

# STABILIZATION OF $\beta$ -CASEIN BY $\kappa$ -CASEIN AGAINST PRECIPITATION BY CALCIUM CHLORIDE

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## SUMMARY

$\beta$ -Casein (0.15%) at 30 C is 80% precipitated by 10 mM calcium chloride.  $\kappa$ -Casein at a  $\kappa/\beta$  ratio of 0.15 will completely prevent the precipitation. This stabilization by  $\kappa$ -casein is dependent on the concentration of  $\beta$ -casein; when the concentration is 0.6%, the  $\kappa$ -casein is considerably less effective. The stabilization is also pH dependent with the maximal effectiveness of  $\kappa$ -casein at pH 7.0.

The casein micelles in milk remain stable in the presence of calcium ions because of the unique properties of  $\kappa$ -casein (5). The ability of the  $\kappa$ -casein to stabilize the component  $\alpha_s$ -casein has been quantitatively evaluated in terms of the amount of  $\kappa$ -casein and the  $\alpha_s$ -casein soluble at a given  $\text{CaCl}_2$  concentration (6). Since  $\beta$ -casein is a major component of the casein micelle in milk, the ability of  $\kappa$ -casein to stabilize the  $\beta$ -casein has now been investigated. Precipitation of  $\beta$ -casein with  $\text{CaCl}_2$ , the influence of the concentration of  $\beta$ -casein on the stabilization, and the influence of pH also have been studied.

## EXPERIMENTAL PROCEDURE

*$\beta$ -Casein.*  $\beta$ -Casein was prepared by the urea method (3). Two preparations were available; one was prepared by Dr. E. B. Kalan, the other by Mr. L. W. Nauman, III. Starch-gel electrophoresis showed the first preparation contained a trace of material moving faster than  $\beta$ -casein, the second a trace of slower material. The major band, the  $\beta$ -casein, was identical in the two preparations. Both gave similar results in the experiments to be described. These  $\beta$ -caseins, prepared from commercial bulk milk obtained in the Philadelphia area, were examined for genetic type (1) and found to be Type A (R. F. Peterson and L. W. Nauman, III, unpublished studies). A few experiments were done with  $\beta$ -caseins Types B and C (these caseins were prepared by Dr. M. P. Thompson). Solutions of both were stabilized by  $\kappa$ -casein in the presence of calcium ions in a way similar to that reported in detail for the  $\beta$ -casein A. There were, however, observed differences in these

caseins.  $\beta$ -Casein B, for example, at a given concentration of calcium chloride, was less soluble than A and C. This solubility difference persisted in the presence of  $\kappa$ -casein and hence more  $\kappa$ -casein was required for complete stabilization (solubilization) of  $\beta$ -casein B than for  $\beta$ -caseins A and C.

*$\kappa$ -Casein.* The  $\kappa$ -casein was prepared by the sulfuric acid method (7), with a final purification by ethanol precipitation (9).

*Stabilization test.* For these tests 0.5 or 1.0% solutions of  $\beta$ -casein and 0.25% solutions of  $\kappa$ -casein were used. The solutions were prepared by weighing the dry caseins, but the actual casein concentration was estimated from the absorption at 280 m $\mu$ , using a value of 1 cm  $E_{0.1\%}$  of 1.22 for  $\kappa$ -casein (9), and 0.48 for  $\beta$ -casein (6). For most of the tests the pH values of these stock solutions were 7.5 to 7.8. On addition of calcium chloride, the final pH value was about 7.0. For the variable pH experiments the pH of the  $\beta$ -casein, the major buffering component, was raised or lowered with 0.1 N NaOH or HCl, as desired. The higher pH values decreased as much as 1.5 units on the addition of calcium chloride; the drop was much less at the lower pH values. Final pH values are given in every case.

