

Crystal structure analysis of CD28 family molecules complexed with signaling proteins

Nobutaka Numoto*

Medical Research Institute, Tokyo Medical and Dental University (TMDU),
Bunkyo-ku, Tokyo 113-8510, Japan

1 Introduction

T cells play a central role in immune system. Two sets of signals are needed to activate T cells. One is the T cell receptor (TCR) signal that is mediated by the major histocompatibility complex (MHC), and the other is the co-signal provided by CD28 family receptors. The intracellular domain of CD28 consists of about 40 amino acids and has no enzymatic activity. A tyrosine kinase is recruited to the intracellular domain of CD28, and then phosphorylated YNM motif (pYNM) is recognized by the SH2 domains of signaling molecules such as Grb2, Grb2-related adaptor downstream of Shc (Gads), and p85 subunit of phosphoinositide 3-kinase (PI3K). The consensus SH2-binding sequence of Grb2 and Gads is pYXNX, whereas that of p85 is pYXXM. Thus, although these three adaptor molecules activate different signaling pathways downstream of CD28, the SH2 domains of all the molecules can bind to CD28 at the same site pYNM. To elucidate the structural bases of the molecular recognition mechanisms between CD28 and these signaling molecules, we have performed crystal structure analyses of three SH2 domains of Gads, N- and C-terminal SH2s of p85, which are complexed with the pY-containing peptide derived from CD28.

2 Experiment

Three SH2 domains (Gads SH2, p85 nSH2, and cSH2) were co-crystallized with the synthesized pY-containing peptide (SDpYMNMTp) derived from CD28. 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 beamlines 5A and 17A at PF, KEK. The data were processed and scaled using program XDS [1] and were truncated using the CCP4 program suite. [2] The structure were solved by the molecular replacement method using PHASER. [3] Several cycles of manual model rebuilding and refinement were performed by using the program COOT [4] and PHENIX, [5] respectively.

3 Results and Discussion

The crystal structures of Gads SH2-CD28 peptide, p85 nSH2-CD28 peptide and p85 cSH2-CD28 peptide were determined at 1.2, 0.9, and 1.1 Å resolution, respectively. [6] A bent conformation of CD28 peptide in the complex with Gads SH2 is observed as previously reported structure of the complex of Grb2 SH2-CD28 peptide. [7] Asn at pY+2 of CD28 peptide in the Gads SH2 complex

forms hydrogen bond with the SH2 protein. In contrast, CD28 peptide reveal a more straight conformation in the structures of p85 nSH2 and cSH2 complexes (Fig. 1a). Met at pY+3 of CD28 peptide in the p85 nSH2 and cSH2 complexes forms hydrophobic contacts with the SH2 protein and Asn at pY+2 loses contact with the protein. These facts are consistent with the difference of the consensus sequence between Gads (pYXNX) and p85 (pYXXM). Moreover, comparison among the three structures reveals the variation of the environments around the bound phosphorylated tyrosine of CD28 peptide (Fig. 1b).

Our detailed models may useful for development of compounds that specifically inhibit the association of CD28 with these SH2 domains of the adaptor proteins to suppress excessive T cell responses, such as in allergies and autoimmune diseases.

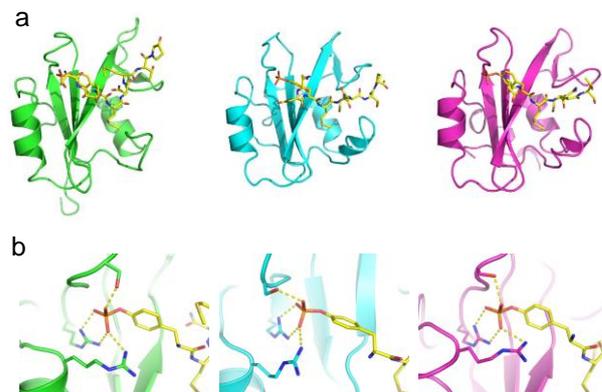


Fig. 1: (a) Overall structures of Gads SH2 (green), p85 nSH2 (cyan), and cSH2 (magenta) complexed with CD28 peptide (yellow, stick model). (b) Close up view of the interactions of the phosphorylated tyrosines. Hydrogen bonds are indicated as yellow dash lines.

References

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* numoto.str@mri.tmd.ac.jp