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Short Communications

Isolation of Crystalline Lactoferrin from Human Milk

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A red protein, lactoferrin,[1] with metal-binding properties similar to those of plasma transferrin. has been isolated from human milk by various investigators.[2–5] The protein differs from transferrin in immunologic properties [2,3,5] and in its amino acid and carbohydrate composition.[6,7] It also occurs in other body secretions,[8–11] but seems to be most readily isolated from human milk. No reports on the crystallisation of lactoferrin has hitherto appeared. A simple procedure for isolating crystalline lactoferrin is described below.

One liter of fresh human milk was diluted with two volumes of distilled water containing 5 mg of Fe^{3+} (in the form of ammonium ferric sulphate). 3 g of dry CM-Sephadex C-50 (Cl^--form) was then added and the mixture stirred for 1 h. The ion exchange gel was allowed to settle. It was distinctly red.

The reddish CM-Sephadex gel was suspended in 500 ml of distilled water, which was siphoned off after the gel had settled. This washing procedure was repeated twice. The gel was then packed into a column, 5 cm in diameter. Washing with 200 ml of 0.05 M tris-HCl, pH 8.0, eluted small amounts of uncoloured protein. The lactoferrin was eluted with 2 M NaCl, buffered at pH 8.0 with 0.05 M tris-HCl, and was obtained as less than 50 ml of a deep red solution, containing 0.5–1 g of protein, mainly lactoferrin. After adjustment of the pH to 7.0 by addition of 1 M HCl the solution was dialysed against 10 volumes of ice-cold 0.005 M sodium phosphate buffer, pH 7.0, which was changed daily. After 2–3 days the solution turned opalescent and a deep red viscous oil was formed, which completely separated from the water phase and was gradually converted into needle-shaped crystals. The crystallisation rate was low (2–4 weeks) and some preparations did not crystallise unless the oil was seeded with a few lactoferrin crystals. The crystals formed were spun down in a centrifuge at +2°C, dissolved in 10 ml of 2 M NaCl, buffered at pH 7.0 with 0.02 M phosphate buffer, and recrystallised by renewed dialysis. Large crystals, 3–5 mm long were obtained by transfer of a partially dialysed lactoferrin solution to a collodium tube (Membrangesellschaft, Göttingen) and continued dialysis of the protein under very slightly reduced pressure. Such crystals are shown in Fig. 1.

Fig. 1. Lactoferrin crystals (×5).

The crystalline preparation was tested for homogeneity by means of urea-starch gel electrophoresis at two pH. Whereas the protein was apparently homogeneous in glycine buffer, pH 8,[12] electrophoresis in formate buffer, pH 3,[13] revealed several minor zones besides the main band. After two recrystallisations only one of these

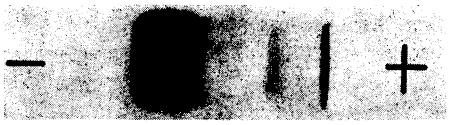

Fig. 2. Urea-starch gel electrophoresis of three times crystallized lactoferrin. The electrophoretic run was performed in 0.035 M sodium formate buffer, pH 2.8, for 7 h at 8 V/cm.[13]

minor bands remained (Fig. 2), which could not be removed by hydroxyapatite chromatography [2] of the preparation The electrophoretic pattern did not change by reduction and alkylation of the protein in 8 M urea.[13] Immunoelectrophoresis of the lactoferrin in a concentration range of 0.1 – 5 % (w/v) against anti-human colostrum serum [14] or anti-transferrin serum did not reveal any impurities.

The light absorption at the maximum of 465 nm was measured to 0.540 for a 1 % solution of lactoferrin at pH 8.2. This value was obatined after addition of bicarbonate ions and is considerably higher than that reported previously.[2] The iron content of the iron-saturated protein was 0.137 % and 0.147 % (w/w) in two preparations, which indicates a minimal molecular weight in the range of 38 000 – 41 000. On thin layer gel filtration in Sephadex G-150 the lactoferrin migrated at the same rate as transferrin. These results suggest a molecular weight of about 80 000 and 2 Fe^{3+} ions bound to each molecule. Similar results have been reported by Masson and Heremans.[10] A single symmetrically sedimenting boundary was observed in the analytical ultracentrifuge with an $s_{20,w}^{\circ}$ of 5.25 ± 0.05 S, a value considerably higher than those reported by other authors.[3–5] It should be noted that a sedimentation coefficient of 5.55 S has been reported for the analogous *bovine* milk protein.[15]

The crystalline lactoferrin seems to be suitable for further studies of the properties of this protein in relation to plasma transferrin.

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