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Crystallographic analysis of apo- and ligand-binding forms of the B-cell inhibitory co-receptor CD72

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1 Introduction

B cells play a key role in the immune system by making antibodies. CD72 is an inhibitory co-receptor that regulates signaling through the B cell receptor (BCR). Activation of CD72 prevent overstimulation of the B cells. Thus, CD72 is necessary to avoid autoimmune diseases and make antibodies properly against exogenous antigens but not autoantigen. CD72 is a type II membrane protein forming a homodimer, primarily expressed in B cells. The ligand binding region of CD72 is located at the C-type lectin-like domain (CTLD) in the C-terminal extracellular region. However, due to the lack of structural information, the mechanism of the ligand recognition of CD72 was unclear. We have demonstrated that CD72 specifically binds to Sm/RNP, an RNA-containing nuclear self-antigen, and inhibit B cell response, preventing production of anti-Sm/RNP antibody crucial for development of systemic lupus erythematosus (SLE) [1]. To elucidate detailed model of the ligand binding site, and obtain structural bases to design novel ligands that regulate CD72 more efficiently, we have initiated crystallographic analysis of the CTLD of mouse CD72 (CD72-CTLD).

2 Experiment

Recombinant mouse CD72-CTLD was overexpressed using BL21(DE3) cells and refolded using His-Accept SF resin (Nacalai Tesque). Crystallization conditions were screened using commercially available screening kits (Hampton Research) with the hanging-drop vapor diffusion method. X-ray diffraction experiments were performed at beamelines 5A and 17A at PF, and NW12A at PF-AR, KEK. The data were processed and scaled using program HKL2000 [2] and were truncated using the CCP4 program suite [3]. The structure were solved by the molecular replacement method using PHASER [4]. Several cycles of manual model rebuilding and refinement were performed by using the program COOT [5] and PHENIX [6], respectively.

3 Results and Discussion

The crystal structure of apo CD72-CTLD was determined at 1.2 Å resolution [1]. The overall structure (Fig. 1a) is very similar to that of the typical CTLD fold but CD72-CTLD lacks the loop region which forms the ligand-binding site in many other CTLDs.

Analysis of the electrostatic potentials of CD72-CTLD reveals that there is a highly positively charged patch on the surface of the molecule (Fig. 1b). Because the molecules with nucleic acids such as Sm/RNP possesses negatively charged region derived from the sugar-phosphate backbone, it is strongly suggested that the positively charged patch of CD72-CTLD is related to recognition of the nucleic acids in Sm/RNP.

In addition, we calculated the homology model of CD72c, which is an allele of CD72 in a model mouse of an autoimmune disease. The model clearly shows that surface charge distribution around the putative ligand binding region is different from that of CD72, substitutions of some residues in this area result in generation of a negative-charge patch. It would be disadvantageous for binding Sm/RNP due to electrostatic repulsion with the negative charge of RNA. In fact, binding affinity to Sm/RNP is decreased in CD72c [1], which is considered to be triggering autoimmune disease. Therefore, the charge distribution on the molecular surface of CD72 is thought to play an important role in controlling the recognition and binding of Sm/RNP.

Further structural analysis of CD72-CTLD-lignad complexes are in progress.

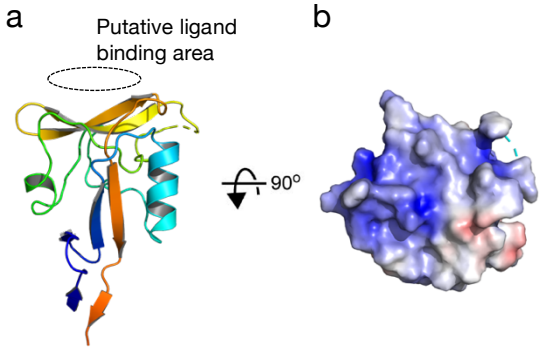

Fig. 1: (a) Overall structures of CD72-CTLD. (b) Electrostatic surface representation around the putative ligand-binding area.

References

[1] C. Akatsu *et al*., *J. Exp. Med*., **213**, 2691 (2016).
[2] Z. Otwinowski and W. Minor, *Methods Enzymol*. **276,** 307 (1997).
[3] M. D. Winn *et al*., *Acta Cryst*. **D67**, 235 (2011).
[4] A. J. McCoy *et al*., *J. Appl. Cryst*. **40**, 658 (2007).
[5] P. Emsley *et al*., *Acta Cryst*. **D66**, 486 (2010).
[6] P. D. Adams *et al*., *Acta Cryst*. **D66**, 213 (2010).

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