

Goat milk with different α_{S2} -casein content: analysis of allergenic potency by REAST-inhibition assay

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Abstract

In goat at CSN1S2 locus, seven alleles have been observed, characterized by different levels of expression, and, in particular, a null allele CSN1S2^O that significantly influences protein composition of milk.

In a local goat breed reared in the North East of Sicily, “Argentata dell'Etna”, the combined use of electrophoretic milk analysis (SDS-PAGE) and 11th exon genomic analysis (PCR-RFLP) made it possible to identify three genotypes respectively normal (CSN1S2^{NN}), heterozygote for the null allele (CSN1S2^{NO}) and homozygote for the null allele (CSN1S2^{OO}). In 214 “Argentata dell'Etna” goats, CSN1S2^O allele has shown a frequency of 0.033.

An immunological test, REAST-inhibition assay (REAST, reverse enzyme allerge sorbent test), was carried out on three lyophilized milk samples (casein and seroprotein fractions) produced by goats with different genotypes at CSN1S2 locus, in order to assess their allergenic potency. By comparing the C₅₀, the casein fraction CSN1S2^{NN} turned out to be the most allergenic, followed by the fraction CSN1S2^{OO} with 60% potency compared to the first one, while the fraction CSN1S2^{NO} presented 54% potency compared to the first one.

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1. Introduction

In goat, a relevant genetic polymorphism has been observed at CSN1S2 locus. This locus is characterized by the presence of seven alleles associated with three different synthesis levels: A, B, C, E and F alleles (Boulanger et al., 1984; Grosclaude et al., 1987; Bouniol et al., 1994; Lagonigro et al., 2001; Ramunno

et al., 2001a) associated with a “normal” α_{S2} -casein content (about 2.5 g/l), D allele associated with a reduced α_{S2} -casein content (Ramunno et al., 2001a) and O allele associated with a non-detectable amount of this casein in milk (Ramunno et al., 2001b).

At molecular level, the CSN1S2^D allele is characterized by a 106 bp deletion, involving the last 11 bp of the exon 11 and the first 95 bp of the following intron. The mutation that characterizes CSN1S2^O allele is a G → A transition at nucleotide 80 of the exon 11, that determines formation of a premature stop-codon in position 110.

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The presence of the CSN1S2^O allele, deeply modifies the distribution of casein fractions in goat milk: whereas in “normal” genotype CSN1S2^{NN} this casein fraction represents about 16% of total casein, in heterozygote CSN1S2^{NO} this percentage decreases to about 9%, and is annulled in CSN1S2^{OO} genotype (Ramunno et al., 2001b).

Until now SDS and UREA-PAGE electrophoresis, RP-HPLC and on-line HPLC electrospray ionization mass-spectrometry (HPLC/ESI-MS) confirmed that CSN1S2^O allele is associated with a null content of this casein in milk and that neither α_{s2} -casein nor a truncated form of 109 residues are produced by its carriers (Ramunno et al., 2001b; Marletta et al., 2002).

Goat milk is of high nutritional interest because of its unique metabolic properties, and therefore its recent revaluation by nutritionists and paediatricians for human consumption is welcome. The use of goat milk in patients with cow milk protein allergy (CMPA) has been widely discussed. The overall judgement on goat milk as a substitute for cow milk is still conflicting: many clinical trials report that a high percentage of patients with CMPA tolerate goat milk (Van der Horst, 1976; Taitz and Armitage, 1984; Reinert and Fabre, 1996; Morand-Fehr, 1996), to the contrary some others report cases of cross-reactions between cow and goat milk (Gjesing et al., 1986; Bellioni-Businco et al., 1999) or even rare cases of goat milk allergy (Wuthrich, 1995), sometimes also in absence of cow milk sensitivity (Umpierrez et al., 1999; Lamblin et al., 2001).

Identification of the rare mutation associated to the absence of α_{s2} -casein in milk of some goats, as occurs in human milk, made it possible to carry out an in vitro immunological test, REAST-inhibition assay, involving sera of patients with CMPA, in order to assess the allergenic potency of goat milk with different content of α_{s2} -casein.

2. Materials and methods

Individual milk samples from 214 goats belonging to “Argentata dell’Etna”, a Sicilian goat breed, reared on 10 farms in a mountainous area in the North East of Sicily, were analyzed by SDS-PAGE (Grosclaude et al., 1987).

