

Identification of the κ -Casein Among the Components of Whole Goat Casein

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Abstract

Goat κ -casein in the reduced state, probably the natural state, has a mobility on acrylamide gel electrophoresis at pH 9, very close to that of the β -caseins. The goat κ -casein was identified by clotting with rennin, by specific changes in the electrophoretic pattern after rennin action, and by nonprecipitation with calcium ions. It stabilized goat α_s -casein so that α_s -casein was not precipitated with calcium ions. Polyacrylamide gel electrophoresis of goat κ -casein in some cases gave a single band (together with identifiable contaminants), and, in other cases, gave a multiplicity of slower-moving bands. In all cases only a single major band was observed when the κ -casein was reduced with mercaptoethanol. Components with the mobilities of para- κ -casein were present in considerable amounts in some preparations of κ -casein.

Goat casein is characterized by two strong bands in the β -casein region¹ on gel electrophoresis at pH 9. On treating whole goat casein with urea-sulfuric acid for the isolation of κ -casein (11), preparations were obtained that on electrophoresis gave a principal band moving in the β -casein region. Other preparations on electrophoresis gave multiple slower-moving bands, but these preparations, too, gave a principal band moving in the β -casein region when treated with mercaptoethanol for electrophoresis. This paper will show that the preparations obtained by the urea-sulfuric acid method are indeed κ -casein and will report some interesting properties of goat κ -casein.

Materials and Methods

Goat milk. Fresh goat milk, both pooled and from two individual goats, was used in the following studies. No major differences were observed in the pooled and in these individual samples. The milk was warmed to 30 to 35 C and skimmed in a separator. Preparation of

the caseins from this milk is described in the next section.

Polyacrylamide gel electrophoresis. Electrophoresis was performed in a vertical cell at pH 9.0 in a 4.5 M urea with an acrylamide concentration of 7.0% (7). In some experiments mercaptoethanol was added (10).

Experimental Procedures and Results

Whole casein was prepared from skimmed goat milk by acidification with HCl. The most effective flocculation and rapid filtration was obtained when the pH was 4.2. The casein was washed twice with water maintained at the same pH. The casein was subsequently washed with ethanol, acetone, and ethyl ether, a process which extracted lipids and dried the casein.

κ -Casein was prepared from whole goat casein by the sulfuric acid-urea procedure (11). Goat casein behaved in this procedure as did bovine casein. The lyophile-dried κ -casein obtained was about 13% of the whole casein used. These impure (see electrophoretic patterns) κ -caseins had the following composition: 8% volatile matter (moisture), 5% fixed ash, 15.5% N, 0.6% P, and 0.8% S, all on a moisture-free basis. The same values for the whole casein were 8, 2, 15.6, 0.8, and 0.8.

The sialic acid content of these preparations was determined by the method of Warren (9). The whole caseins contained 0.13%, the κ -caseins 0.3% sialic acid. Although these goat κ -caseins still contain other components, it is evident that they contain considerably less sialic acid than bovine κ -casein (2.0%). Sialic acid content of goat and cow whole casein, is respectively, 0.13 and 0.36%. Alais and Jollès (1) have found goat casein to contain 0.13% sialic acid, the glycopeptide 3.0%.

The precipitate obtained in the κ -casein isolation, termed α_s - β casein, was suspended in water, dissolved with NaOH, dialyzed for 18 hr against H₂O, precipitated with 0.1 N HCl, and dried with organic solvents, as was the whole casein. Polyacrylamide gel electrophoresis of

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¹ M. P. Thompson, unpublished studies; R. Aschaffenburg, personal word to M. P. Thompson.

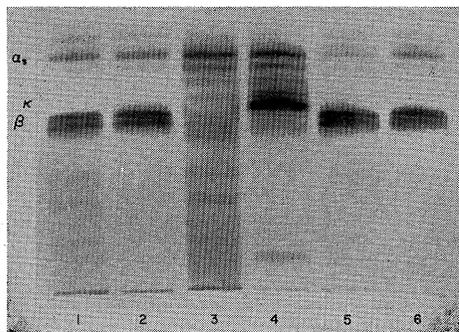


FIG. 1. Polyacrylamide gel electrophoresis of whole goat casein, κ -casein, and α_s - β casein. 1, 2 Whole casein; 3, 4 κ -casein; 5, 6 α_s - β casein. Patterns 2, 4, and 6 were obtained with mercaptoethanol present. The doublet of intermediate mobility, very evident in Patterns 1, 2, 5, and 6, is β -casein. The components of greatest mobility are α_s -casein. The major band of the mercaptoethanol-treated κ -casein, presumably reduced κ -casein, moves just ahead of the β -casein. This preparation without mercaptoethanol gives the multiple bands, presumably corresponding to oxidized κ -casein, showing between the β -caseins and the origin.

