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Structural basis for regulation of binding between the CD28 family and signaling molecules

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1 Introduction

T cell regulation is pivotal in the human immune system, necessitating two distinct signals for activation. Firstly, an antigen-specific signal is delivered through the T cell receptor (TCR) upon interaction with the major histocompatibility complex (MHC). Secondly, an antigen non-specific signal is triggered by the CD28 family molecule binding to the B7 molecule group. Within the intracellular domain of CD28, comprising approximately 40 residues, signal transmission occurs by recruiting additional molecules. The tyrosine residue within the YMNM amino acid sequence of CD28's intracellular domain undergoes phosphorylation by Tyr kinase, leading to the formation of phosphotyrosine (pY) and the pYMNM sequence. This pYMNM sequence is then recognized by signaling molecules such as Growth factor receptor-bound protein 2 (Grb2). Grb2's SH2 domain selectively binds to the consensus sequence of pYXNX, which aligns with the pYMNM sequence in CD28. Our previous study unveiled the crystal structure of the Grb2 SH2 domain bound to the CD28 derived phosphopeptide SDpYMNMTP [1], shedding light on the mechanism underlying pY recognition and the significance of the Asn residue within the consensus sequence.

We have recently succeeded in synthesizing a low-molecular compound that controls the binding between Grb2 and the CD28 family, opening the possibility of drug discovery that does not rely on expensive biological agents. In this study, we aimed to analyze the structures of these compounds in complex with the SH2 domain of Grb2 to obtain a structural basis for the rational design of compounds for further functionalization.

2 Experiment

Grb2-SH2 was overexpressed in *Escherichia coli* and purified as described previously. 1 mM Grb2 SH2 with 5 times molar ratio of CD28 derived phosphopeptide (SDpYMNMTP) and 5 times molar ratio of the synthesized compound were co-crystallized by the sitting-drop vapor-diffusion method at 20ºC. Prior to data collection, the crystals were soaked in cryo-protectant solutions containing 20% (v/v) glycerol along with their respective reservoir buffers and flash-frozen using nitrogen gas stream at 95 K.

X-ray diffraction experiments were performed at the beamlines BL-17A and NE3A at PF and PF-AR, KEK. All data were processed and scaled using XDS [2]. The initial phases were determined by the molecular replacement method using the program PHASER [3]. Several cycles of manual model rebuilding by using COOT [4] and refinement by using PHENIX [5] were performed.

3 Results and Discussion

Crystals of the complexed Grb2 SH2 were obtained in the buffer containing 0.1 M Tris-HCl pH 8.5, 0.2 M MgCl2, and 30% PEG4,000. The structure was determined at 1.6 Å resolution.

A blob electron density was clearly observed (Fig. 1) far from the CD28 derived peptide binding site in several data sets. This suggests the presence of an allosteric regulatory mechanism for binding to the CD28. However, by careful refinement, we concluded that the observed densities are too small to consider as a whole of the bound ligand. In parallel, we attempted to prepare crystals by the soaking method, but the results were similar, and although there was indeed a blob electron density, we could not determine that it was from a synthetic compound. Although there is a possibility that the observed densities are the part of the ligand, there is another possibility that the densities are for the small molecule included in the crystallization conditions. The use of compounds that contain heavy atoms or other clear marker atoms may make it easier to determine which compound the electron density is derived from.

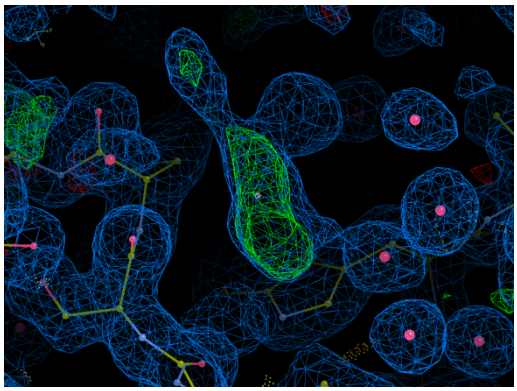

Fig. 1: A blob density in the crystal structure.

References

[1] K. Higo *et al*., *PLoS One* **8**, e74482 (2013).
[2] W. Kabsh, *Acta Cryst.* **D66**, 125 (2010).
[3] A. J. McCoy *et al*., *J. Appl. Crystallogr.* **40**, 658 (2007).
[4] P. Emsley *et al., Acta Cryst.* **D66**, 486 (2010).
[5] P. D. Adams *et al., Acta Cryst.* **D66**, 213 (2010).

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