On the genomic DNA, extracted from the leukocytes according to Goossens and Kan (1981), molecular analysis was performed at the CSN1S2 locus by PCR-RFLP to identify the carriers of CSN1S2^O and CSN1S2^D alleles (Ramunno et al., 2001a).

Three milk samples with different α_{s2} -casein content (genotypes NN, NO and OO with N = A, B, C, E or F) were ultracentrifugated to separate total casein (C-NN, C-NO and C-OO) and seroproteins (S-NN, S-NO and S-OO), according to Iametti et al. (2000).

The separated protein fractions (seroproteins and total casein) were lyophilized and used in an in vitro REAST-inhibition assay (REAST, reverse enzyme allergeo sorbent test), as described by Falagiani et al. (1994). The choice of this method, instead of the more common RAST-inhibition, is based on the fact that preliminary investigations on human sera used for analysis revealed a very low reactivity in the RAST system, probably due to the presence of allergen-specific IgG antibodies, that exercise an inhibiting effect through competition with IgE antibodies.

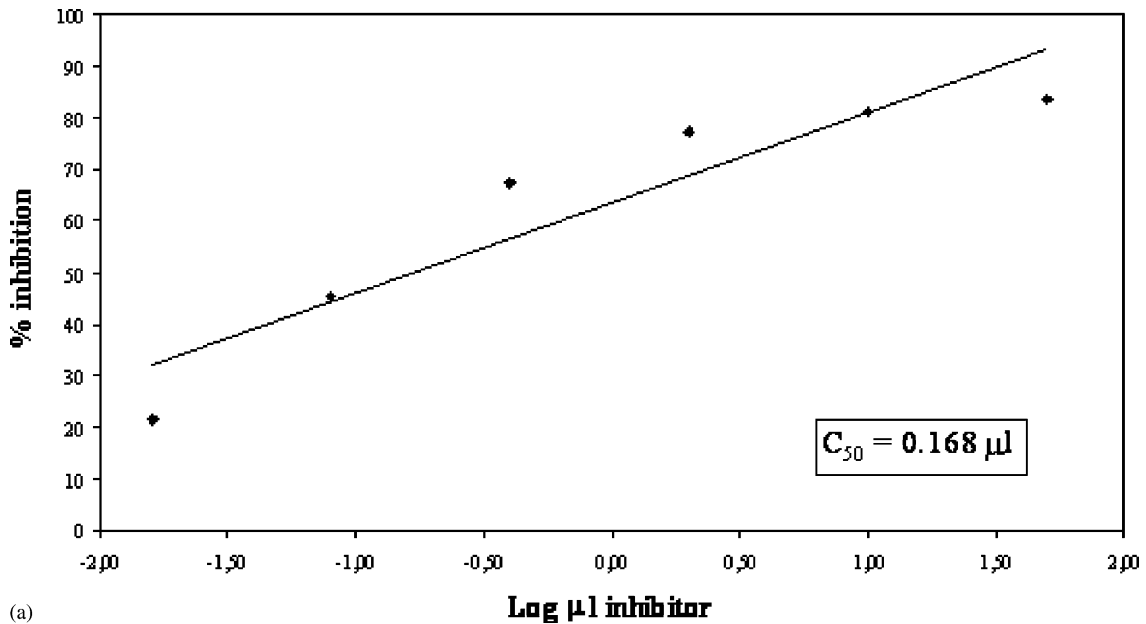
REAST-inhibition is based on the capture of allergen-specific IgE by an anti-human IgE antibody bound to ELISA microtiter wells, and on the subsequent reaction with the biotinylated reference allergen (in this case cow milk), pre-mixed with an equal aliquot of inhibiting allergen (in this case goat milk protein fractions) serially diluted. Streptavidin-peroxidase, chromogenic substrate (TMB-H₂O₂) and stopping solution (1N HCl) were added in sequence and the OD at 450 nm was determined using a Spectra ELISA reader (SLT Labinstruments, Milan, Italy). The test is conducted as a dose-response curve: the program calculates the inhibition percentages for each sample and its dilution and traces regression curves. The C₂₀, C₅₀ and C₈₀ values are calculated, that are the sample quantity that inhibits the reaction by 20, 50 and 80%, respectively. The allergenic potency is inversely proportional to the volume required to inhibit the test. C₅₀ value, which is located in the central part of the curve that is highly linear, is normally used to express the allergenic potency.