The calcium precipitation and the stabilization tests were performed in a final volume of 5.0 ml in 15-ml centrifuge tubes. In the case of the former the required amount of  $\beta$ -casein was added plus sufficient water to make the final volume 5 ml after the addition of the variable amounts of  $\text{CaCl}_2$  (0.1 M). In the stabilization tests, the required amounts of the  $\kappa$ -casein, together with the  $\beta$ -casein, were added, water to make the volume 4.5 ml, and finally 0.5 ml of 0.1 M calcium chloride. In both tests the solutions were thoroughly mixed and the mixture placed in a 30 C water-bath for 15 min. The tubes were centrifuged at room tem-

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perature (22-25 C) at about  $3,000 \times G$  for 5 min. A 1-ml portion of the supernatant solution was removed, diluted with 4 ml of water, clarified with one drop of 10 N NaOH, and the absorption measured at 280  $m\mu$ . The solubilization of the  $\beta$ -casein was calculated from these absorption values, after subtracting that due to the  $\kappa$ -casein in the mixture, on the assumption that all of the  $\kappa$ -casein remained soluble.

## RESULTS

The precipitation of  $\beta$ -casein (0.15%) by calcium chloride at pH 6.7 and 30 C is shown in Figure 1. Precipitation has leveled off at 80%

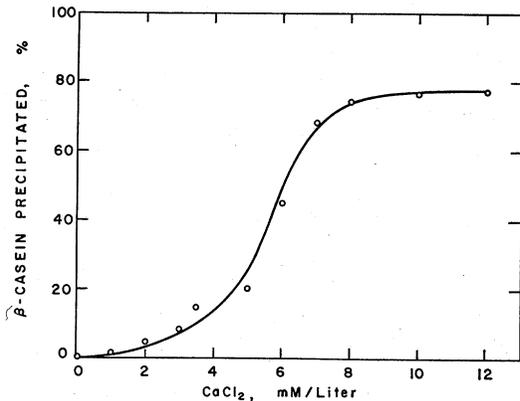


FIG. 1. Precipitation of  $\beta$ -casein (0.15%) by  $\text{CaCl}_2$  at pH 6.7 and 30 C.

in the range of 8 to 10 mM  $\text{CaCl}_2$ . A calcium chloride concentration of 10 mM was chosen for the subsequent stabilization tests.

The solubility (stabilization) of  $\beta$ -casein with calcium chloride when  $\kappa$ -casein is present is shown in Figure 2. These experiments were done with three concentrations of  $\beta$ -casein (0.15, 0.30, and 0.60%). The greatest stabilization by  $\kappa$ -casein is exerted in the most dilute system.

The influence of pH on the solubility of  $\beta$ -casein with calcium chloride, with and without  $\kappa$ -casein, is shown in Figure 3. Three pH ranges are distinguishable. Below pH 7.2 the influence of  $\kappa$ -casein on maintaining the solubility of  $\beta$ -casein is decreasing; also, above this pH the solubility of  $\beta$ -casein with  $\kappa$ -casein decreases and, finally, above pH 8.0 the solubility increases. This latter trend occurs roughly in parallel with a comparable increase in the solubility of  $\beta$ -casein when no  $\kappa$ -casein is present.

## DISCUSSION

The present experiments show that  $\beta$ -casein at 0.15% concentration is precipitated by 8-10

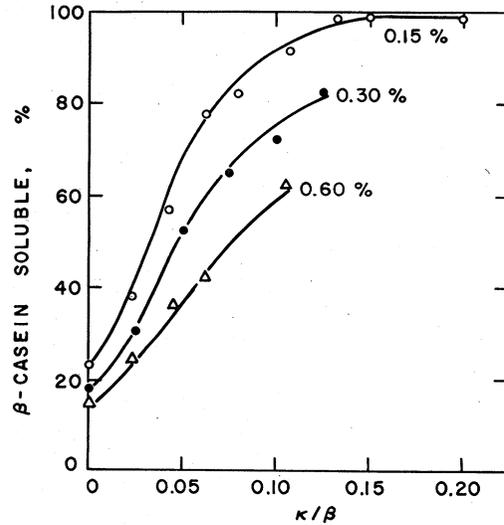


FIG. 2. Solubility of  $\beta$ -casein at several concentrations (0.15, 0.30, and 0.60%) with  $\kappa$ -casein and a  $\text{CaCl}_2$  concentration of 0.01 M.

