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Crystal Structure of Fab Fragment of Anti-Osteocalcin Antibody KTM219 with the Addition of Fluorophore TAMRA

Shuma YAZAKI[1], Misaki KOMATSU[1], Jinhua DONG[2], Hiroshi UEDA[2], and Ryoichi ARAI[1,*]

[1]Fac. of Tex. Sci. Tech. and Inst. for Biomed. Sci., Shinshu Univ., Ueda, Nagano 386-8567, Japan
[2]Lab. for Chem. Life Sci., Tokyo Inst. of Tech., Yokohama, Kanagawa 226-8503, Japan

1 Introduction

Abe *et al.* developed a novel reagentless fluorescent biosensor strategy named Quenchbody (Q-body), which functions via the antigen-dependent removal of the quenching effect on a fluorophore that is attached to a single-chain antibody variable region [1]. Also, Ultra-Quenchbody (UQ-body), the antibody Fab fragment that were fluorolabeled at two of the N-terminal regions, was developed, and its improved response was observed [2]. An anti-osteocalcin C-terminal peptide antibody KTM219 is one of the antibody Fab fragments suitable for UQ-body. Recently, we solved a crystal structure of the Fab fragment of the antibody KTM219 (PDB ID: 5X5X). In this study, for the purpose of further analysis for UQ-body, a crystal was prepared with the addition of a fluorophore TAMRA.

2 Experiments

The Fab fragment of the anti-osteocalcin C-terminal peptide antibody KTM219 was expressed with His_6-tag in *Escherichia coli*, and purified by the following steps: immobilized-metal affinity chromatography, anion exchange chromatography, and gel filtration chromatography. The KTM219 Fab were crystallized at 20ºC with the addition of TAMRA using the hanging drop vapor diffusion method. The KTM219 Fab and TAMRA were mixed with the same volume of reservoir solution (0.3 M Ammonium Acetate, 0.1 M HEPES, pH 7.5, 25% PEG 3350). X-ray diffraction data were collected at KEK Photon Factory Structural Biology Beamline BL-5A at 95 K with 25% glycerol as a cryoprotectant. The structure was solved by molecular replacement method using Phaser with a model structure of KTM219 Fab (PDB ID: 5X5X). The crystal structure was refined using COOT and REFMAC5.

3 Results and Discussion

The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit cell constants of $a = 66.19$ Å, $b = 69.52$ Å, $c = 96.65$ Å, and contains one Fab antibody molecule per asymmetric unit. The structure was refined to 2.14 Å resolution (R_{work} = 20.5%, R_{free} = 27.4%). The crystal structure shows that the KTM219 antibody Fab fragment comprises a light chain (V_L–C_L) and a heavy chain (V_H–C_{H1}) with typical immunoglobulin folds (Fig. 1A). However, we could not find the electron density assigned to TAMRA. A deep pocket exists between V_H and V_L and probably provides the potential binding site for the antigen osteocalcin C-terminal peptide (BGP-C7: RRFYGPV). There is a potential space for TAMRA to be present in the binding pocket. Remarkably, two loops of V_H near the binding pocket have different structures compared to the previously solved structure (PDB ID: 5X5X) of the KTM219 Fab without TAMURA (Fig. 1B and 1C). Further analysis is still needed to understand mechanisms for UQ-body in detail.

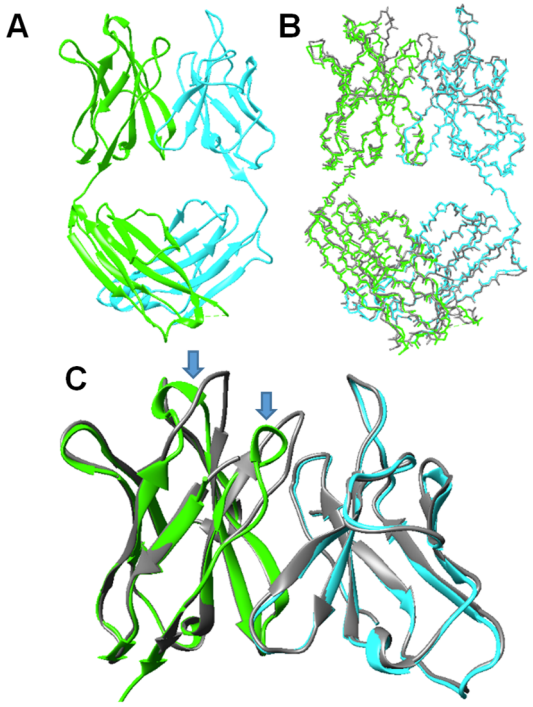

Fig. 1: Crystal structure of the Fab fragment of the anti-osteocalcin antibody KTM219 with the addition of TAMRA. (A) Ribbon representation of the overall structure. The heavy and light chains are shown in green and cyan, respectively. (B) Structural alignment of main chain structures of KTM219 Fab (green/cyan) with TAMRA and KTM219 Fab (gray; PDB ID: 5X5X). (C) Closeup view of (B) around the potential binding pocket (ribbon representation). The arrows indicate the loops that have the distinct structures.

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*rarai@shinshu-u.ac.jp