3. Results and discussion

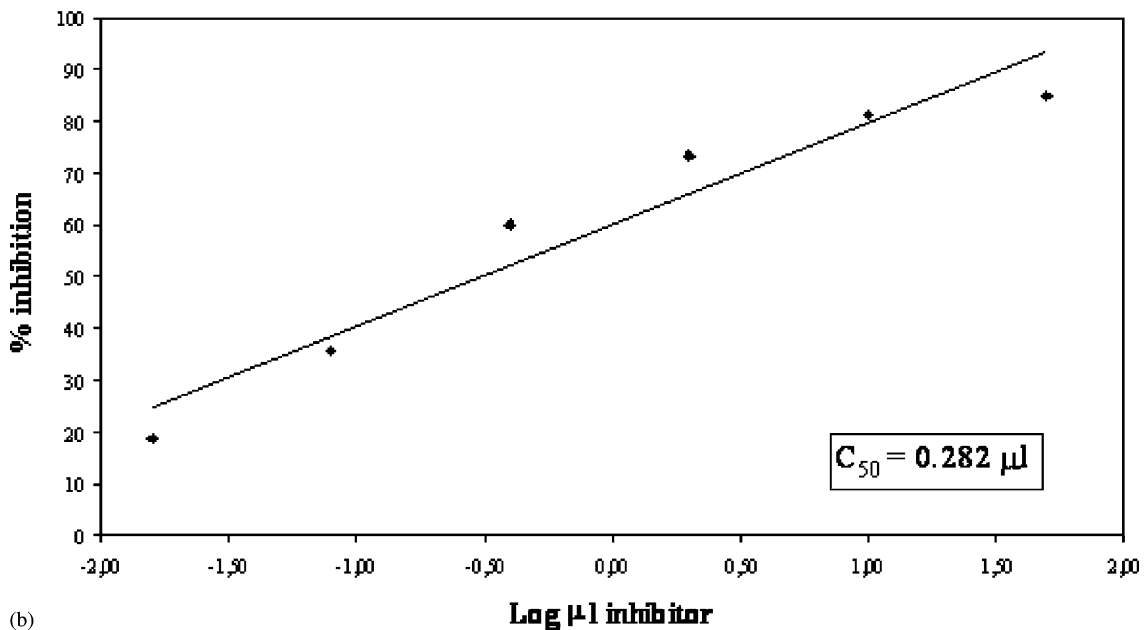
SDS-PAGE analysis made it possible to identify 13 individual milk samples with an anomalous pattern:

a single milk sample did not contain the band corresponding to α_{s2} -casein, whereas 12 milk samples showed a less intense band (reduced to about half) of this fraction.

The genomic analysis at exon 11 revealed that these goats were all carriers of the CSN1S2^O allele, 1 homozygote and 12 heterozygotes, whereas the CSN1S2^D allele was not identified. In the sample



(a)



(b)

Fig. 1. REAST-inhibition assay: (a) analysis of CSN1S2^{NN} casein fraction; (b) analysis of CSN1S2^{OO} casein fraction; (c) analysis of CSN1S2^{NO} casein fraction. Regression line and C₅₀ value, corresponding to 50% inhibiting dose, are reported.

