INTERACTION OF FLUORIDE IONS WITH MILK PROTEINS STUDIED BY GEL FILTRATION

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SUMMARY: The interaction of fluoride ions with the bovine milk protein a-lactalbumin, type I α-casein, β-casein, and κ-casein was studied at pH 6.6, 5.5 and 3.9. At pH 6.6 and 5.5 fluoride ions do not combine with any of these protein. However, at pH 3.9 they combine with α-lactalbumin.

Key words: Fluoride; Gel filtration; Milk proteins.

Introduction

Earlier work has shown that fluoride ions may combine with serum albumin (1). In enzymatic proteins such an interaction frequently leads to changes in activity and may be monitored by kinetic methods (2,3). Numerous studies have also revealed that non-enzymatic proteins, such as hen egg albumin and bovine albumin bind fluoride ions (4,5). Methods widely used in these investigations are the gel filtration technique, fluoride electrode implementation, and the equilibrating dialysis method.

A separate problem in nutrition is posed by the presence of fluoride in milk products. Duff ascertained that the fluoride content of milk of cows drinking fluoridated water is greater than in the milk of cows drinking non-fluoridated water (6). This author also noted that part of the fluoride in milk was in a bound state - with organic milk components or with calcium - and that the percentage goes up with increased time of storage.

The aim of the present research was to find out whether fluoride ions combine with the milk proteins α-lactalbumin, type I α-casein, β-casein and κ-casein. Since the pH of fresh milk is 6.6-6.8 and decreases during storage, the investigations were carried out at pH 6.6, 5.5 and 3..9.

Material and Methods

Bovine α-lactalbumin, type I α-casein, β-casein and κ-casein (all from Sigma) were dissolved in 0.02 M phosphate buffer with pH 6.6 and 0.02 M acetate buffer with pH 5.5. Additionally, a solution of α-lactabumin was prepared in 0.02 M acetate buffer with pH 3.9 (at this pH caseins fail to dissolve). To avoid eventual contamination by fluoride ions, these solutions were purified by passing them through a column of "Sephadex G-25" (Pharmacia, Uppsala) equilibrated by protracted rinsing in the afore-mentioned buffers. Protein concentrations in the resulting solutions were determined by measuring the light absorption at 280 nm.

In the studies on the binding of fluoride by proteins, Hummels and Dreyer's technique (7) was employed with minor modification. For this purpose a column (0.8 cm -70 cm) was prepared and filled with Sephadex G-25 fine and equilibrated with the appropriate buffer with added NaF (terminal concentration of fluoride ion was 3.0×10^{-4} M). Subsequently, 1.0 ml of previously purified protein solution (1.0×10^{-4} M) suspended in the same buffer, but without fluoride, was transferred onto the column, which was then rinsed with equilibrating buffer. The flow rate was adjusted to 0.3 ml/min, and 1.0 ml fractions were collected. The protein content in respective fractions was defined by measuring the light absorption at 280 nm.

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The fluoride concentration was measured with the aid of an ion-selective fluoride electrode (Radelkis, Hungary). For this purpose 0.5 ml of each fraction was mixed with 0.5 ml of TISAB buffer (μ = 5.0 M, pH 5.5). Under these conditions it was found that fluoride disssociates from the protein, resulting in measurement of total fluoride concentration.

Results and Discussion

The results presented in Figure 1 indicate that at pH 6.6 and 5.5 none of the proteins bind the fluoride ions. If fluoride combined with protein under the conditions used, the fluoride concentration in fractions containing protein should rise over the concentration value of fluoride in buffer equibrilated with the column, and thereafter fall below this value.

However, we observe a drop in fluoride concentration only in later fractions already deprived of protein. This decrease was due to the fact that a protein sample free of fluoride ions was transformed onto the fluoride-equilibrated column. In the acetate buffer with pH 3.9 the profile for fluoride assumes a characteristic course showing that fluoride ions do combine with lactalbumin (Figure 2).

