Physicochemical Properties of Human Lactalbumin

By MASAHISA MAENO AND ISAO KIYOSAWA

Central Research Laboratory, Morinaga Milk Industry Co. Ltd., Tokyo, Japan

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Proteins of the same kind in human milk and cow's milk have different properties. Mellander (1947) reports differences in phosphorus content and the amount of carbohydrates of casein and that the casein of cow's milk is more easily digested by proteolytic enzymes *in vitro* than that of human milk. Kiyosawa, Ryoki & Maeno (1961) observed a marked difference in the solubility of casein and the turbidity created by binding inorganic salts to casein. Deutsch (1947) and Wegelin (1952) showed that there were different electrophoresis patterns of whey protein in human milk and cow's milk. Physical and chemical properties of crystalline β -lactoglobulin (Palmer, 1934; Sørensen & Sørensen, 1939; Deutsch & Bain, 1948) and α -lactalbumin (Gordon & Semett, 1953) of cow's milk have been studied. Johansson (1958) separated lactalbumin from human milk by calcium phosphate-column chromatography. Casein and lactoglobulins in the whey were removed with ammonium sulphate at 50% saturation, the lactalbumin fraction was then precipitated by saturation with ammonium sulphate and separated into three components (serum albumin, lactalbumin and salmon-coloured component) by calcium phosphate-column chromatography.

In the present study, the lactalbumin was prepared from human-milk whey by a method different from that adopted by Johansson.

EXPERIMENTAL

Materials. Human milk collected from women more than 10 days after parturition was used. Immediately after its collection, the milk was frozen and stored. It was then heated to 30°, and fat was removed two or three times by a cream-separator (Alpha Laval).

Method. A portion (10 l.) of the skim milk described above was cooled to 5° and the pH was adjusted to 4.7 by addition of 0.1N-HCl, with glass-electrode pH meter (Beckman, model H2). After storage at about 2° overnight, skim milk was warmed to room temperature (about 25°) and centrifuged for 30 min. at 34 850g (Spinco model L ultracentrifuge, rotor 30) to precipitate casein. A small amount of fat was removed from the surface and a clear supernatant (whey) was obtained. The whey (9.4 l.) was adjusted with aq. N-NH₃ soln. to pH 6.6 and then (NH₄)₂SO₄ was added slowly, with stirring, to a final concentration of 2.3M. After about 1 hr., the precipitate was filtered off with Toyo filter paper (no. 2). When 0.1N-HCl was added

to the filtrate, a precipitate began to appear at about pH 5.0 and the albumin fraction was completely precipitated at pH 4.8. The precipitate obtained on filtration had a pale salmon colour and was suspended in a small quantity of water and dialysed against water at about 2°. After dialysis for 4 days by changing water two or three times a day, the solution became clear and salmon-pink and contained lactalbumin, red protein and serum albumin (Fig. 1a). The solution was warmed to room temperature and adjusted to pH 4.8 with 0.1N-HCl, and the lactalbumin formed a white precipitate (Fig. 1b). The precipitate was dissolved in water by the addition of aq. 0.1N-NH₃ soln. at the protein concentration of about 2% and pH 7.0. Lactalbumin was precipitated on addition of 0.1N-HCl to pH 4.8. This procedure was repeated two or three times at room temperature. The lactalbumin used for electrophoresis and ultracentrifuge experiments was thoroughly washed with water and dried carefully at -2° with ethanol and ether cooled to -10°. Denaturation of the lactalbumin was not observed electrophoretically by the above-mentioned procedure. For the solubility measurements, the lactalbumin precipitated was suspended in water at the protein concentration of about 5% and freeze-dried (Kyowa, model RL-500s). About 7.1 g. of electrophoretically pure lactalbumin was obtained in this experiment.

RESULTS

Solubility. Solubility measurements were carried out by the method of Northrop, Kunitz & Herriot (1948). A test tube containing 0.05 g. of lactalbumin, 5 ml. of McIlvaine's buffer and one small glass bead was closed with a rubber bung and fixed to a pulley rotating at 40 rev./min. in water at 25°. After 1 hr., the solution was centrifuged for 30 min. at 6590g (rotor 40) and nitrogen in the

Fig. 1. Paper-electrophoresis patterns of the albumin fraction in veronal buffer (pH 8.5, *I* 0.05) at 5° after electrophoresis for 5 hr. at a field strength of 0.3 mA/cm. (a) Albumin fraction; (b) lactalbumin isolated from albumin fraction.

supernatant was determined by the micro-Kjeldahl method. As shown in Fig. 2, lactalbumin is dissolved completely below pH 3.20 and above pH 6.00, but not completely between pH 3.20 and 6.00. Only about 10% of lactalbumin is dissolved at pH 4.6, which is near the isoelectric point of lactalbumin.

Electrophoresis. Electrophoresis experiments were carried out in the glycine, acetate, phosphate

Fig. 2. Effect of pH on mobility (○) and solubility (●) of lactalbumin. Mobility was measured at 0°, I 0.1 and at a concentration of about 1.0% lactalbumin. Veronal buffer, pH 8.5, phosphate buffer, pH 6.0 and 7.0, sodium acetate buffer, pH 5.5, and glycine buffer, pH 2.0, 3.0 and 3.5, were used. McIlvaine's buffer was used for the solubility experiment at 25°.