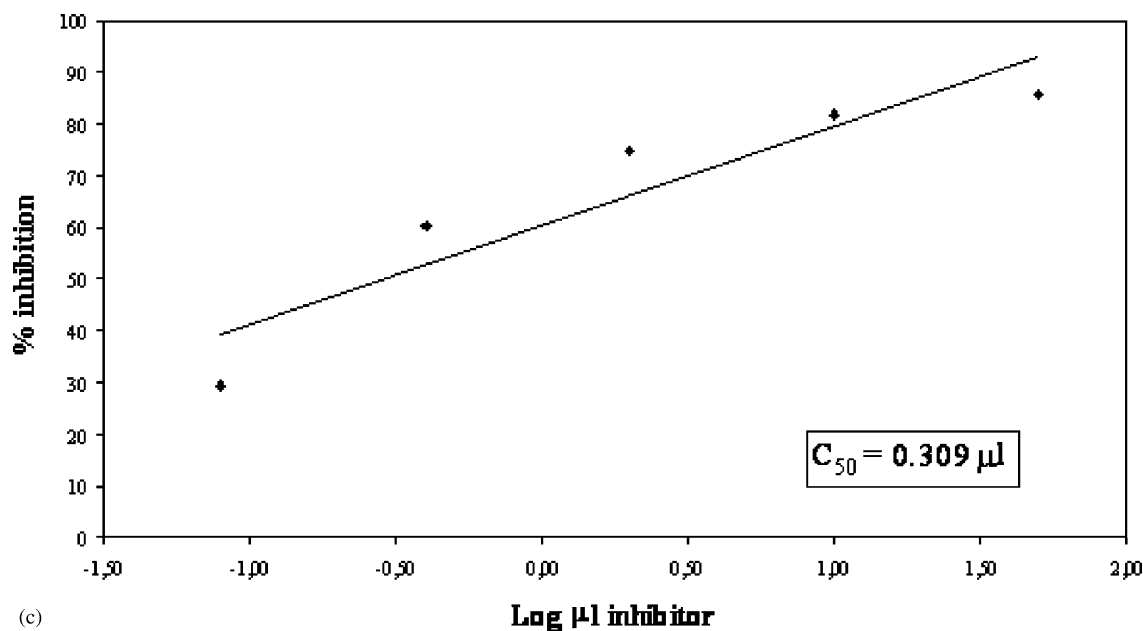


Fig. 1. (Continued).

tested (214) of the “Argentata dell’Etna” breed, the CSN1S2^O allele had a frequency of 0.033 (Marletta et al., in press).

So far the CSN1S2^O allele has only been identified in small goat populations of Southern Italy (Ramunno et al., 1997; Marletta et al., 2000) always at low fre-

quencies (0.049 and 0.017) and it could be a genetic peculiarity of these populations.

The allergenic potency of the three casein fractions was similar. In terms of C_{50} , the most allergenic was the CSN1S2^{NN} fraction (Fig. 1a), followed by the CSN1S2^{OO} fraction (Fig. 1b) with a capacity of 60%

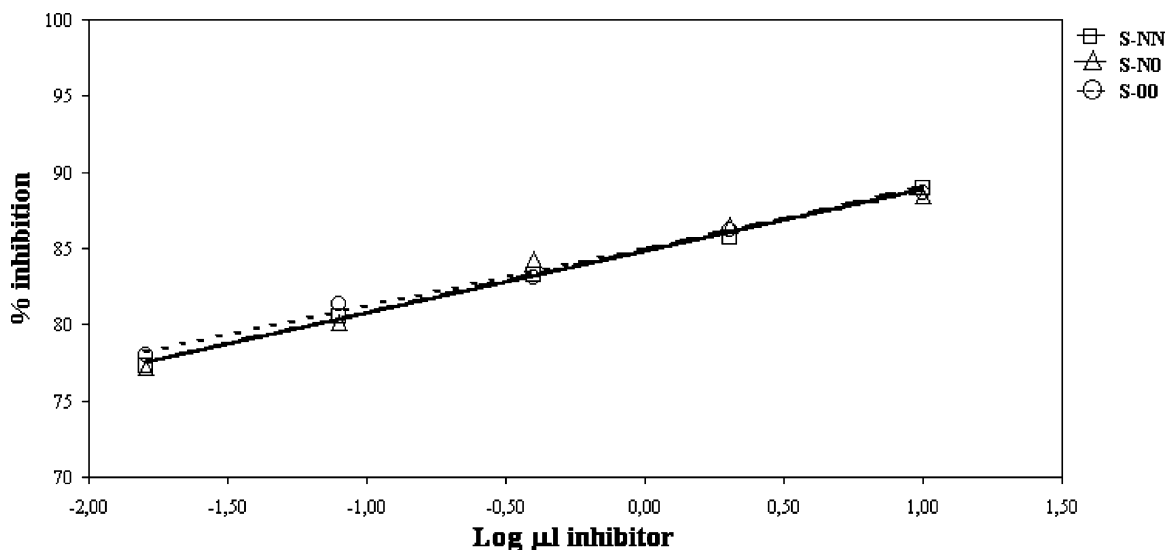


Fig. 2. REAST-inhibition assay. Analysis of the seroprotein (S-NN, S-NO and S-OO) fractions.

