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Structural analysis of NZ-1 Fab complexed with PA peptide

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1 Introduction

PA tag system is a novel affinity tag system consisting of a rat monoclonal antibody NZ-1 and its epitope peptide dubbed "PA tag" [1]. NZ-1 was raised against 14-residue peptide derived from human podoplanin PLAG domain, where PA tag sequence (GVAMPGAEDDVV) is present [2]. PA tag system proved to outperform many existing affinity tag systems (e.g. FLAG, Myc, HA) owing to its high affinity, high selectivity, and extended reusability. Therefore, to understand the structural basis of these features, we have determined the crystal structures of NZ-1 with and without bound PA peptide.

2 Experiment

The PA14 peptide (EGGVAMPGAEDDVV) was synthesized by a standard solid-phase synthesis and purified by reverse-phase HPLC (Scrum Inc.). The rat anti-PA monoclonal antibody NZ-1 (IgG_{2a}, λ) was produced in mice ascitic fluid. The NZ-1 was purified using Protein G Sepharose 4 Fast Flow. To prepare NZ-1 Fab fragment, NZ-1 IgG was digested with immobilized papain, and purified using the PA peptide-immobilized Sepharose 4 Fast Flow. The Fab fragment was further purified from the contaminating uncleaved IgG by gel filtration chromatography, and concentrated to approximately 10.0 mg/mL. The ligand free protein crystal was grown under the condition of 0.1 M sodium citrate buffer pH 5.6 and 15% (w/v) polyethylene glycol 4000 and gave 1.96 Å resolution diffraction using a cryoprotectant solution containing 10% (v/v) glycerol. To make a co-crystal of NZ-1 Fab and the antigen, PA14 peptide was added at approximately 3-fold molar excess to the concentrated NZ-1 Fab. The co-crystal was grown under the condition of 0.1 M sodium citrate buffer pH 5.6 and 24% (w/v) polyethylene glycol 4000 and gave 1.70 Å resolution diffraction using cryoprotectant solution containing 10% (v/v) ethylene glycol.

X-ray diffraction data sets were collected at the beam line BL-1A or BL-17A of Photon Factory. The ligand free data were processed using *HKL2000* program package [3], and the co-crystal data were processed using *XDS* program package [4]. Initial phase was determined by molecular replacement method using *Phaser-MR* [5]. A rat anti-NGF AD11 antibody Fab (IgG_{2a}) structure (1ZAN) was used as a search model for the ligand free NZ-1 Fab structure. The resultant NZ-1 Fab structure was then used for the PA peptide complex as a search model. The structure models were built using *COOT* [6] with model refinement cycle with *REFMAC5* [7]. The structure models were validated using the program *MOLPROBITY* [8].

3 Results and Discussion

The conformation of the Fv region of NZ-1 Fab was similar between the uncomplexed and the PA peptide-complexed versions, with root-mean-square deviation (RMSD) of 0.420 Å. Furthermore, these structures were essentially identical at the level of the side chains, even in the CDR regions. Therefore, we only discuss about complex structure hereafter. The crystal of NZ-1 Fab/PA peptide complex contains two Fabs per asymmetric unit, and their structures are almost indistinguishable with 0.237 Å RMSD. Therefore, we will describe the structure of one pair (chains C and D) in the following sections.

In the structure, the PA peptide was docked in the cleft formed between V_H and V_L of NZ-1, and good electron density was visible for 11 out of 14 residues (except for the N-terminal Glu1-Gly2-Gly3). There are numerous contacts between the peptide and the Fab, particularly at the region encompassing Met6-Asp12. The side chain of Met6 is deeply inserted into the cleft and makes many van der Waals interactions. The side chain of Asp12 is also inserted into the cleft and makes multiple hydrogen bonds, with Gly33, Thr99, Ser100, and Arg101 within the Fab heavy chain *via* direct hydrogen bonds. It was suggested that NZ-1 achieves the very high affinity toward PA peptide using a combination of many interactions to the central core segment (Met6-Asp12), with the contribution of Met6 and Asp12 being dominant. Additionally, the main chain of Val4 and Val13 interacted with the main chain of Fab *via* direct hydrogen bond. It is suggested from these observations that at least 10-residue portion of the PA peptide (Val4-Val13) is required for the high affinity. In fact, many amino acid residues (Met6, Pro7, Gly8, Glu10, Asp11 and Asp12) were confirmed to be essential for the binding through a series of SPR binding assays in which twelve non-alanine residues in PA peptide were mutated to alanine. Moreover, M6A and D12A mutations in the tag sequence nearly eliminated the binding toward NZ-1, indicating that these are the most important residues.

Within the binding pocket of NZ-1, the PA peptide assumed a tight 2-residue type II β-turn conformation, with Pro7 and Gly8 being the tip of the hairpin. As it is known that Pro-Gly sequence have strong propensity to form tight β-turn, this conformation is likely to be a preferred one in the solution even before the binding by NZ-1. This means that the loss of entropic freedom upon the binding is relatively small, which may be the reason for the high affinity. This property also suggests an interesting possibility that PA tag may be inserted into a tip of turn or in the middle of a loop region of proteins,

without losing its high affinity toward NZ-1. As it is generally difficult to graft linear epitope tag in a structured protein domain, the PA tag system may provide unique opportunity to attach purification/labeling handle to a target protein.

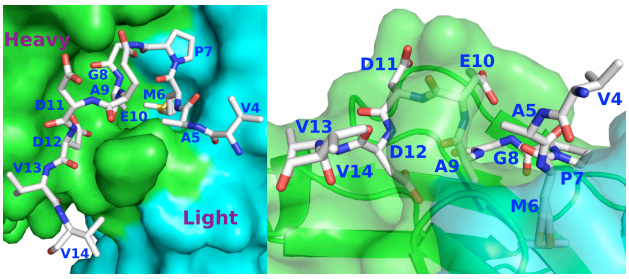

Fig. 1: NZ-1 Fab/PA peptide complex structure. The bound PA peptide (stick model) is viewed from the top (left) or the side (right) of the antigen-binding pocket, shown as surface presentation.

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Research Achievements

1. Young Scientist Award, The 13th Annual Meeting of the Protein Science Society of Japan
2. Journal of Structural and Functional Genomics Poster Prize (SILVER), International Conference on Structural Genomics 2013 –Structural Life Science– (ICSG2013-SLS)

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