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Development of a novel antibody fragment format "Fv-clasp"

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1 Introduction

Antibody fragments such as Fab and single-chain Fv (scFv) fragments have been widely used as research tools. In the structural biology field, antibody fragments are used as "crystallization chaperone" to aid crystallization of crystallization-resistant proteins. So far, many crystal structures of biologically and medically important proteins have been determined by using Fab or scFv fragments [1, 2]. However, each of these antibody fragments has potential problems to use as crystallization chaperon. For example, it is known that Fab fragment has structural flexibility between variable (Fv: V_H, V_L) and constant (C_H1, C_L) domains, which may contribute negatively to crystallization [3]. ScFv lacks the constant domains, resulting in instability of Fv region, and hence, some antibodies are not compatible with the scFv format. To overcome these problems, we designed a novel antibody fragment format termed "Fv-clasp". In the Fv-clasp format, SARAH domain derived from human Mst1 protein that forms an antiparallel coiled-coil structure was fused with C-termini of V_H and V_L to stabilize Fv fragment (Fig. 1). In addition, for further stabilization of the heterodimer conformation, we introduced Cys residues into the SARAH domains to form an inter-chain disulfide bond.

To know whether Fv-clasp assumes the expected structure, we tried to determine the crystal structure of Fv-clasp derived from 12CA5, a commercially available anti-HA tag antibody.

2 Experiment

V_H and V_L domains of 12CA5 were fused with the SARAH domain from human Mst1 via a Gly-Ser linker. These chimeric proteins were separately produced in *E. coli* cells and were expressed as inclusion bodies. After the solubilization of the inclusion bodies with 6 M guanidine hydrochloride, V_H-SARAH and V_L-SARAH were mixed with each other, and 12CA5 Fv-clasp was refolded using a stepwise dilution method. The refolded sample was purified by size-exclusion and anion-exchange chromatographies. For crystallization trial, 12CA5 Fv-clasp was concentrated to approximately 8 mg/mL and mixed with antigen peptide (HA peptide) at a molar ratio of 1 : 5.

A total of 288 conditions were screened for crystallization using commercially available screening kits, and after a few days, crystals had appeared in 51 conditions. However, as a result of preliminary X-ray diffraction experiment for the several initial crystals, they all showed poor diffraction quality. To produce crystals suitable for X-ray analysis, crystallization conditions were optimized, and diffraction quality crystals were obtained under the condition of 0.1 M Na/K phosphate (pH6.2) and 1.4 M ammonium sulfate.

X-ray diffraction data were obtained at the beam line BL-17A of Photon Factory. The data was processed and scaled to maximum resolution of 3.0 Å using HKL2000 [4]. The crystal belonged to space group *C*2 and contained one 12CA5 Fv-clasp molecule in the asymmetric unit. Initial phase was determined by molecular replacement analysis with PHASER [5] using crystal structures of an Fab fragment of mouse IgG (PDB ID: 1HIL) and the Mst1 SARAH domain (PDB ID: 4NR2) as search models. Structural model was refined with REFMAC 5 [6] and the model was modified with COOT [7].

3 Results and Discussion

Overall structure of the 12CA5 Fv-clasp is shown in Fig. 2. The SARAH domains formed disulfide-bridged anti-parallel coiled-coil structure at the bottom of the Fv opposite from the antigen binding site as our design concept shown in Fig. 1. V_H and V_L domains of 12CA5 Fv-clasp formed a heterodimer with a general configuration observed in many antibody structures. Electron density attributable to the antigen peptide was observed in the antigen binding cleft between the V_H and V_L domains, and all 9 residues of HA peptide could be assigned in the map (Fig. 2). The HA peptide was specifically recognized by 12CA5 Fv-clasp through extensive hydrogen bonding and hydrophobic interactions. These facts indicate that, although crystal structure of 12CA5 has not been reported to date, our structure represents genuine antigen-binding conformation of the antibody. Furthermore, formation of the functional structure of the Fv region demonstrated that the fusion of the SARAH domains did not affect the conformation of the Fv region.

The crystal structure of 12CA5 Fv-clasp illustrated that our attempt to develop a novel antibody fragment using the SARAH domain was successful. However, it still has a problem, because diffraction powers of the crystals were not enough to be used as crystallization chaperones even after the optimization of the crystallization condition. Therefore, further optimization of Fv-clasp design must be sought. Structural information of 12CA5 Fv-clasp obtained here will help us to re-design of Fv-clasp.

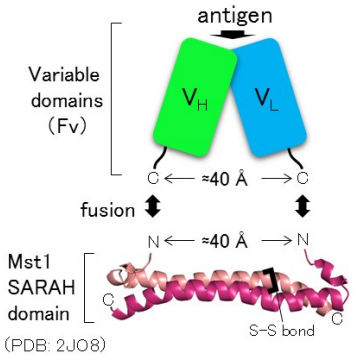

Fig. 1 Design of Fv-clasp

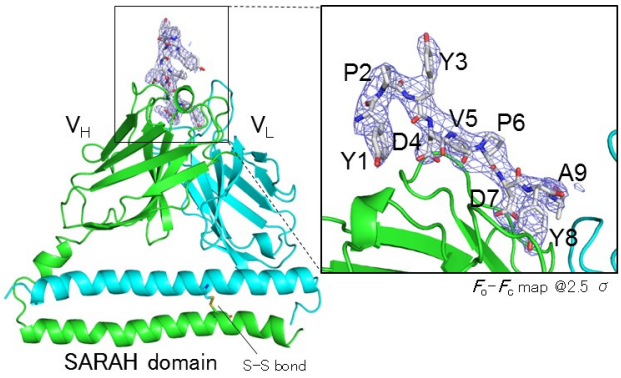

Fig. 2 Crystal structure of 12CA5 Fv-clasp

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