

## Preliminary SAXS study of protease-tolerant form of human galectin-9

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### 1 Introduction

Human Galectin-9 (hG9) has two different carbohydrate recognition domains (N- and C-terminal CRDs) which are specific to beta-galactosides. The physiological function of hG9 is miscellaneous and known to be an eosinophil chemoattractant, a ligand of T-cell immunoglobulin domain and mucin protein 3, and inducer of T helper 1 cell apoptosis. There are isoforms depending on the length of the linker region between N- and C-CRDs. Two isoforms, hG9L (355 a.a.) and hG9M (323 a.a.), are protease sensitive due to their long linker with ca. 30-60 a.a. As a protease resistant hG9 mutant, hG9Null with short linker has been reported, and it retains the biological function of native hG9, pleiotropic immune response [1]. The structure of each CRD had been already available, however the whole structure of hG9 with two CRDs was not reported before we determined the crystal structure of a mutant form of G9Null. In the crystal structure of the mutant form, N- and C-CRD interact with each other via a metal ion, making a rigid and stable structure, although any metal ion was not added in the process of the sample preparation. Thus, it is intriguing for understanding of interaction of N- and C-CRDs in the presence/absence of any metal ion in solution.

In this study, we attempted SAXS analysis of the mutant form of hG9Null to determine the solution structure.

### 2 Experiment

The mutant form of hG9Null was prepared in the solution of 10 mM Tris-HCl pH 7.0, 150 mM NaCl, 2 mM DTT. SAXS measurements were done in the presence of additional 5 mM DTT or 5 % glycerol or 0.5 mM EDTA at 293 K on BL10C in the KEK using PILATUS3-300KW detector. The X-ray wavelength was 1.488 Å and camera length was 929 mm.

### 3 Results and Discussion

The low concentration of the mutant sample was relatively measurable, however it was impossible to measure using the concentrated sample up to the protein concentration of 1 mg/ml, indicating the state of aggregation.

The mutant hG9Null is easily aggregated depending on the concentration. To avoid the aggregation, various condition were tested. We are currently optimizing the measurement condition to compare the solution structure in the presence/absence of metal ion.

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### References

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