mm  $\text{CaCl}_2$  to the extent of 80%. This apparently is a characteristic solubility of  $\beta$ -casein at 30 C.  $\beta$ -Casein is completely soluble at low temperature (0-5 C) with calcium chloride (4, 10). Waugh (4) has reported the solubility of  $\beta$ -casein in 0.03 M  $\text{CaCl}_2$  at 25-37 C as 0.2 g per liter. The present data at 30 C indicate that the solubility in 0.01 M  $\text{CaCl}_2$  is 0.3 g per liter.

The precipitation of  $\beta$ -casein by calcium chloride is prevented by  $\kappa$ -casein, presumably in consequence of association of the  $\kappa$ -casein with the  $\beta$ -casein, similar to that which occurs with  $\alpha_s$ -casein (4, 5). Waugh and von Hippel have presented qualitative evidence (4, 5) that  $\kappa$ -casein and  $\beta$ -casein associate also.

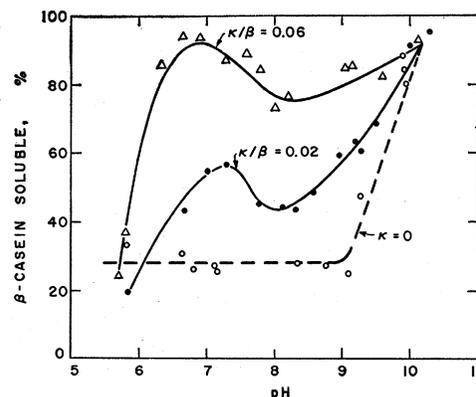


FIG. 3. Solubility of  $\beta$ -casein (0.15%) at pH 5.7 to 10.3 with 0.01 M  $\text{CaCl}_2$  with no  $\kappa$ -casein and with  $\kappa/\beta$  ratios of 0.02 and 0.06.

The concentration dependence of the  $\kappa$ -casein:  $\beta$ -casein stabilization is very marked and similar to that observed for  $\alpha_s$ -casein (8). This concentration dependence may be related to the occurrence of  $\kappa$ -casein in polymeric form at pH 7.0 and suggests that if the amount of the monomeric form is increased on dilution the monomer is the more effective stabilizer. This hypothetical appearance of monomer on dilution may be difficult to demonstrate by physicochemical means, because of the low concentration of the casein and the requirement for buffering salts, which aggregate the caseins.

The stabilization of  $\beta$ -casein by  $\kappa$ -casein is pH dependent, just as it is with  $\alpha_s$ -casein (8). With  $\beta$ -casein the optimal pH is at 7.2, with  $\alpha_s$ -casein B it is at pH 8.2, and with  $\alpha_s$ -casein A it is at pH 6.3. Tentatively, the three areas of pH influence are explained as follows: In the pH range below pH 7.2, the pH of maximum stabilization for  $\beta$ -casein, the decrease in solubility is ascribed to the decrease in the net negative charge on the  $\beta$ -casein: $\kappa$ -casein complex as the pH approaches the isoelectric pH of maximum precipitation. The decrease in solubility above pH 7.2 is ascribed to a decrease in the association between  $\beta$ -casein and  $\kappa$ -casein, due perhaps to electrostatic repulsion between these caseins because of the increase in net negative charge. The subsequent increase in the solubility at higher pH values parallels the increase in the solubility of the  $\beta$ -casein alone.  $\beta$ -Casein: $\kappa$ -casein complex and dissociated  $\beta$ -casein cannot be distinguished here, but in view of the dissociation trend between pH 7.2 and 8.0 this probably represents

the solubilization of dissociated  $\beta$ -casein at these higher pH values.

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