the goat whole casein, κ -casein, and the α_s - β casein preparation are shown in Figure 1, both without and with mercaptoethanol. Some of the κ -casein preparations gave a pattern almost identical to no. 4 without the use of mercaptoethanol. The urea-sulfuric acid method excluded most of the β -casein, but considerable α_s -casein is still present in this κ -casein preparation. Other κ -casein preparations have been considerably freer of α_s -casein (see Figure 2), with most of this component in the α_s - β casein preparation. An electrophoretic comparison of goat whole casein with the bovine casein is shown in Figure 2. Although the β -caseins have

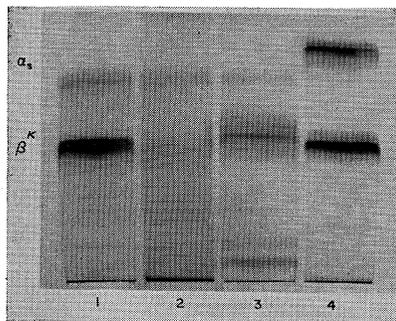


FIG. 2. Electrophoretic comparison of goat and bovine whole caseins and goat κ -casein without and with mercaptoethanol present. 1. Whole goat casein. 2. Goat κ -casein. 3. Goat κ -casein with mercaptoethanol present. 4. Whole bovine casein. The two slow-moving bands in no. 3 correspond to para- κ -casein.

about the same mobility, the goat α_s -casein has a lower mobility than the bovine α_s -casein. Also shown is another κ -casein preparation, without and with mercaptoethanol. This preparation shows the presence of some spontaneously formed para- κ -casein (see later). The influence of mercaptoethanol on another, more typical preparation (sharp bands, little of the diffuse staining, only trace of para- κ -casein) of κ -casein is shown in Figure 3.

Since the κ -casein preparation gave the strong band in the β -casein region, tests were performed to determine whether the preparation had the characteristics of κ -casein. Neutral 1% solutions of goat whole, κ -, and α_s -caseins were mixed with 0.1 M CaCl_2 , final concentration 0.01 M. The solution of whole casein became white, the κ -casein solution remained clear, and the α_s -casein precipitated. The same caseins at pH 6.5 with 0.05 M NaCl (no CaCl_2) present were treated with rennin (1:100) at 37 C. The whole casein and the α_s -casein remained clear; the κ -casein became white. The goat α_s -casein, 80% precipitated by 0.020 M CaCl_2 , was stabilized by the κ -casein at a weight ratio of 1:7, so that only 20% of the α_s -casein was precipitated.

All these properties are characteristic of κ -casein, based on what is known about bovine κ -casein. The most striking demonstration that the sulfuric acid-urea preparation is indeed mainly κ -casein was obtained by following the action of rennin with polyacrylamide gel electrophoresis. The casein was treated with rennin, as described above, but in this experiment

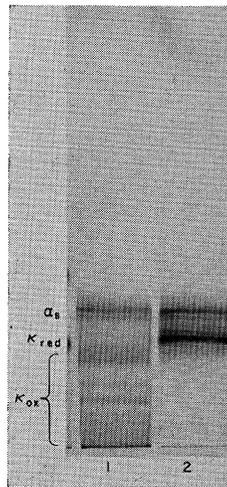


FIG. 3. Influence of mercaptoethanol on polyacrylamide gel electrophoretic pattern of goat κ -casein. 1. κ -Casein without mercaptoethanol. 2. κ -Casein with mercaptoethanol.

no NaCl was added. Small samples were removed at intervals and NaCl added to determine the course of rennin action. The action appeared to be complete at 30 min (treatment for 70 min gave the same electrophoretic results) and the sample was frozen and freeze-dried. The electrophoretic results are shown in Figure 4. The strong band in the β -casein region, the κ -casein band, is totally transformed by rennin to two bands of much less mobility. These results are reminiscent of the action of rennin on bovine κ -casein (4, 5), although both goat κ - and para- κ -caseins have greater positive mobilities than the corresponding bovine components. Identical patterns were obtained whether the rennin acted for 30 or 70 min. The complex pattern of the contaminating α_s -casein remained unchanged, suggesting that during the period the rennin acted no nonspecific proteolysis occurred.

