Crystallography

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Structural studies of Rituximab in complex with its epitope peptide

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Introduction

The CD20 targeted chimeric monoclonal antibody (mAb) Rituximab (Rituxan®, IDEC-C2B8) was the first FDA approved mAb drug for the treatment of malignancy. Though it was originally used for treating low-grade non-Hodgkin's lymphoma (NHL), Rituximab has been proven to be also effective against other types of lymphomas and some autoimmune diseases [1]. To understand the molecular mechanism of Rituximab recognition of human CD20, the crystal structure of the Rituximab Fab in complex with a synthesized peptide encompassing the epitope of human CD20 has been determined at 2.6 Å. Analysis of the complex structure explains very well biochemical data from the epitope mapping studies and provides useful hints for the designing and development of improved antibody drugs with higher affinity and better specificity [2].

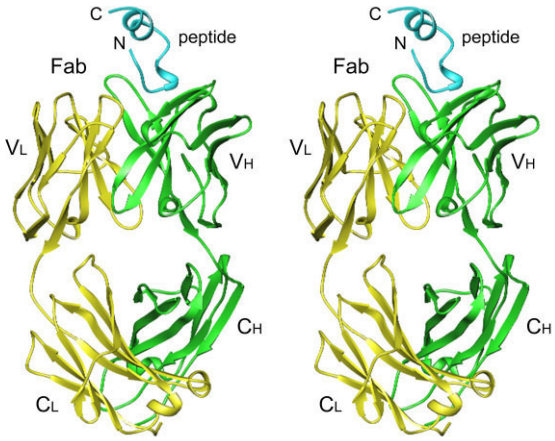

Fig 1. The overall structure of the complex

Method and Results

Rituximab was purchased from Roche. For co-crystallization experiments, the purified Rituximab Fab and the epitope peptide (corresponding to residues 163-187 of the large extracellular loop of human CD20) were mixed at a molar ratio of 1:5 at 4 °C for 12 hours. Co-crystallization was carried out using the hanging drop vapor diffusion method by mixing equal volumes of the protein-peptide mixture solution and a reservoir solution (0.2 M calcium acetate, 0.1 M sodium cacodylate, pH 6.5, and 18% PEG8000). For diffraction data collection, crystals were cryostabilized by Paratone-N (Hampton Research) and then flash-cooled to -170 °C. Diffraction data were collected to 2.6 Å resolution at beamline NW12 of Photon Factory, Japan and processed using program HKL2000. The structure was solved by molecular replacement with Phaser and refined with CNS in program O (Fig 1).

The structure analysis reflects that the peptide fits the CDR regions of the Rituximab Fab quite well with a high degree of structural and chemical complementarity, especially the important ANPS motif. (Fig2).

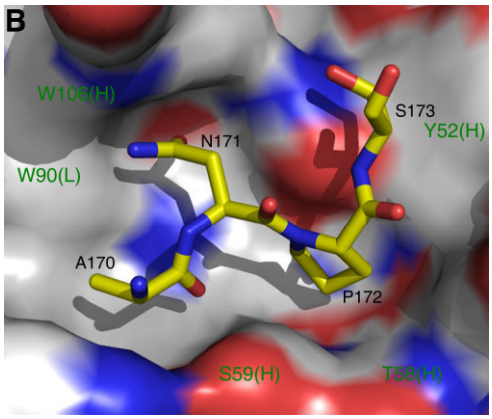

Fig2. Interactions between the Fab and peptide

References

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[2] Du, J. *et al.* Structural Basis for Recognition of CD20 by Therapeutic Antibody Rituximab. ***J. Biol. Chem***. 282, 15073-80 (2007)

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