

## Association Mechanism of Lactoferrin with Multiferric Ions

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### 1 Introduction

Bovine Lactoferrin (Lf) from milk is an iron binding protein, and its biological function such as antibacterial activity, regulation of iron absorption, and regulation of the production of macrophages and granulocytes has been well studied. Lf is folded in two lobes, each of which contains a binding site for an Fe<sup>3+</sup> ion [1].

On the other hand, Lf can also bind more Fe<sup>3+</sup> ions (about 70 ions per protein molecule) at other sites in the presence of bicarbonate. Various physicochemical investigations for this form of Lf (70FeLf) have revealed that 70FeLf is more stable than intact Lf and that 70FeLf contains 15 or 16 protomers whereas native Lf is a monomer [2]. It is also suggested that the quaternary structure of 70FeLf can be stabilized through the conjunction of CO<sub>3</sub><sup>2-</sup> with excessive Fe<sup>3+</sup> ions, although the detailed mechanism of the association of Lf with multiferric ions is still unclear.

In this study, we investigated the architecture of 70FeLf by small-angle X-ray scattering (SAXS).

### 2 Experiment

Native Lf and 70FeLf were purified in the same manner as previously reported [2]. The protein solution was prepared at various concentrations from 0.5 to 2 mg/mL at pH 1.0 – 6.0.

All the experiments were performed at BL10C and BL15A. SAXS data were measured at a sample-to-detector length of 1 m and at 20°C with PILATUS detector. The exposure time was 60 s in one measurement. The sample in the cuvette was exchanged every three times. Bovine serum albumin was also measured for calibration.

Guinier analyses and singular value decomposition (SVD) were performed with our in-house program.

### 3 Results and Discussion

SAXS data of 70FeLf showed multidisperse profiles in a wide range of pHs, whereas native Lf was shown as monodisperse, which was confirmed by SVD analysis. It was suggested that the system may consist of randomly aggregated form and Fe<sup>3+</sup>-bound complex. Therefore we performed Guinier analysis with two-exponential fitting, and characterized structural properties of the latter major component.

The radius of gyration (Rg) of Fe-Lf complex was ranged from 40 to 50 angstrom, and hardly changed at various pHs. On the other hand, molecular weight (Mw) estimated from forward scattering was changed around

pH 3, which suggested the contribution of CO<sub>3</sub><sup>2-</sup> ion upon the formation of Fe-Lf complex.

Table 1: pH profile of 70FeLf

pH	1	3	6
Rg (Å)	49.2	45.7	43.8
Mw (kDa)	439	525	543

Based on these results, we are now constructing the molecular model of the complex that can elucidate the association mechanism of Lf and multiferric ions.

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### References

- [1] S. Moore *et al.*, *J. Mol. Biol.* **274**, 222 (1997).  
 [2] F. Hu *et al.*, *International Dairy Journal* **18**, 1051 (2008).

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