One preparation of κ -casein, obtained from the whole casein of a second lot of pooled goat milk, showed some differences from the other preparations. These differences are shown in the electrophoretic patterns in Figure 5. The pattern (no. 1) is more diffuse and, when treated with mercaptoethanol (no. 2), two slow-moving bands appear near the origin, together with the faster-moving κ -casein band. The major of these two bands appears in other preparations, but not to the same extent. Results obtained with rennin (Figure 4) suggested that these bands corresponded to para- κ -casein. Treatment of this preparation with rennin, followed by mercaptoethanol reduction, transformed all of the κ -casein to components (no. 4) whose mobilities coincided with the slow-moving bands, strongly suggesting that these bands corresponded to para- κ -casein that had been spontaneously formed.

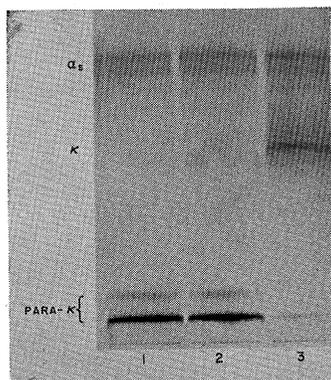


FIG. 4. Electrophoretic patterns illustrating the action of rennin on κ -casein. 1. 70-min treatment. 2. 30-min treatment. 3. Untreated κ -casein.

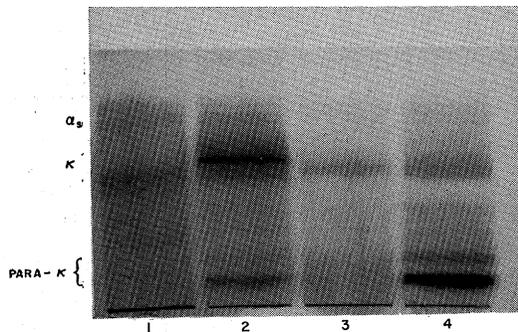


FIG. 5. Polyacrylamide gel electrophoretic patterns of goat κ -casein containing considerable para- κ -casein-like material initially, without and with mercaptoethanol, before and after treatment with rennin. 1. κ -Casein. 2. κ -Casein treated with mercaptoethanol. 3. κ -Casein treated with rennin. 4. κ -Casein treated with rennin and reduced with mercaptoethanol.

Discussion

In goat whole casein two strong bands appear in the β -casein region on acrylamide gel electrophoresis. In none of many samples examined has a single band been observed in this region.¹ Present results indicate that these bands obscure the reduced κ -casein band or that one of these bands is κ -casein. The position of the κ -casein band in the purified preparation relative to the position of the two strong bands in the whole casein, as well as the position of the two strong bands remaining in the α_s - β casein preparation, suggest that the κ -casein is obscured by these β -casein components. The identity of the component separated by urea-sulfuric acid as κ -casein was established by a number of properties. The most striking is the change in electrophoretic mobility brought about by the action of rennin. Major and minor bands of much less mobility are obtained, reminiscent of bovine κ -casein (4, 5). Studies with pure goat κ -casein are needed to show whether these para- κ -casein bands arise from one or two κ -casein components [see Reference (5)].

Apparently, goat κ -casein can change to para- κ -casein without treatment with rennin. This change was very marked in one preparation of goat κ -casein studied. This transformation has been observed with bovine κ -casein, but only to a small extent. This change has been attributed (3) to spontaneous breakdown of the rennin-sensitive bond or to the action of microorganisms. The naturally occurring milk protease would appear to be a possible cause also, although assay (12) of the goat whole casein giving the κ -casein just mentioned showed relatively little protease.

The two types of electrophoretic patterns obtained with κ -casein, a single band and a multiplicity of bands, have interesting implications. In view of the single band obtained when reduction is accomplished with mercaptoethanol and from analogy with bovine κ -casein, the single band is interpreted to represent the reduced κ -casein. Presumably, this is the form synthesized by the goat, since some preparations show this electrophoretic pattern even without reduction. When oxidation occurs, and this apparently can occur spontaneously, a multiplicity of components results, as shown in the electrophoretic patterns. The result of oxidation of goat κ -casein differs from that of the cow. In cow κ -casein, a size heterogeneity apparently results that is apparent as a smudge on gel electrophoresis, whereas in the goat κ -casein, apparently polymers of discrete sizes result preferentially.

The nature of the interactions that lead to the apparent great heterogeneity in bovine κ -casein is not clear, although formation of disulfid bonds must have a major role. The formation of polymers of discrete sizes postulated for goat κ -casein has a counterpart in the human serum haptoglobulins (2). The haptoglobulin polymers are transformed to monomers by treatment with mercaptoethanol (8).

These observations with goat κ -casein, and the recent report on the heat stability of goat milk (6), indicate that goat κ -casein is an interesting casein for further study, particularly comparative studies with the bovine κ -casein.

Acknowledgments

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