

RESEARCH PAPERS

INFLUENCE OF SODIUM CHLORIDE ON THE STABILITY OF THE CALCIUM CASEINATE MICELLE

SUMMARY

Sodium chloride of relatively low concentration (0.17 M) destabilizes colloidal calcium caseinate (whole casein) so that part of it precipitates. This occurs also with colloidal calcium caseinate prepared from calcium-sensitive (α_s)-casein and kappa-casein. In the first system the optimal concentration of sodium chloride for destabilization is independent of the calcium chloride concentration, whereas in the second system the optimal concentration of sodium chloride increased with calcium chloride concentration. The dissociation of the α_s -casein-kappa-casein complex, manifest by precipitation of the dissociated α_s -casein by calcium chloride, was increased by decreasing the temperature from 30 to 7 C, and increased further by the addition of sodium chloride (0.15 M).

Sodium chloride (at saturation, about 5 M) will not precipitate sodium caseinate, but it will precipitate calcium caseinate. Investigation of the influence of calcium concentration and pH on this precipitation revealed that casein is partly precipitated at much lower concentrations of sodium chloride (0.17 M). Details of this observation are reported. In addition, the influence of sodium chloride on the formation of the calcium caseinate micelle at 7 C is reported, a reaction that presumably involves the association between the calcium-sensitive and the kappa-caseins. These latter results have been reported briefly (3).

MATERIALS AND METHODS

Whole casein. This was acid-precipitated casein prepared in the usual way (6). Neutral solutions of sodium caseinate were prepared by adding NaOH to an aqueous suspension of casein, avoiding an excess of the alkali.

Kappa-casein. Prepared by fractionation in ethanol, essentially by the method of McKenzie and Wake (1).

Calcium-sensitive (α_s) casein. Prepared by fractionation in urea (5).

The influence of sodium chloride on calcium caseinate was determined at room temperature (22 to 25 C). The calcium caseinate was prepared by the addition of calcium chloride to a neutral solution of sodium caseinate and the

pH adjusted to the required value of pH 6 or 7 with 0.1 N HCl. For the higher concentrations of sodium chloride, the solid was added to the 10-ml volume of the test solutions. Since the sodium chloride contributed very little in volume, the molarities were calculated from the weight of sodium chloride and the volume of the test solution. For the lower concentrations of sodium chloride, the required volume of a 30% solution was added and the total volume adjusted to 10 ml.

The test mixtures were held at 30 C for 15 min, then centrifuged at about $3,000 \times G$ for 5 min. A portion of the supernatant solution was withdrawn for determination of the protein remaining in solution. After dilution, one drop of 0.5 N NaOH was added for clarification, and the protein concentration was determined from the light absorbance (optical density) at 280 $m\mu$ with a 1-cm light path. A factor of 1.0 was used for converting light absorbance to milligrams of casein per milliliter.

Other experimental conditions are described under each experiment.

RESULTS

The influence of sodium chloride on the calcium caseinate micelle was determined for 0.02, 0.03, and 0.04 M calcium chloride. The results are shown in Figure 1 for pH 6.0. Results were the same at pH 7.0. The concentration of calcium chloride determined the level of solubility with no sodium chloride. In each case, the addition of sodium chloride destabilized the micelle. Maximum destabilization of the calcium caseinate micelle occurred with a sodium chlo-

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ride concentration of 0.17 M, irrespective of the calcium chloride concentration. This was also found with a calcium chloride concentration of 0.010 M, not shown in the figure. Somewhat larger concentrations of sodium chloride brought about solubilization of the casein in nonmicellar form, that is, the solutions were clear. Whole casein precipitated (not shown) quantitatively, as did α -casein, at near-saturated concentrations of sodium chloride (about 5 M) with the concentrations of calcium chloride used in the above experiments. Precipitation did not occur with sodium caseinate. Kappa-casein with 0.020 M calcium chloride precipitated slowly and incompletely with 5 M sodium chloride; with 0.20 M calcium chloride precipitation with sodium chloride was rapid and complete.

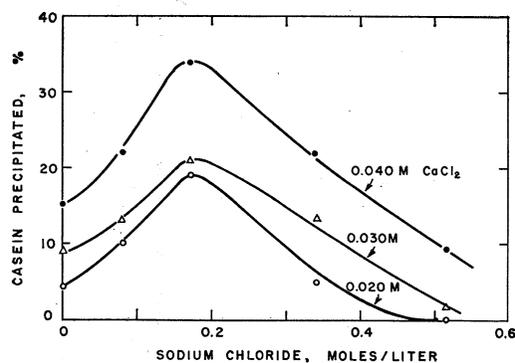


Fig. 1. Destabilization of calcium caseinate micelles by sodium chloride. Concentration of whole casein was 1%.

Similar experiments were performed with the reconstituted casein complex obtained by mixing α -casein and kappa-casein. Calcium ions were added to obtain micelles and the influence of sodium chloride determined. The results are shown in Figure 2. Sodium chloride destabilized this simplified casein system also. In this instance the concentration of sodium chloride giving maximum destabilization was influenced by the calcium chloride concentration.