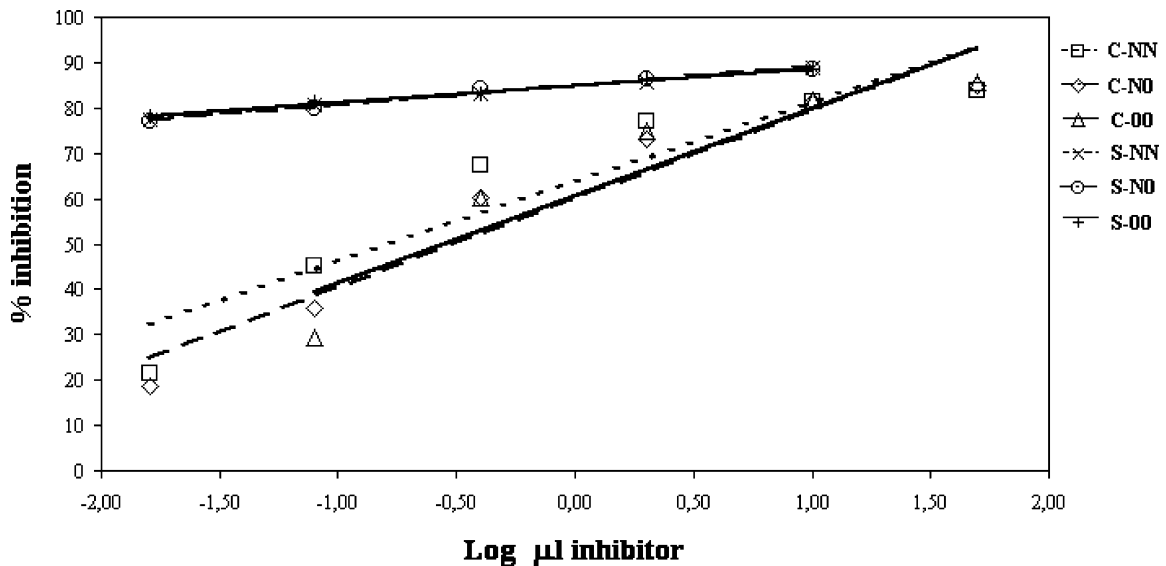


Fig. 3. REAST-inhibition assay. Analysis of the casein (C-NN, C-NO and C-OO) and of seroprotein (S-NN, S-NO and S-OO) fractions.

compared to the CSN1S2^{NN}, and by the CSN1S2^{NO} fraction (Fig. 1c) with a potency of 54% compared to the first one. For each curve, there were at least four experimental points in the interval between the 10 and 90% inhibition rates, which is the range of acceptance of data according to the Food and Drug Administration (1985). Correlation coefficients (*r*) were very high, being in all cases greater than 0.92.

As concerns the seroproteins (Fig. 2) from the three samples of different genotype at CSN1S2 locus, due to their very high allergenic activity, it was not possible to refer to the C₅₀ and, consequently, the C₈₀ had to be assessed that were 0.067 µl for S-NN, 0.063 µl for S-NO and 0.048 µl for S-OO.

By comparing C₈₀, the allergenic potency of the three seroprotein fractions, in any case much more allergenic than caseins, was similar. The fraction S-NN was 128 times more potent than the corresponding casein fraction (C-NN), the S-NO was 136 times more potent than the corresponding casein fraction, whereas the S-OO fraction was 179 times more potent than the C-OO fraction.

Results obtained in the REAST-inhibition assay have indicated that the protein fraction that plays a role in allergic reactions is first and foremost than that of the seroproteins (Fig. 3), according to several investigations (Kitagawa et al., 1995; Gall et al.,

1996), whereas some other studies point out that both protein fractions (seroproteins and casein) can elicit allergic reactions in sensitized individuals (Savilahti and Kuitunen, 1992; Wal, 2001) or that caseins have been found to be major allergens of milk (Docena et al., 1996).

4. Conclusions

REAST-inhibition data indicate that the absence or the reduced content of α_{s2}-casein in goat milk determines only a small decrease in the allergenic potency of the casein fraction.

However, the allergenic potency of goat milk without α_{s2}-casein has to be further investigated “in vivo” by clinical trials on patients with CMPA.

The rare mutation associated with a null content of α_{s2}-casein could be used to exploit dairy productions in goat breeds and population of Southern Italy, with a view to improve their economical viability and their preservation.

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