STUDIES OF CASEIN

IV. THE ISOLATION OF κ-CASEIN*

By R. G. WAKE†

The preparation of second-cycle casein—fraction S—from skim milk has been described in Part I of this series (McKenzie and Wake 1959*a*) and by Waugh and von Hippel (1956). This fraction contains mainly κ-casein, as well as β- and γ-casein, and a number of minor components. In the present communication a method is described for the separation and purification of the κ-casein. Some observations on its chemical and physical properties are also included.

Experimental

A solution of second-cycle casein—fraction S (Fig. 1(*a*)) was prepared from skim milk according to the earlier procedure except for centrifugation at 50,000 r.p.m. (using a Sharples supercentrifuge with a type T-9-46 clarifier bowl) to spin out and wash the micelles. The pH of this solution (containing approx. 0·3 per cent. protein) was adjusted to pH 4·6 at 2°C with 0·5N HCl. On subsequent warming to 35°C most of the protein was precipitated. After collecting the precipitate by centrifugation for 20 min at 1000 *g* and room temperature in a M.S.E. laboratory centrifuge, it was dissolved in water at pH 7·5 by the slow addition of 1N NH_3 to give a 2 per cent. solution approximately. This was made 0·4M with ammonium acetate, and 95 per cent. ethanol was added slowly, with stirring, to a final concentration of 50 per cent. The solution was adjusted to pH 5·7 (see Part I for pH measurements) by the slow addition of 2N acetic acid in 50 per cent. ethanol. Stirring was continued for 1 hr and the precipitate removed by filtration on a Whatman No. 1 paper. This material contained approximately 90 per cent. κ-casein (Fig. 1(*b*)). Most of the slowly sedimenting impurity, probably β-casein, was removed as follows: the crude κ-casein was dissolved at pH 12 by the slow addition of 2N NaOH to give a 0·4 per cent. solution approximately, adjusted to pH 7 with 1N HCl, cooled to 2°C, and finally precipitated at pH 4·4 by the addition of cold 0·2N HCl (β-casein is more soluble than κ-casein under these conditions of temperature and pH). The precipitate was obtained by centrifugation for 10 min at 2°C and 895 *g* in an International type PR-2 refrigerated centrifuge. It was essentially pure κ-casein with only a trace, approximately 2 per cent., of β-casein, (Fig. 1(*c*)). Attempts to remove this impurity, which could only just be detected on paper electrophoresis, by dissolution to 0·2 per cent. protein concentration and precipitation at pH 4·4 and 2°C were unsuccessful. This acid-precipitated κ-casein was dissolved at pH 12 (the material is soluble with difficulty at lower pH), adjusted to pH 7, and freeze-dried. Approximately 3 g κ-casein was obtained from 14 l. skim milk.

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Properties

Freeze-dried κ-casein was readily soluble in water at neutral pH and gave no precipitate on the addition of calcium chloride. With α-casein and in the presence of 0·06M $CaCl_2$ it gave micelles which could be clotted by rennin. Preliminary analyses have indicated the presence of phosphorus and neuraminic acid, as well as carbohydrate. No significant quantities of free α-amino end-groups could be detected in κ-casein by means of the fluorodinitrobenzene method of Sanger (1945). The possibility of arginine being present as an end-group has not been settled (see Part V, Wake 1959).

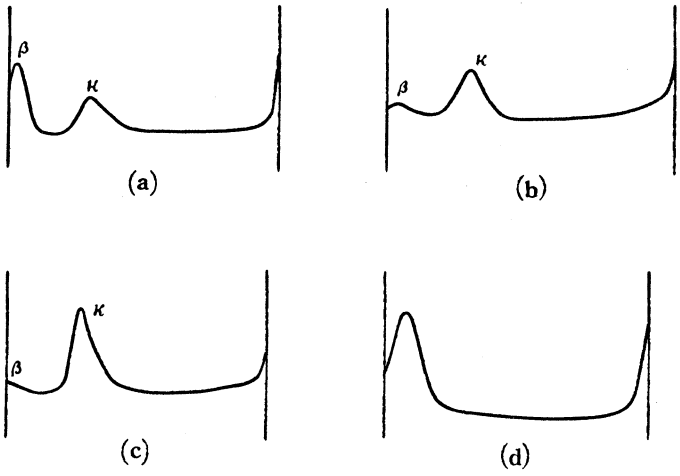

Fig. 1.—Sedimentation (at 59,780 r.p.m.) of fractions obtained during the purification of κ-casein. (*a*), (*b*), and (*c*) were carried out in sodium phosphate buffer, pH 7·0, ionic strength 0·1 (0·05M NaCl) at 2–5°C, using 0·5–1 per cent. protein: (*a*) second-cycle casein—fraction S, 34 min, $\theta = 65°$; (*b*) crude κ-casein, 36 min, $\theta = 55°$; (*c*) purified κ-casein, 26 min, $\theta = 60°$; (*d*) 1 per cent. κ-casein in sodium phosphate buffer, pH 12·0, ionic strength 0·19, at room temperature, 59 min, $\theta = 65°$. The preparation of the pH 7·0 buffer is described in Part I (McKenzie and Wake 1959*a*), and that of the pH 12·0 buffer in Part III (McKenzie and Wake 1959*b*).

Moving-boundary electrophoresis of 0·7 per cent. κ-casein in sodium phosphate buffer at pH 7·1, ionic strength 0·1 (0·08M NaCl), showed a single peak in both the ascending and descending limbs. The mobility was $-6{\cdot}80\times10^{-5}$ cm^2 V^{-1} sec^{-1}. Sedimentation under similar conditions (pH 7·0, low temperature) showed essentially a single peak with S_{20} approximately 13 S (Fig. 1(*c*)). This peak represents an aggregate of very high molecular weight. In sodium phosphate buffer at pH 12·0, ionic strength 0·19 (von Hippel and Waugh 1955), 1·0 per cent. κ-casein showed a single, well-defined peak, with $S_{20} = 1{\cdot}0$ S (Fig. 1(*d*)). This probably represents the κ-casein monomer.

An estimate of the molecular weight of κ-casein at pH 12·0 has been obtained by application of the Archibald ultracentrifugal procedure (see Part III, McKenzie and Wake 1959*b*). The molecular weight is in the vicinity of 26,000.

κ-casein can thus be prepared in essentially pure form from soluble casein. It is a mucoprotein containing phosphorus, neuraminic acid, and probably other sugars (cf. bovine submaxillary mucin (Gottschalk 1958)) and has no detectable α-amino end-groups in significant quantities. It is highly aggregated at neutral pH but shows single peaks on electrophoresis and sedimentation. It can be disaggregated into monomers at pH 12 with a molecular weight of approximately 26,000.

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