LINKAGE OF THE FOUR BOVINE CASEIN GENES AS DEMONSTRATED BY PULSED FIELD GEL ELECTROPHORESIS

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SUMMARY

Pulsed field gel electrophoresis was used to demonstrate the association of the genes for the κ, α_{s1}, α_{s2}, and β bovine caseins. All the four genes lie on a single SalI fragment of about 600 Kb and on two adjacent XhoI fragments: the κ and α_{s2} reside on the larger, 210 Kb fragment, the β and α_{s1} on the smaller, 120 Kb fragment. The analysis of hybridization patterns generated with XhoI, ClaI, SmaI indicate the order κ, α_{s2}, β, α_{s1}, and suggest that they are located on a stretch of approximately 330 Kb.

INTRODUCTION

The caseins represent the major protein component in the milk of most animal species. More than 20 polypeptides can be identified in whole milk that are the primary translation products of four genes, α_{s1}, α_{s2}, β, and κ. Both post-translational modifications and genetic variation account for this abundance. Genetic variants of the caseins have been described and the pattern of inheritance of alleles has demonstrated that all the four genes are linked in a cluster, Grosclaude et al. (1973), Grosclaude et al. (1979). Recently the localization of the casein genes has been reported by in situ hybridization to chromosome 6, Womack et al. (1989). In this paper pulsed field gel electrophoresis was used to demonstrate the physical linkage of the casein genes and to study their order and spacing in the locus.

MATERIALS AND METHODS

Cells: Fibroblasts from bovine kidney (RBE) were cultured in Ham's F12 medium supplemented with 20% fetal calf serum.
Genomic DNA preparation and digestion: High molecular weight DNA in agarose plugs was prepared from RBE cells at a concentration of 10^7/ml, Anand and Southern (1989). Individual plugs (0.1 ml) contained approximately 10 ug of DNA. For the digestions, plugs were equilibrated overnight at 4°C in 20 fold excess of TrisCl 10 mM EDTA 1 mM (TE) and then each plug was washed 3 times in 10 fold excess of TE, on ice, for 30 min., with gentle shaking. 1/2 plugs (5 ug DNA) were subsequently incubated on ice in 1 ml of restriction endonuclease buffer for 30 min., the buffer was then removed and replaced with 60 ul of the same buffer containing BSA (100 ug/ml) spermidine (2 or 5 mM with 50 mM or 100 mM NaCl respectively) and enzyme (40 units). Single digestions were terminated by removing the enzyme mix and replacing it with 1 ml of ice-cold 0.5X TAE 10 mM EDTA (1X TAE is 40 mM Tris acetate 2 mM EDTA). In the case of double digestions, plugs from the first enzyme treatment were washed briefly with 1 ml of cold TE,

equilibrated 30 min. in ice with the new restriction buffer and digested as described above with the second endonuclease. Digested plugs were kept on ice for 30 min. in 0.5X TAE 10 mM EDTA, prior to loading on gel.

Pulsed field gel electrophoresis: A CHEF, Chu et al. (1986), hexagonal-array apparatus was used (Pulsaphor, LKB). Parameters of the runs are given in the legend of Fig.1 . DNA was transfered on to nitrocellulose (Hybond-C Extra, Amersham) using standard protocols.

Probes and hybridizations: cDNAs corresponding to the different bovine casein genes (courtesy of A.G.Mackinlay and S.I.Gorodetsky), were used as probes. Fragments containing the entire cDNA, or portions of, were purified from the vector prior to labeling with the random priming method. Hybridization in formamide was performed according to standard protocols.

RESULTS

Eight "rare-cutters" (ClaI, NotI, SalI, XhoI, SfiI, NarI, SmaI, PvuI) were used to digest plugs from RBE cells. In the samples digested with NotI, SfiI, PvuI and NarI, all the casein probes identified fragments of very high molecular weight, that is well above 1,000 Kb (not shown). The patterns generated by ClaI, XhoI, SalI and SmaI, were more informative, see Fig.1. With SalI at least five fragments were visible with all the casein probes; the smallest, 600 Kb, appears in Fig.1 just below the region where the unseparated DNA accumulates. The strong signal in this region is due to fragments of 780, 860, 970 and >1,000 Kb which were separated under different electrophoretic conditions (not shown).

XhoI reduced the maximum size of the region to 330 Kb: two major fragments, 120 and 210 Kb, were apparent in the autoradiographs, the former hybridizing to the β/α_{s1} probes and the latter to the κ/α_{s2}. Minor bands were visible in the XhoI digests, probably attributable to incomplete cleavage by the endonuclease as a result of site-methylation. In particular, all the casein probes hybridized to a 330 Kb fragment, which represents the combination of the two end products of digestion. Analysis of the ClaI patterns confirmed that the 120 and 210 Kb XhoI fragments were adjacent. Although their interpretation was complicated by the invariable presence of fragments ranging from 90 to more than 400 Kb with all the probes, the autoradiographs for the β and α_{s2} caseins, Fig.1, showed that the two cDNAs identified the same ClaI fragments, *i.e.* 125, 210, 280, 320, 350 Kb. This was considered as a strong indication that the κ and α_{s2} genes were present on the same 125 Kb fragment. Considering that the two probes hybridized to different XhoI fragments, a ClaI-XhoI double digest was carried out in order to position the extremes of the 125 Kb ClaI fragment with respect to the XhoI site that joins the 120 and 210 Kb XhoI fragments. As expected, each probe hybridized to a single ClaI-XhoI fragment, approximately 70 Kb for the β and 55 Kb for the α_{s2}. The hybridization of the α_{s1} cDNA to a 50 Kb ClaI-XhoI fragment (not shown) confirmed the correct positioning of the 125 Kb ClaI fragment, as anticipated from the hybridization of the α_{s1} and β to the same 120 Kb XhoI fragment. The Cla I patterns produced by the four casein probes were subsequently compared to look for shared fragments, as the majority of the autoradiographic signals were the result of incomplete digestion of the samples, which was probably caused by methylation of the sites.

