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DENATURATION OF HUMAN LACTOFERRIN IN ACIDIC SOLUTIONS

Gregory Bezkorovainy and Anatoly Bezkorovainy[1]
Biochemistry Department
Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois 60612

ABSTRACT

Iron-saturated human lactoferrin and transferrin and hen's egg conalbumin were dissolved in buffer solutions at pH 1 to 8.3, and the optical rotation at 589 nm, absorbance at 465 nm, and viscosity of the solutions were measured. It was found that some loss of iron from lactoferrin first occurred at about pH 4, whereas gross denaturation did not become manifest until pH 3. Since digestion of iron-saturated lactoferrin by pepsin at pH 3 results in the destruction of only the N-terminal segment of the protein, it is proposed that the denaturation observed at pH 3 was restricted to this portion of the protein. Conalbumin and transferrin lost their iron at pH 6, and became denatured below pH 4.

INTRODUCTION

The non-heme iron-binding proteins serum transferrin, milk lactoferrin, and hen's egg conalbumin play an important role in iron metabolism and host resistance to disease (Zschocke and Bezkorovainy, 1974; Weinberg, 1978). It is well established that these iron-binding proteins lose iron at pH values below 7, though lactoferrin appears to be much more resistant to acid pH than is either transferrin or conalbumin (Bezkorovainy and Zschocke, 1974). Advantage has recently been taken of this property of lactoferrin; when the agent was subjected to proteolysis by pepsin at pH 3, the C-terminus was isolated intact and the N-terminus was destroyed (Line et al., 1976; Bluard-Deconinck et al., 1978). It seemed of interest to determine the gross physical status of lactoferrin and the other two proteins at varying pH, so

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[1] To whom reprint requests should be directed

that the basis for such behavior of lactoferrin could be ascertained.

MATERIALS AND METHODS

Lactoferrin saturated with iron was prepared from defatted human milk by the procedure of Johanssen (1969). Iron-saturated conalbumin was prepared as previously described (Bezkorovainy et al., 1968). Iron-free transferrin was purchased from Behringwerke Corp., Marburg, West Germany, and was saturated with iron using ferric citrate (Line et al., 1967). Buffer solutions used were as follows: pH 8.3, 0.1 M $NaHCO_3$; pH 7 and pH 6, 0.05 M cacodylate-0.1 M NaCl; pH 5 and pH 4, 0.05 M acetate-0.1 M NaCl; pH 3 and pH 2, 0.1 M glycine-HCl; and pH 1, 0.1 M HCl.

Viscosity measurements were done in an Ostwald-type viscometer in a water bath maintained at 31.5°. Volume of fluid used was 4.0 ml, and protein concentrations were kept at 4 mg/ml. Flow time for water was between 222 and 223 sec in this system. Optical rotation measurements at 589 nm were made at 25° in 10 cm cells using the Perkin-Elmer Model 141 instrument, and absorbance measurements were made in the Coleman Junior II spectrophotometer at 465 nm (maximum absorption for iron-containing transferrins, as per Bezkorovainy and Zschocke, 1974).

RESULTS AND DISCUSSION

Proteins were dissolved in the iron-saturated state in the buffer solutions specified, and permitted to stand for 2 hr at 25° before being analyzed. The results are depicted in Figure 1.

Loss of iron as reflected by the decrease in A_{465} took place at pH 6 in case of conalbumin and transferrin, whereas with lactoferrin the first loss was observed at pH 4. Concomitant with the loss of iron, there was an increased (more positive) optical rotation in all proteins (pH 5 and 6 for transferrin and conalbumin, and pH 4 for lactoferrin). This finding is consistent with previous observations (Line et al., 1967). The loss of iron by transferrin and conalbumin was not accompanied by gross denaturation, which became manifest in these proteins at or below pH 4. In lactoferrin, denaturation, as indicated by increased reduced viscosity and levorotation, took place at pH 3 and lower. Hence, it would appear that the observed partial loss of iron in lactoferrin at pH 4 was not due to gross protein denaturation, but to other factors such as the loss of the synergistic anion (for definition see Bates and Schlabach, 1975), or by protonation of an essential histidyl residue. At pH 3, considerable amounts of iron were still present in lactoferrin, though physical parameters pointed to an

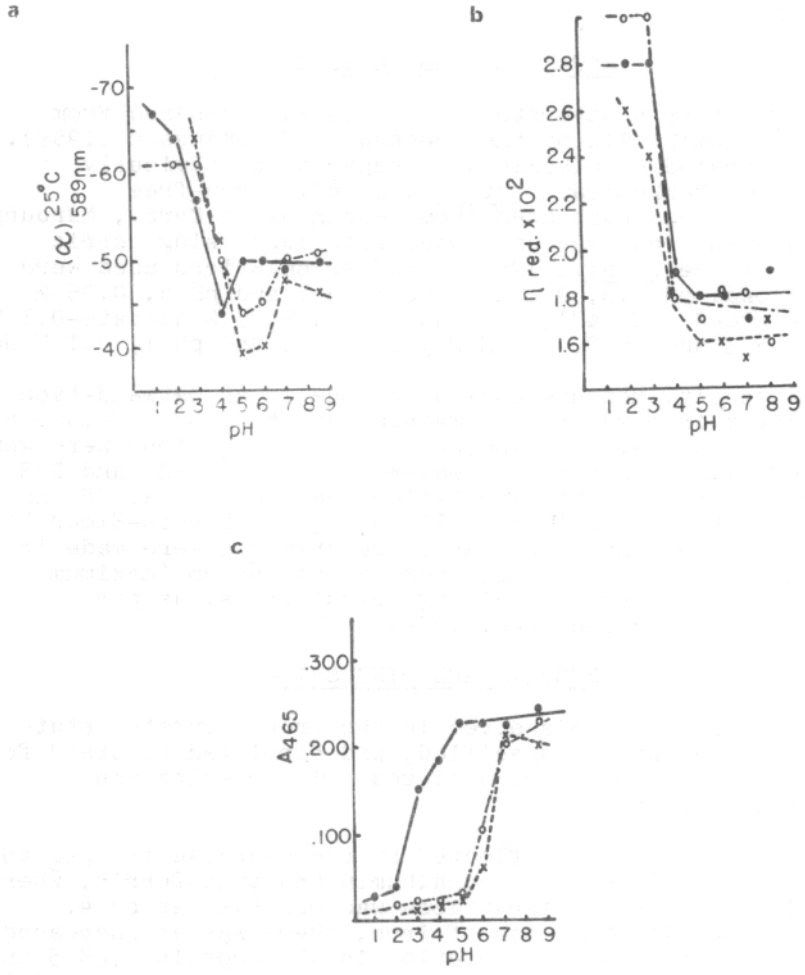

Figure 1. Physical parameters of lactoferrin (solid circles), transferrin (open circles) and conalbumin (crosses) as a function of pH at protein concentrations of 4 mg/ml. a. Optical rotation, b. Reduced viscosity (average of 6 determinations), and c. Absorbance.

extensive degree of denaturation. It is therefore possible that the reason why pepsin at pH 3 can destroy the N-terminal segment of iron-containing lactoferrin while leaving the C-terminus intact and bound up with iron is that the N-terminus is more susceptible to denaturation and thus subject to attack by proteolytic enzymes.

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