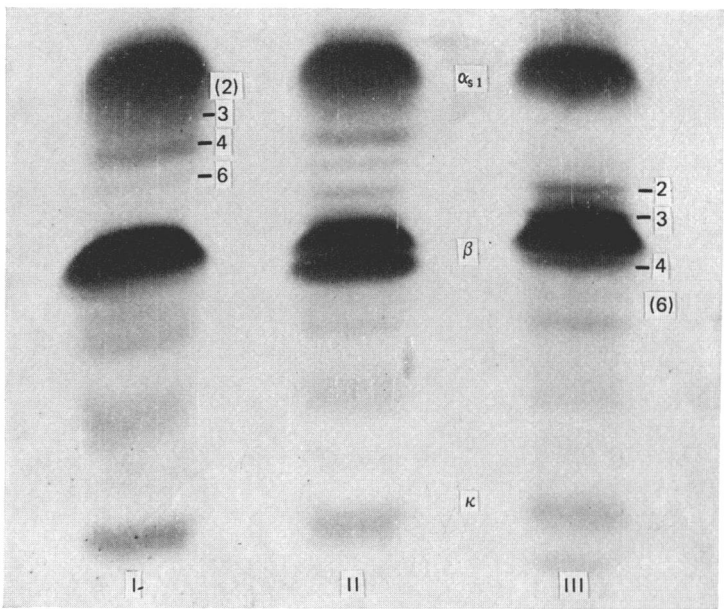
Biochemical study of variant α_{s2} -Cn D

Variant α_{s2} -Cn D was prepared using whole casein from the milk of a cow homozygous for α_{s2} -Cn^D (type III). Whole casein was dissolved in 6.6 M-urea at pH 4.9 and then diluted to 3.3 M-urea (Thompson & Kiddy, 1964). In these conditions band 3 (Plate 1) is found with β -casein in the supernatant, but bands 2 and 4 precipitate with α_{s1} - and κ -caseins: these bands were purified on a DEAE-cellulose column according to Mercier *et al.* (1968).

CNBr and tryptic peptides were purified from α_{s2} -Cn D (band 4) and their amino acid compositions were compared with those of the homologous peptides of variant α_{s2} -Cn A, whose complete sequence is known (Brignon *et al.* 1977). The N-terminal sequence of α_{s2} -Cn D, and the sequence of peptide CN1 were established using automatic Edman degradation. The only difference was found in peptide CN5. In this part of the chain, peptide T1 of α_{s2} -Cn A is replaced by peptide T4 b2 whose sequence was completely elucidated by manual Edman degradation. This peptide differs from peptide α_{s2} -Cn A T1 by a deletion of 9 residues, including 2 Glu and a cluster of 3 SerP. Due to peculiarities of the amino acid sequence in this part of the casein chain, this deletion cannot be located unambiguously but it involves positions 50–58, or 51–59, or 52–60 (Fig. 1).

Linkage of the α_{s2} -Cn locus to the cluster α_{s1} -Cn- β -Cn- κ -Cn

Allelic segregations were studied in the progeny of a bull heterozygous at the α_{s2} -Cn, α_{s1} -Cn and κ -Cn loci, and of another bull heterozygous at the α_{s2} -Cn, β -Cn and κ -Cn loci. Among 49 daughters of these bulls, only 2 allelic combinations were transmitted instead of the 4 expected in the case of genetic independence. The locus α_{s2} -Cn is thus closely linked to the cluster α_{s1} -Cn- β -Cn- κ -Cn. In both breeds a strong



linkage disequilibrium was observed, the main combination with α_{s2} -Cn^D being α_{s1} -Cn^B- α_{s2} -Cn^D- β -Cn^{A2}- κ -Cn^B. The 4 casein species are thus synthesized by 4 closely linked loci.

DISCUSSION AND CONCLUSIONS

Variant α_{s2} -Cn D is, after α_{s1} -Cn A (Grosclaude *et al.* 1970), the second example of a casein with a deletion. In this case, the deleted peptide is a very acidic one, including 2 glutamyl and a cluster of 3 phosphoseryl residues. Such a peptide is found twice in the reference sequence of α_{s2} -Cn. It is also found in α_{s1} - and β -Cn. The observed deletion is another example of the genetic instability of this peptide in caseins.

The physico-chemical properties of variant α_{s2} -Cn D were not investigated; nevertheless, observations made in preparing α_{s2} -Cn fractions indicate possible differences in at least some properties of α_{s2} -Cn A.

The genetic analysis of this α_{s2} -Cn polymorphism found in France shows that locus α_{s2} -Cn is closely linked to the cluster α_{s1} -Cn- β -Cn- κ -Cn. All 4 bovine casein species are thus synthesized by a cluster of 4 closely linked genes.

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EXPLANATION OF PLATE

The 3 phenotypes of α_{s2} -casein (Cn) found in the Vosgienne breed. I, α_{s2} -Cn^A/ α_{s2} -Cn^A (common or reference type); II, α_{s1} -Cn^A/ α_{s2} -Cn^D; III, α_{s2} -Cn^D/ α_{s2} -Cn^D. Nomenclature of α_{s2} -Cn bands of type I according to Annan & Manson (1969); the bands assumed to be homologous in type III are given the same nomenclature. Starch-gel electrophoresis at pH 8.6 according to Wake & Baldwin (1961), with addition of 2-mercaptoethanol.