Influence of Heat on κ-Casein: Effect of α_s-Casein and Concentration of Calcium Chloride and Sodium Chloride

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Abstract

κ-Casein is precipitated when heated at 100 C for 5 min at pH 7 with 0.005 to 0.05 M concentrations of $CaCl_2$. At higher concentrations of $CaCl_2$ precipitation decreases and at 0.15 M $CaCl_2$ it is completely prevented. Sodium chloride will also decrease or prevent $CaCl_2$-heat precipitation of κ-casein. The effect of NaCl is markedly dependent on the $CaCl_2$ concentration; with 0.05 M $CaCl_2$, 0.25 M NaCl is needed to prevent precipitation; with 0.005 M $CaCl_2$ only 0.05 M NaCl is needed. The effect of excess CaCl or $NaCl_2$ shows little pH dependence. Some comparable experiments with $CaCl_2$ precipitation of α_s-casein (unheated) shows a marked pH dependence of the effect of excess NaCl. κ-Casein, heated in the presence of salts and mercaptoethanol, is heat-labile, as measured by its inability to stabilize α_s-casein against $CaCl_2$ precipitation. The presence of α_s-casein in a 1:1 weight ratio when the κ-casein is heated prevents the heat lability.

In a previous paper (5) the conditions were described (salts, sulfhydryl compound) for maximum alteration of κ-casein by heating at 100 C for 5 min, judged by its loss of ability to stabilize α_s-casein in the presence of calcium chloride. The deleterious effect of heat on κ-casein is not evident in milk, since this would be expected to lead to physical instability which does not occur. Since κ-casein is associated principally with α_s-casein in milk, the influence of α_s-casein on the heat lability of κ-casein has been investigated and shown in the present report to prevent the heat lability of the κ-casein. Previous studies (5) showed that the presence of calcium chloride (0.01 M) in the κ-casein solutions when heated caused precipitation. In the present studies concentrations of calcium chloride were extended to 0.20 M. In some of the experiments part of the calcium chloride was replaced by sodium chloride. Higher concentrations of either salt prevented the precipitation of the κ-casein by heat. Comparative experiments were done with α_s-casein solutions (unheated) with the same concentration range of these salts.

Materials and Methods

κ- and α_s-Caseins. The casein preparations used throughout these studies were described in the previous paper (5).

Stabilization test. This test has been described in detail (5). It differs from the previous method (4) only in the use of imidazole buffer, pH 7.3, to dissolve the α_s-casein and effectively buffer the test system.

Heating experiments. A stock 0.5% solution of the κ-casein at pH 7.5 was used. This was diluted to 0.25% concentration with a buffer mixture (final concentration 0.025 M buffer–0.025 M NaCl) plus added salts or the α_s-casein under study. The details are given in reporting the results of each experiment. Fifteen-milliliter tubes containing the test mixtures of usually 4.0 ml were immersed in a boiling-water bath to the 10-ml mark and heated for 5 min.

Results

The effect of increasing the concentration of $CaCl_2$ on the precipitation of κ-casein by heat is shown in Figure 1. Very low concentrations (0.005 M) of $CaCl_2$ bring about precipitation, but when the concentration exceeds 0.05 M (0.15 ionic strength) a preventive or solubilizing effect is apparent. This effect continues to increase with $CaCl_2$ concentration until at about 0.15 M (0.45 ionic strength) no precipitation occurs. This prevention of precipitation by excess $CaCl_2$ is to a degree a nonspecific ionic effect, for in systems containing sufficient $CaCl_2$ (0.005 to 0.05 M) to precipitate κ-casein when heated, additions of NaCl lessen or prevent precipitation. The effective NaCl concentration for preventing precipitation is markedly influenced by the concentration of $CaCl_2$. When $CaCl_2$ is 0.05 M, a 0.25 M concentration of NaCl (total ionic strength of 0.4) is required, but when the $CaCl_2$ concentration is 0.005 M a 0.05 M concentration of NaCl is sufficient (Figure 1).