The assignement of SmaI sites was complicated for two reasons. Firstly the enzyme produced more than six fragments for the β, α_{s1}

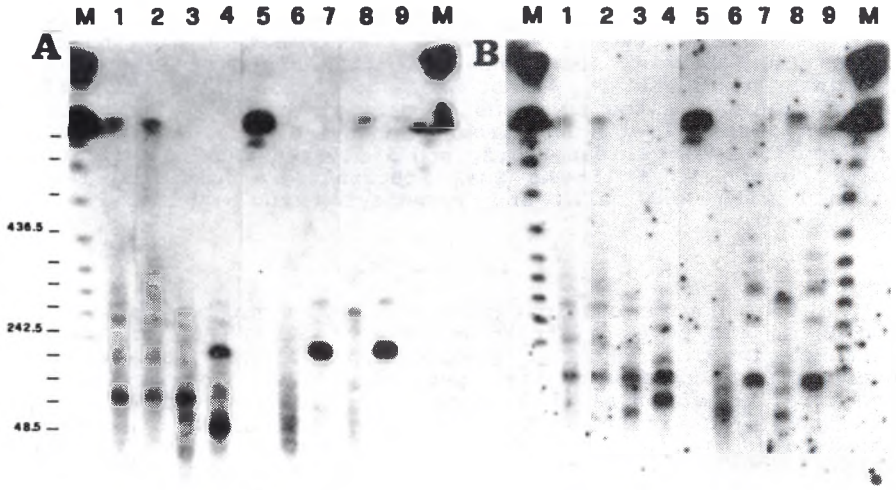

Fig. 1. Long range restriction analysis of the bovine casein region. Digests of DNA from fibroblast cells were separated in a CHEF exagonal-array apparatus. A 1.5% agarose gel was run 18 hours at 190 V, 230 mA, 16°C, in 0.5X TAE with pulses of 30 sec., transferred to nitrocellulose paper and hybridized with an α_{s2} (A) and β (B) probe. Numbers indicate, in the following order, the digestion with ClaI, ClaI+SalI, ClaI+SmaI, ClaI+XhoI, SalI, SalI+SmaI, SalI+XhoI, SmaI and XhoI. M are λ concatamers with size in Kb on the left.

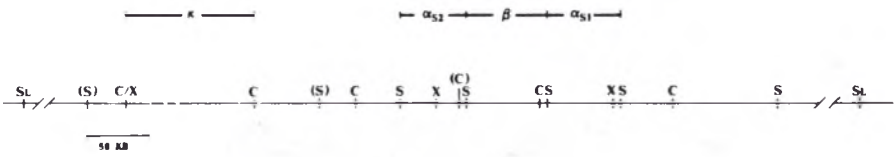

Fig. 2. Physical map of the bovine casein region. Restriction sites: C, ClaI; S_L, SalI; S, SmaI; X, XhoI. Brackets mark the sites that were cleaved only in lymphocytes. The bars above the map indicate the smallest fragments to which hybridization of the casein genes was observed.

and α_{s2} casein. And secondly, the hybridization of the κ casein to DNA from fibroblasts was weak with a concommitant high background noise. The little difference in the size of the smallest fragments, α_{s1} 50 Kb, α_{s2} 45 Kb, and β 55 Kb made it quite difficult to position the sites by means of partial digestion products. The patterns obtained with DNA from lymphocytes elucidated additional SmaI and ClaI sites, see Fig.2, and indicated the hybridization of the κ casein to a 155 Kb SmaI fragment. By combining the data obtained with RBE cells and lymphocytes the map of Fig.2 was established.

DISCUSSION

The four bovine casein genes were mapped on two adjacent XhoI fragments, and cover a stretch of DNA of 330 Kb at most. The map presented in this paper is preliminary but some features are of interest and consistent with published data. In particular the demonstration of physical linkage of the caseins is in agreement with the localization of the four genes to chromosome 6 demonstrated by in situ hybridization, Womack et al. (1989). In addition, our data is consistent with the well established absence of relationship between the κ and the Ca-sensitive caseins, Alexander et al. (1988) if one assumes that a possible explanation is the distance between the two set of genes. In our map the κ-casein gene is separated by the next gene, the α_{s2}, by at least 60-70 Kb, which could be 120 Kb if the gene is close to the leftmost XhoI site. On the contrary the α_{s2}, β, and α_{s1} genes are more clustered and might span a 120-140 Kb stretch of DNA. Finally the size of the smallest (SmaI) fragments identified with the casein probes is in accordance with the restriction maps and sequence reported recently in the literature.

Work is in progress to isolate the casein region by cloning in to YAC vectors, Sgaramella et al. (1990).

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