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Multiferric ion-induced bovine lactoferrin as a new antianemic material assembly from acidic to neutral pH

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1 Introduction

Bovine lactoferrin (bLf) was first isolated from bovine milk [1]. Kawakami *et al*. found that bLf was able to bind multiferric ions, and the multiferric-bound-bLf was soluble at neutral pH [2]. Hu *et al*. reported that bLf in the presence of 70 mol ferric ions (70FeLf) forms a stable structure [3]. They suggest that multiferric-bound-bLf could be a new antianemic edible material.

However, the conformation of multiferric-bound-bLf, 70FeLf, was yet unknown. So, we have studied the conformational features of 70FeLf at pH 1-6 by small-angle X-ray scattering (SAXS).

2 Experiment

70FeLf was prepared as described in ref. 3. Buffer was prepared acetic acid instead of phosphate buffer.

SAXS experiments were done at the beamline of 10C. The measurements were done, keeping the sample-to-detector distance at ca. 1 m with a PILATUS. Equilibrium experiments were performed at 20°C. For the analysis of Guinier plots, both single and double exponential analyses were used [4]. When irregular aggregates was included in the protein solution, the double exponential analysis is very convenient and useful analysis method to obtain the structural properties of the major component in the solution as are the cases of previous reported by other proteins [4]. Bovine serum albumin was employed for scaling scattering intensities. Concentrations of 70FeLf used in the SAXS experiments were less than 1.0 mg/mL.

3 Results and Discussion

The radius of gyration (R_g) of the major component of 70FeLf values are not changed so much by pH (Table). This suggests that the molecular size of 70FeLf was independent of pH. In contrast, the number of bLf monomer contained in the major component of 70FeLf shows pH-dependence (Table). Why was the number of bLf monomer in 70FeLf changed with pH? Hu *et al*. suggested that the structure model of 70FeLf was described by electrostatic interactions of ferric and bicarbonate ion among bLf molecules. As the apparent pK_a of bicarbonate ion is 6.3, it is difficult to interpret dependence of the number of bLf monomer in 70FeLf against pH only by pK_a of bicarbonate ion. However, it is known that the apparent pK_a is dependent on surroundings. In another study, it is reported that two ferric ions on one lactoferrin molecule was released below pH 4 [5]. These suggest that the change of the number of bLf monomer in 70FeLf by pH might be correlated with the internal conformational change in one bLf monomer molecule or the change of the apparent pK_a of bicarbonate ion.

Table. Estimated parameters of the major component of 70FeLf by SAXS measurements.

pH	R_g (Å)	number of bLf monomer
1	57.6 ± 1.9	8.9 ± 0.3
2	62.8 ± 0.1	11.0 ± 0.2
3	60.9 ± 3.5	13.3 ± 1.1
4	61.4 ± 1.7	13.2 ± 0.7
5	59.5 ± 0.3	13.6 ± 0.1
6	59.9 ± 0.1	15.9 ± 0.3

All parameters are average value of 0.5 and 1.0 mg/mL. Number of monomer was calculated by zero scattering intensity at each pH and molecular weight of bLf monomer (76 kDa)

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