The influence of sodium chloride on the dissociation of the α -casein and kappa-casein complex also was studied. The results shown in Figure 3 for 7 and 30 C were obtained with a relatively crude preparation of kappa-casein containing considerable of the λ -casein fraction. These results have, however, been confirmed with the purified kappa-casein used for the other experiments in this paper. The complex dissociates strongly at the lower temperature which makes possible the precipitation of the α -casein by the calcium ions. This process is

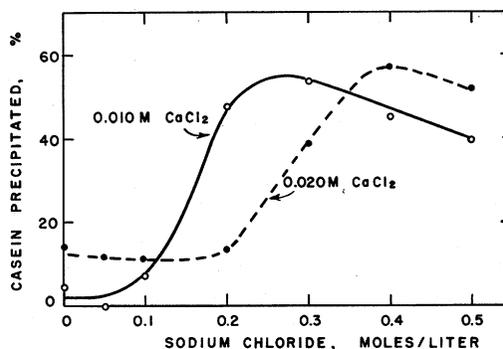


Fig. 2. Destabilization of caseinate micelles, formed from calcium-sensitive (α -)casein, kappa-casein and calcium chloride, by sodium chloride. Concentration of α -casein 0.3%, of kappa-casein 0.03%, concentration of calcium chloride as indicated.

not reversed at higher temperatures, for the test is completed by warming to 30 C (4). The presence of sodium chloride increases the dissociation at 7 C, and this is manifested by increased precipitation of the α -casein. Addition of sodium chloride at 30 C, however, had a negligible effect on the results. The slight tendency to enhance the casein solubilization shown here was less pronounced in other experiments. This appeared to be contrary to the destabilization results reported in Figure 2. The methods differed in detail, but the principal difference seemed to be the order in which the sodium chloride and the calcium chloride were added. Consequently, the type of experiment shown in Figure 3, in which the calcium chloride was added last, was performed with the sodium chloride (final concentration 0.15 M,

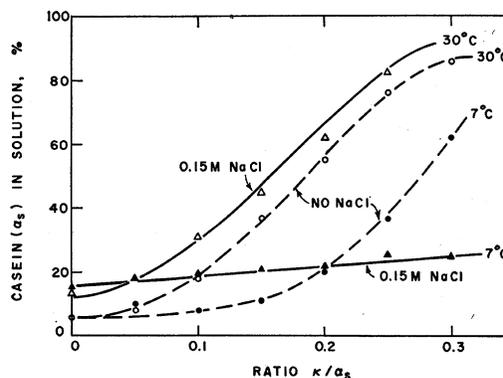


Fig. 3. Influence of sodium chloride concentration (none, and 0.15 M), and temperature (7, 30 C) when mixed, on the stabilization of calcium-sensitive casein (α -) by a preparation of kappa-casein. Concentration of calcium chloride was 0.02 M.

added in a volume of 0.35 ml) added last. In this case a destabilization did occur. With a K/α_s ratio at which 75% of the α_s -casein was soluble, addition of the sodium chloride decreased the solubility to 53%.

DISCUSSION

Colloidal calcium caseinate is negatively charged. This charge helps to maintain the calcium caseinate as a physically stable colloid. Presumably, the low concentration of sodium chloride (0.17 M) reduces the stabilizing repulsive effects between the negatively charged ions and destabilization leads to precipitation of a part of the casein. Electrophoretic examination of the precipitate from whole casein showed that no fractionation had occurred. With the addition of still larger concentrations of sodium chloride a considerable ion atmosphere forms about the casein molecule, which leads to almost complete solubilization of the casein. At concentrations of sodium chloride approaching saturation the salt ions compete with the casein for water molecules, so that a total precipitation of the calcium caseinate occurs. That this occurs with calcium caseinate and not with sodium caseinate emphasizes the strong binding of calcium to casein and its marked influence on the properties of the product. It is expected that the observed destabilizing action of low concentrations of sodium chloride on calcium caseinate will also be evident in more concentrated systems and would have a marked influence on the physical state of milk products.

The destabilizing action of sodium chloride on calcium caseinate is also obtained with a mixture of calcium-sensitive (α_s)-casein and kappa-casein. Since in this case the concentration of sodium chloride giving maximum destabilization shifted with the concentration of calcium chloride, other components of whole casein, particularly β -casein, probably have a role in the stability of the total system also. The fact that the results are influenced by the

order in which the sodium chloride and the calcium chloride are added suggest that there are time (aging) effects or that the ionic components are not in equilibrium.

The influence of sodium chloride on the stability of the α_s -casein-kappa-casein mixture at 7 C when calcium chloride is added reflects its influence on the dissociation of the α_s -casein-kappa-casein complex. When the complex is dissociated the calcium ion precipitates the α_s -casein, an interaction that is not reversed at higher temperatures. The results in Figure 3 show that a decrease in temperature from 30 to 7 C facilitates the dissociation and this is enhanced by the presence of sodium chloride. Methods for the separation of the α_s -casein from the kappa-casein utilize this effect of low temperature; the solution of whole casein is chilled (1, 2) before the calcium chloride is added; subsequently, the temperature is raised to obtain complete precipitation of the α_s -casein.

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