Fig. 3. Electrophoresis patterns of lactalbumin at 4°, I 0.1 and a protein concentration of about 1.0% after 1 hr. (a) Veronal buffer, pH 8.5, with a field strength of 7.24 v/cm.; (b) glycine buffer, pH 3.5, with 6.26 v/cm.; (c) glycine buffer, pH 2.0, with 5.32 v/cm.

and veronal buffers of Miller & Golder (1950), with the Tiselius apparatus (Hitachi, model HT-D), and mobility was calculated by the method described by Warner (1944). Fig. 3 shows the electrophoretic patterns of lactalbumin at different pH values. The patterns of lactalbumin were homogeneous at pH 2.0, 5.5 and 8.5, but separated into two components at pH 3.5. When mobility was plotted against pH at I 0.1 (Fig. 2), the isoelectric point was found to be about pH 4.8. The mobility of α -lactalbumin of cow's milk is

$$-4.2 \times 10^{-5} \text{ cm.}^2 \text{ v}^{-1} \text{ sec.}^{-1}$$

(veronal buffer, pH 8.5, I 0.1) and that of β -lactoglobulin is $-5.1 \times 10^{-5} \text{ cm.}^2 \text{ v}^{-1} \text{ sec.}^{-1}$ (veronal buffer, pH 8.4, I 0.1) (Larson & Jenness, 1955). The mobility of lactalbumin of human milk is different from that of these proteins of cow's milk.

Ultracentrifuge experiments. Sedimentation studies were made with the Hitachi model UCA-1 ultracentrifuge, synthetic boundary cells being used to determine the homogeneity of lactalbumin at 54 780 rev./min. (220 000g). Sodium phosphate buffer containing 4.64 g. of NaCl, 0.81 g. of Na_2HPO_4 and 0.38 g. of NaH_2PO_4 /l., pH 7.0, I 0.1, was used. After dialysis, with stirring, against buffer at 2° overnight, the protein solution was used for the sedimentation experiments. A single symmetrical boundary was observed for lactalbumin (Fig. 4). The sedimentation coefficient (at about 1.0% concentration) is 1.7s, which is very similar to the sedimentation coefficient of 1.73s for

Fig. 4. Sedimentation diagram of lactalbumin obtained in phosphate buffer (pH 7.0, I 0.1) at 18.9° and 220 000g after 1 hr.

Table 1. *Molecular weight of the lactalbumin*

Concentration was 0.82 %.

Time (min.)	Top portion of cell	Bottom portion of cell
20	22 900	22 800
30	23 000	23 100
40	23 200	23 100
50	22 800	23 000

lactalbumin isolated by Johansson (1958). The partial specific volume of a 1 % soln. of lactalbumin was found to be 0.72. The molecular weight was calculated by the method of Klainer & Kegeles [1956; based upon Archibald's (1947) suggestions]. The sedimentation experiment for this purpose was performed at 32 000 rev./min. The molecular-weight results are summarized in Table 1. Klainer & Kegeles reported no significant difference in the molecular weight of bovine plasma albumin with the concentration of the solute, and we did not study the variation of the molecular weight with concentration of lactalbumin. We calculated the molecular weight of lactalbumin to be 23 000.

DISCUSSION

Human casein is not as easily precipitated by acids as is cow's casein. In order to prepare the whey protein in human milk, Deutsch (1947) removed casein by adding rennet to human milk and adjusting to pH 4.8. Mellander (1947) also adjusted human milk to pH 4.6 by adding hydrochloric acid and diluted the acidified milk with water to precipitate casein. But the denaturation of whey proteins must be avoided during the treatment with rennet and the dilution of whey proteins is unsuitable for the preparation of lactalbumin. It is therefore desirable to remove the casein from acidified milk by ultracentrifuging. There is no detailed description of the preparation and characterization of lactalbumin by Johansson's method. It is therefore impossible to compare his method and characterization of lactalbumin with ours. On the point of the heterogeneous electrophoresis patterns of purified protein, Polis, Schmuckler, Custer & McMeekin (1950) and Gordon & Semett (1953) reported that β -lactoglobulin and α -lactalbumin prepared from cow's-milk whey were divided into two components at certain low pH values. The reason is thought to be

the intermolecular interactions between components. The electrophoresis pattern of human lactalbumin is similar to that observed for whey proteins of cow's milk. The molecular weight of lactalbumin, 23 000, was higher than that of cow's α -lactalbumin, 17 000, measured by Svedberg (1937) by the sedimentation method.

SUMMARY

1. The separation of lactalbumin from the whey of human milk and its physicochemical properties are described.

2. The mobility of lactalbumin was -4.7×10^{-5} cm.²v⁻¹ sec.⁻¹ at pH 8.5, I 0.1, and the electrophoresis patterns were heterogeneous at pH 3.5, but homogeneous at pH 3.0 and 5.5.

3. A single symmetrical sedimentation boundary of lactalbumin was obtained. The sedimentation coefficient was 1.7s and the molecular weight 23 000.

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