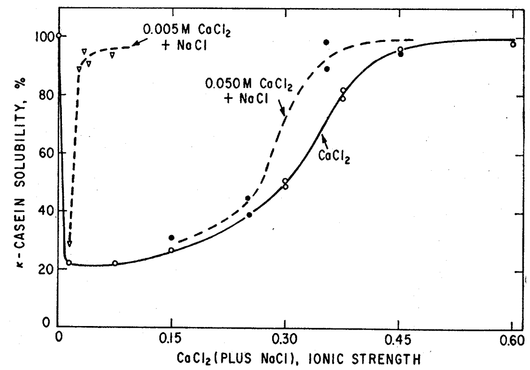

FIG. 1. Influence of $CaCl_2$ and $CaCl_2$, plus NaCl, on the precipitation of 0.25% κ-casein by heat (100 C for 5 min) at pH 6.2 (cacodylate-NaCl) with the salt concentration expressed as ionic strength (the ionic strength of NaCl equals the molarity, that of $CaCl_2$ equals three times the molarity). The first point on the NaCl curves represents $CaCl_2$ alone and this concentration is present in all the NaCl solutions. The ionic strength given does not include the 0.025 M buffer and 0.025 M NaCl present in all experiments.

These experiments were done at pH 6.2. An experiment with 0.05 M $CaCl_2$ and increasing concentrations of NaCl at pH 7.2 (imidazole-NaCl) gave a precipitation curve identical with that for pH 6.2. κ-Casein heated with sufficient $CaCl_2$ (0.2 M) to prevent precipitation was not a good stabilizer of α_s-casein; the stabilizing ability of such solutions was about the same as in systems containing low concentrations (0.020 to 0.005 M) of $CaCl_2$ (5) in which precipitation occurred. (The ionic strength of milk based on the major free salts is about 0.08.)

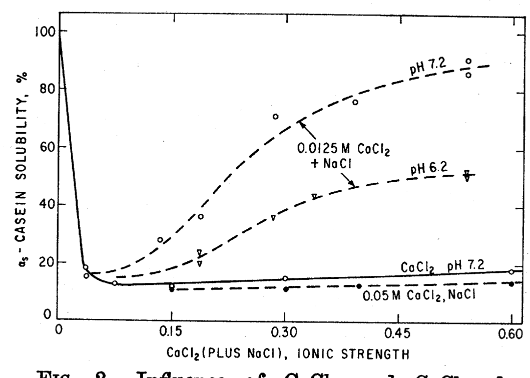

FIG. 2. Influence of $CaCl_2$, and $CaCl_2$ plus NaCl, on the precipitation of 0.25% α_s-casein (no heat) at pH 6.2 and 7.2 (imidazole-NaCl). Salt concentrations are expressed as ionic strength. The first point of the NaCl curve represents $CaCl_2$ alone, and this concentration is present in all the NaCl solutions. The ionic strength given does not include the 0.25 M buffer and 0.025 M NaCl present in all experiments.

To determine whether the effect of excess salt was principally a salting-in effect, some similar experiments were done with unheated α_s-casein (0.25% concentration), which is precipitated by low concentrations of $CaCl_2$. The effect of excess salts in this system is shown in Figure 2. The effect of excess $CaCl_2$ alone is slight. The curve shown is for pH 7.2, imidazole buffer, but comparable results were obtained at pH 6.2 either in imidazole or in cacodylate buffer. When the $CaCl_2$ concentration was 0.05 M (0.15 ionic strength), addition of NaCl had little effect either at pH 7.2 or 6.2. When, however, the concentration of $CaCl_2$ was 0.0125 M, a concentration sufficient to precipitate over 80% of the α_s-casein, then NaCl was effective in decreasing precipitation. The effect was more marked at pH 7.2 (imidazole) than at pH 6.2 (imidazole or cacodylate). The order of adding the salts made a difference. In these experiments the $CaCl_2$ was added last. When the NaCl was added last (the α_s-casein already precipitated by the $CaCl_2$) then the solubility was at a lower level, thus indicating that all of the components were not in true equilibrium.