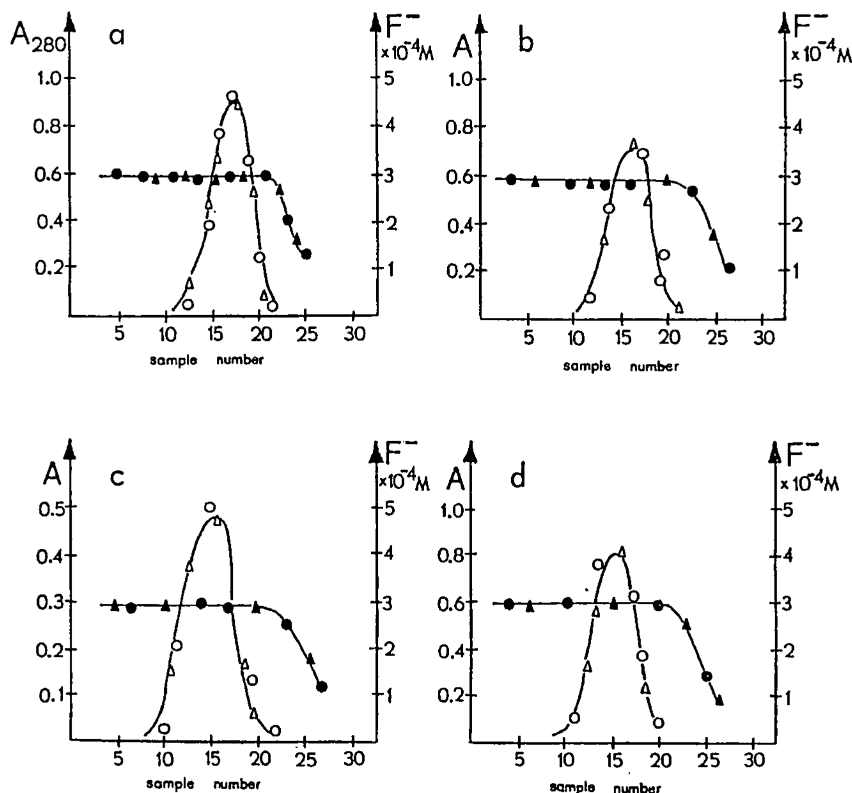

Figure 1. Elution profile of the 280 nm absorbancy (o,Δ) and F^- concentration (●,▲) accompanying the passage of: a) α-lactalbumin, b) type I α-casein, c) β-casein and d) κ-casein through a column of Sephadex G-25 gel which was equilabrated with 0.02 M phosphate buffer, pH 6.6 (o,●) and 0.02 M acetate buffer, pH 5.5 (Δ,▲). Both buffer contained 3.0 x 10^{-4} M NaF. All experiments were carried out at 20°C.

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α- and β-Caseins are hydrophobic acid proteins with isoelectric points at pH 4.1 and 4.6, respectively (8). At both pH 6.0 and 5.5 they appear in anion form, which can account for their lack of interaction with fluoride anions. κ-Casein is also a slightly acidic protein (pK 6.0), and at pH 6.0 exclusively negative charges are present on its surface (9).

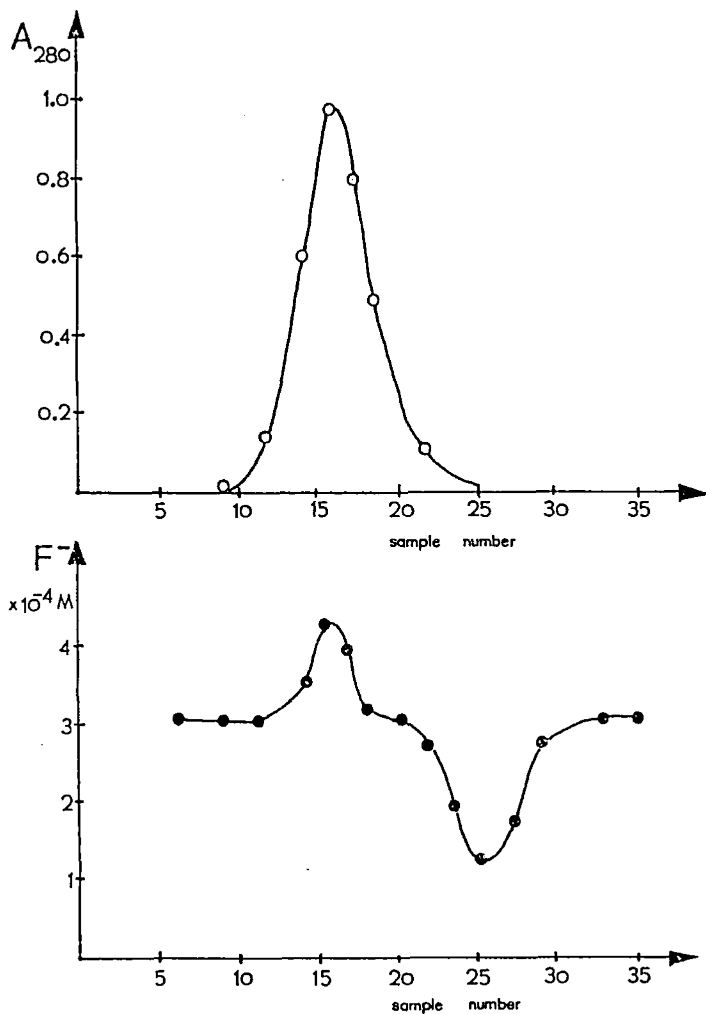

Figure 2. Elution profile of the 280 nm absorbency (o) and F^- concentration (•) accompanying the passage of α-lactalbumin through a column of Sephadex G-25 gel which was equilibrated with 0.02 M acetate buffer pH 3.9 containing 3.0 x 10^{-4} M NaF.

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It is surprising why α-lactalbumin (pK 6.5) does not bind fluoride ions at pH 5.5 but only on reaching pH 3.9. Apparently this pH dependence stems from the residue of asparaginic acid (side chain pK 3.9). Only a pH below this value effects dissociation of the carboxylic groups in the side chain of this amino acid and makes the fluoride ion binding possible by positively charged side chains of other amino acid residues e.g. lysine. α-Lactalbumin also contains in its molecule one atom of calcium (10). It has been shown that the primary agent binding calcium in milk is the residue of asparaginate as well as lysine, and that calcium dissociates from the protein below pH 4 (11,12).

In the light of the above studies, the results established by Duff seem to bear out the idea that fluoride is bound in milk by calcium rather than by proteins. α- and β-Caseins are phosphoproteins with a considerable content of calcium, which passes from the proteins into the solution as pH decreases (13). This fact could also explain the drop in free fluoride ion content in milk during storage, which is accompanied by a lowering of pH.

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