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Crystal structure of an antibody specific for methylated-lysine in complex with the cognate peptide.

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Methylation is an important posttranslational modification in proteins. We have prepared a number of methylysine-specific antibodies and characterized their mechanism of recognition. Herein we report the structure of the Fab region of the antibody termed D6 at a resolution of 1.8 Å. The mechanism of recognition involves a cage of three Trp residues surrounding the trimethylated portion of the modified lysine residue of the peptide.

1 Introduction

Protein modification play critical roles governing their biological function. Among them, methylation is often observed in histones, producing changes in the control of DNA transcription [1]. Antibodies are molecules or protein origin that specifically recognize their target antigens, and for that reason they have been widely employed in basic and applied research [2]. Methylation-specific antibodies are still rare, and their development and characterization may contribute to the functional studies involving methylation [3].

By using a methylated fragment (peptide) of the protein MAP3K2, we could generate a trimethylated lysine-specific antibody [4]. Herein we describe the crystal structure of the Fab region of the antibody (termed D6) in complex with the cognate tri-methylated peptide at 1.8 Å resolution. The crystal structure revealed that the trimethylated lysine is tightly held to the antibody by a cage of three Trp residues [5].

2 Experiment

DNA encoding the heavy and light chains of D6 Fab were cloned into expression vector pcDNA3.4 for expression in Expi293 cells by co-transfection. Protein was purified by immobilized metal affinity chromatography, anion exchange chromatography, and size exclusion chromatography in a final buffer composed of 20 mM Tris-HCl (pH 8.0) and 20 mM NaCl. The purified Fab was mixed with methylated peptide and concentrated to 4.3 mg/ml. Crystallization screening with an Oryx8 protein crystallization robot (Douglas Instruments) was carried out.

Single crystals of C9 were obtained by mixing the protein with a solution of 200 mM calcium chloride and 20% (w/w) PEG-3,350. Suitable crystals were cryo-protected with mother liquor supplemented with 20% glycerol, and transferred to liquid N_2 until data collection.

Diffraction data were collected in beamline BL5A of the Photon Factory (Tsukuba, Japan) under cryogenic conditions (100 K). The data was processed, and the structure determined by the method of molecular replacement and refined all procedures as reported earlier in detail [5].

3 Results and Discussion

The crystal structure of the Fab region of the antibody D6 in complex with cognate peptide was determined to a resolution of 1.8 Å in spacegroup P1. The asymmetric unit contained two units, each one comprising a heavy chain, a light chain, and the cognate peptide (Figure 1). The peptide was modified with a triple-methylated lysine residue.

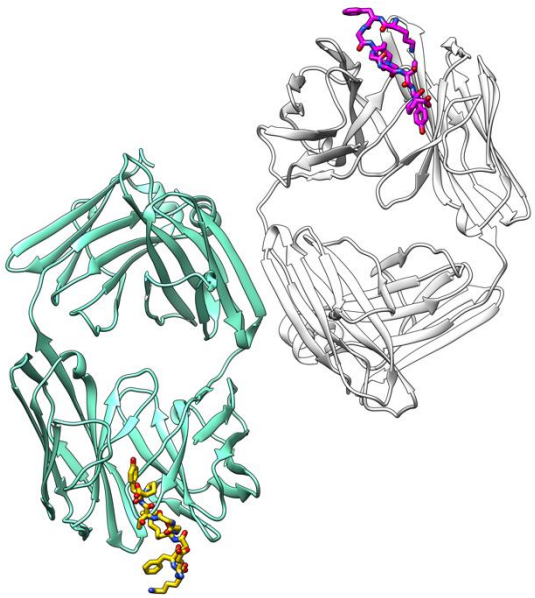

Fig. 1: Crystal structure of D6 Fab (gray and light green) with cognate peptide bound (yellow and magenta)

The differences between these two Fab units were below an RMSD of 1 Å. The peptide was tightly attached to the antibody by a combination of non-covalent interactions such as hydrogen-bonds, and electrostatic and van der Waals interactions, all of them typically found in protein-protein complexes. In addition, we noted an interested feature. The trimethyl-lysine, a key element for the recognition if this peptide by the antibody, is surrounded by three Trp residues and a Glu residue. In particular the aromatic residues are arranged in a very characteristic fashion reminiscent of a cage, and that is why we have proposed to term this motif "aromatic cage" [5].

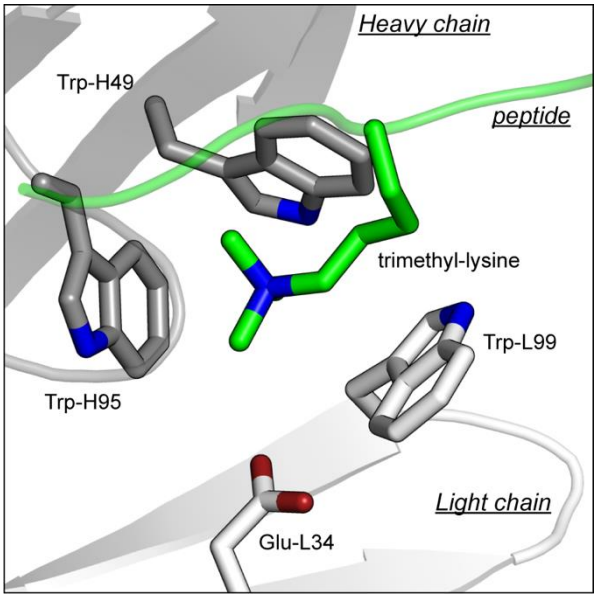

Fig. 2: Close-up view of the interaction between D9 antibody and the trimethyl lysine of the cognate peptide. Heavy chain, light chain, and peptide are shown in gray, white and green, respectively. Key residues are depicted with sticks.

In summary, we report the crystal structure of an anti-methylated-lysine antibody at high resolution (1.8 Å). The recognition mechanism of this antibody reminds involves a variety of non-covalent interactions, among which the aromatic cage is the most distinctive element.

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References

[1] T. Jenuwein and C.D. Allis, *Science* **293**, 1074 (2001).
[2] J.M.M. Caaveiro and K. Tsumoto, *eLS Immunology* a0001117 (2016).
[3] T. Hattori and S. Koide, *Curr. Opin. Struct. Biol.* **51**, 141 (2018).
[4] P.K. Mazur *et al.*, *Nature* **510**, 283 (2014).
[5] Ishii *et al.*, *J. Biol. Chem.* **296**,100176 (2021).

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