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Crystal structure of Reg IV protein from cancer gene

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1 Introduction

The mortallity of gastric cancer locates in the second (the first one is lung cancer), and genomics of gastric cancer became to be studied. Yasui *et al*. [1] developed the method of such genomics called SAGE (Serial Analysis of Gene Expression). Regenerating islet-derived family, member 4 (Reg IV) was identified in such gastric cancer genomics

By the results of primary structure comparison, the Reg IV is classified to the C-type lectin, which is widely found in the immunology or signal transduction. Concerning the C-type lectin, we have already determined the NMR structure of LOX-1 which is also classified as the C-type lectin family.

The molecular mechanism of gastic cancer formation is unknown and the structure determination of Reg IV is expected to enhance the cancer study. The protein is also available as a marker of gastric cancer.

Therefore, we have crystallized Reg IV protein and intended to clarify the structural relation of the formation of gastric cancer.

2 Experiment

Although the overexpression of Reg IV was found in several cancer cells, the expression in *Eschellicia coli* is not found yet. In order to provide enough amount of protein for crystallization, screening of expression was performed such as His-tag protein or GST protein. In both cases, the protein was expressed as inclusion body. Thus, refolding process was also searched. Finally, a stable sample was obtained as His-tag protein and refolded using Arginine method. As the 22 residues of N-terminal were signal region, the recombinant protein was constituted omitting this N terminal region.

In solution, we confirmed that Reg IV molecule is in monomer by using gel filtration, and it forms the β sheet structure by CD spectra.

Single crystals of Reg IV were obtained in both microbatch method and vapor diffusion sitting drop method. The largest crystal was 0.3 x 0.2 x 0.2 mm in size. This crystal belongs to the spacegroup of $P3_121$, and the lattice parameters were a = b = 145.86 Å, and c = 66.06 Å. The number of molecules in the asymmetric unit was 4, which gave the V_m value as 2.8. The diffractions were observed up to 2.5 Å resolution. Diffraction images were processed and scaled using the program HKL2000 and SCALEPACK, which gave the R_{merge} of 0.081.

We could not find good derivatives, therefore the initial phase was determined by the molecular replacement method using the coordinates of Reg I α monomer as a search model. The initial R-factor was 0.41. After the crystallographic refinement and model rebuilding with REFMAC5 and COOT, respectively, the R-factor converged to 0.25 at 2.5 Å resolution.

Another type of crystal, which belong to the spacegroup *C*2, was also obtained (a=114.70 Å, b=90.17 Å, c=66.60 Å, β =99.79°). But the R_{merge} was not so good.

3 Results and Discussion

Now we can observe the whole electron density map of Reg IV, but the several loops close to the neighbor molecule were still invisible. So we are now trying to apply several density modifications to improve the electron densities of these loops. As one of these invisible loop is supposed to bind saccharides, structure determination of the complex is also in progress.

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Reference

[1] Yasui *et al*., *Pathology International* **59**, 121 (2009).

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