Sequence variations in genes coding for bovine β-lactoglobulin and αS_1-casein

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ABSTRACT

The techniques involved in searching for bovine milk protein variants by the direct sequencing of genes are discussed. Examples are drawn from our studies of two major milk protein genes, β-lactoglobulin and αS_1-casein. In the former case it was possible to subdivide the A and B variants even further according to point mutations found in the promoter region of the gene, while in the latter case, a New Zealand αS_1 casein type A variant in Friesians was shown to arise from a point deletion in the gene which differs significantly from a mutation reported to give rise to the same type A variants in the German Red breed. The potential of this analytical approach for analysing milk protein genes is discussed.

Keywords: bovine; milk protein variants; genes; DNA sequencing.

INTRODUCTION

Several variant forms of virtually all of the major milk proteins have been found in the dairy cow. Some of these variants are very common, others are much rarer. Thus, for a protein such as β-lactoglobulin, there are two common variants (A and B) in the New Zealand herd while a third (C) variant is quite rare. The same is true of αS_1 -casein for which the B and C variants are common and the A variant rare. Once considered somewhat of an academic curiosity, specific variant proteins are now believed to have properties which enhance the value of milk in specific processing applications.

We have used molecular techniques to examine the genes which code for these variant proteins with the aim of further characterising subtle variations at both the gene expression and amino acid level.

MATERIALS AND METHODS

For β-lactoglobulin, straws of frozen semen from bulls previously shown to be homozygous for either the A variant or B variant gene (Wilkins and Kuys, 1992) were supplied by the Livestock Improvement Corporation Inc. and DNA was extracted according to the method of Lien *et al* ., (1989). The regions of milk protein genes of interest were amplified using standard polymerase chain reaction (PCR) conditions with the primers shown in Table 1. Manual sequencing using a Sequenase kit was used either directly on the PCR products or after subcloning into a Bluescript vector. For the αS_1 -casein work, blood samples were supplied from cows that the Dairy Research Insitute had analysed as having A/A αS_1 -casein milk. The DNA was isolated from leukocyte nuclei using standard proteinase K and phenol protocols (Sambrook *et al*., 1989).

TABLE 1: PCR Primers used in this study.The βLG primers amplify a 600bp segment in the promoter region of the βLG gene and the αS_1 primers a 214 bp region spanning exon 4 of the αS_1 gene.

LG	Forward primer	5' TTTTGCTACCCTAACTGGGCAGC 3'
LG	Reverse primer	5' GCAAGCAGGAGGCACTTCAT 3'
αS_1	Forward primer	5' CTCCTTTTCTGACTGTGTTTTTCAC 3'
αS_1	Reverse primer	5' GTGGTTGCTTGGGTGAGTAAATGAA 3'

RESULTS AND DISCUSSION

The promoter regions of the β-lactoglobulin gene from 12 A or B alleles were sequenced. One restriction enzyme sequence polymorphism was found which was tightly linked to the B genotype, a *Rsa* 1 site. This site has proved the basis of a useful alternative PCR test for A and B genotypes of β-lactoglobulin as it is in a region of the gene which is more amenable to analysis than the regions of the gene in which the two amino acid point mutations occur (Wilkins and Kuys, 1992). Additionally, a number of other DNA sequence variations were identified that correlated with the A or B genotype some of which were in regions presumed to bind transcription factors. The significance of these last variations are yet to be established.

In the case of the αS_1 -casein gene, only one A variant is known in New Zealand, so the aim of the investigation was purely to detect, at the DNA level, the mutation which gave rise to the truncated αS_1 -casein protein which has lost 13 amino acids due to the deletion of exon 4 (39 nucleotides) from the mRNA (McKnight *et al*., 1989). Analysis of PCR fragments clearly showed that the αS_1 -casein A gene was the same size as the B gene (data not shown) indicating that exon 4 is still present in the gene but has been skipped during mRNA processing. When this region of the A gene was sequenced and compared with the B gene, only one base change was seen - the loss of an A residue 3 bases into

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the splice donor site of intron 4 (Table 2b). Loss of this base destroys the splice donor consensus sequence and, without a doubt, would prevent exon 4 being recoginised as a *bona fide* exon. In otherwords, during processing, the entire intron 3 - exon 4 - intro 4 segment of DNA will be processed out of the mRNA. It is of interest to contrast this mutation with that found in a German Red αS_1 -casein A variant (Mohr *et al*., 1994, see Table 2c). In this case a point mutaton (T -> A) occurs at position 6 of the splice donor sequence and this would be expected to have a minor effect on mRNA processing. Nevertheless, this minor mutation does seem sufficient to cause exon skipping although it is not clear if some normal length mRNA is also processed in this case. Clearly, there could be a range of different αS_1 -casein mutations which all appear as phenotypically identical A variants at the protein level.

TABLE 2: Variations in the sequence of αS_1 -casein genes at the exon 4 - intron 4 boundary.

a. Variant B (Normal)

exon 4 intron 4

.....*ttttgtggca* gtaagtattatctacttcttcttcaatgacaa.....

b. Variant A (DRI)

exon 4 intron 4

.....*ttttgtggca* gt_agtattatctacttcttcttcaatgacaa.....

c. Variant A. (German Red)

exon 4 intron 4

.....*ttttgtggca* gtaagaattatctacttcttcttcaatgacaa.....

CONCLUSIONS

The two examples discussed in this paper clearly demonstrate that while relatively few variations are detected in milk proteins when milk itself is analysed, there are considerably more variations at the DNA sequence level. As well as potentially providing more genetic information on various sires, these DNA investigations also offer valuable insights into how various DNA mutations can influence gene expression.

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