κ-Casein heated in the presence of salts and mercaptoethanol is heat-labile, as measured by its inability to stabilize α_s-casein (5). The effect of having α_s-casein present when the κ-casein was heated was investigated and the results are shown in Figure 3. For these stabilization tests the calculations were based on total α_s-casein, including that usually in the test system plus

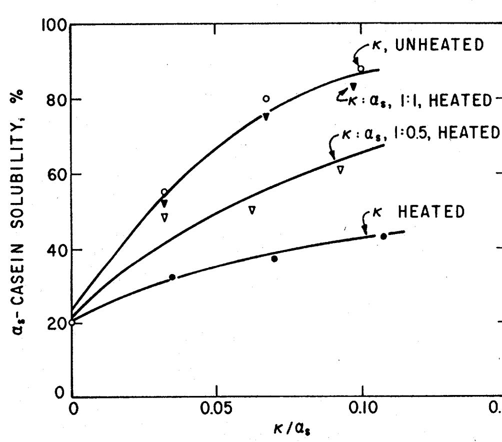

FIG. 3. Stabilization curves of κ-casein heated (100 C for 5 min) with α_s-casein present and subsequently tested in the standard stabilization test. The solutions heated contained 0.25% κ-casein in 0.025 M imidazole-0.025 M NaCl, pH 7.2, with 0.032 M mercaptoethanol. The influence of α_s-casein in this mixture was studied at 0.12% or 0.25% concentration. κ-Casein, unheated O and κ-casein, heated •; κ-casein—0.12% α_s-casein, heated ▽; κ-casein—0.25% α_s-casein, heated ▼.

that contributed by the α_s-casein in the heated mixture. It is evident that α_s-casein imparts heat-stability to κ-casein and it is fully effective at a $\kappa:\alpha_s$ weight ratio of 1:1.

Since the concentration of α_s-casein required to stabilize κ-casein is rather large, it was desirable to determine whether proteins in general exert a similar protective effect. For this purpose α-lactalbumin was used. The experiments were done without mercaptoethanol, to avoid sulfhydryl-disulfide interactions between the proteins, at pH 6.2 for maximum heat lability (5). The α-lactalbumin was ineffective in preventing heat lability of κ-casein even at a 1:1 ratio by weight.

Discussion

The ability of excess $CaCl_2$ or NaCl to prevent the precipitation of κ-casein which occurs when heated with low concentrations (0.005 to 0.050 M) of $CaCl_2$ explains the results of Hayes et al. (1) referred to previously (5). These workers reported that a 5% concentration of κ-casein containing 0.5 M $CaCl_2$ did not form a gel or precipitate even on gentle boiling. The present results indicate that this was due to the high concentration of $CaCl_2$ used and shows that the effect extends to high concentrations of κ-casein. In spite of the suppression of gross aggregation by excess salts, the heated κ-casein has little ability to stabilize α_s-casein. It had previously (5) been surmised that the decreased stabilizing ability of heated κ-casein might be due to cross bonding leading to a degree of molecular aggregation.

Although the effects of excess $CaCl_2$ and NaCl are qualitatively similar, the marked influence of the $CaCl_2$ concentration in the NaCl experiments indicates that the calcium ion has a certain degree of specificity, perhaps because of calcium ion binding exerted even in the presence of excess NaCl or $CaCl_2$. The solubilizing effect of NaCl on $CaCl_2$ precipitates of whole casein has been reported in another paper (2).

The effect of excess salts on heated κ-casein precipitate is probably not due alone to a salting-in effect on a κ-casein precipitate which potentially can still be formed in this system, but rather to a direct effect of the salts on the heat-treated aggregation process itself. This conclusion seems evident from the markedly different effect of salts on the $CaCl_2$ precipitation of α_s-casein. In this case excess $CaCl_2$ exerts very little effect on the precipitation of α_s-casein, and NaCl is not effective unless the concentration of $CaCl_2$ is very low (0.0125 M). The components of these mixtures are evidently not in equilibrium, because when the $CaCl_2$ was added first, subsequent addition of NaCl did not decrease the solubility level to that obtained when $CaCl_2$ was added last. Similar observations have been made with systems containing both α_s- and κ-caseins (3).

The ability of $\alpha_s$casein to prevent heat damage to the κ-casein probably results from the specific association between these two proteins. The need for the high ratio of $\alpha_s:\kappa$ of 1:1 for protection is not clear. The negative experiments with α-lactalbumin support the conclusion that the results with α_s-casein are specific and not simply a general protein effect.

References

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