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Structural and thermodynamic analyses of interaction between a humanized antibody and its antigen: The case of anti-lysozyme antibody, HyHEL-10

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Introduction

Antibody humanization is generally used technique for reducing the immunogenicity of murine monoclonal antibodies in humans, but it is known that humanization of murine antibodies often leads to reduction of their affinity for targets. The mechanisms for problems of antibody humanization remain poorly understood. To address the problems, we investigated the interaction between humanized anti-hen egg white lysozyme (HEL) antibody HyHEL-10 (hHyHEL-10) and HEL as a model.

Although hHyHEL-10 Fv has the specificity to its cognate antigen, HEL, it showed approximately 10-fold reduced affinity, compared with the parental murine antibody. Thermodynamic analysis showed that the increase of negative entropic change resulted in the reduced affinity. We then focused on the interface between the variable domains of heavy chain (VH) and light chain (VL). Several mutants of which the amino acid residues at VH-VL interface were substituted with those from murine antibody (i.e. HQ39K, HW47Y and HQ39KW47Y) were prepared and characterized. Affinity of HQ39KW47Y for HEL was almost identical to that of the parental murine antibody, and the increase of negative entropic change via humanization was compensated for by the mutations.

Here, we report the crystal structure of complex of humanized antibodies, including two mutants, with HEL, and discuss the critical factors for functional improvement of the humanized antibodies.

Experimental

The gene encoding hHyHEL-10 Fv was chemically synthesized, expressed in *E. coli*, and purified from the culture supernatant. The crystal of the wild-type Fv–HEL complex was grown in 100 mM MES (pH 6.4), 10 mM $ZnSO_4$, and 28–31% (w/v) polyethylene glycol monomethyl ester 550. The crystals of the mutant Fv–HEL complexes were also obtained under similar conditions.

Data for wild-type Fv–HEL complex were collected at the Photon Factory beamline BL-5A, and data for mutant Fv–HEL complexes were collected at the Photon Factory beamline NW12A. Initially, the structure of the HW47Y mutant Fv–HEL complex was determined by a molecular replacement (MR) method. The search model for MR was derived from the structure of parental murine Fv–HEL complex. The structures of the other Fv–HEL complexes (wild-type and HQ39KW47Y) were determined by a MR method using the structure of HW47Y mutant Fv–HEL complex as a search model. The atomic coordinates and structural factors for each Fv-HEL complex have been deposited in the Protein Data Bank (ID codes hHyHEL-10, HW47Y, and HQ39KW47Y for 2EKS, 2EIZ, and 2YSS, respectively).

Results and Discussion

The crystal structure of complex of humanized antibodies with HEL demonstrated that the structures of the antigen binding site in the complexes were nearly identical to that of the complex of the parental murine antibody. On the other hand, relative orientation of VH, VL, and HEL has been changed via humanization (Fig. 1). However, two mutations into interfacial residues between variable domains led to recovery of affinity for the target, due to reduction of unfavorable entropy change. Structural analysis suggested that the double mutant (i.e. HQ39KW47Y) complex has a tertiary structure almost identical to that of the parental murine antibody complex, except for some interactions of the Fv-HEL interface. These results have indicated that the interfacial structures of variable domains significantly influence the interaction between a humanized antibody and a target, suggesting that appropriate association of variable domains is critical for humanization of murine antibodies.

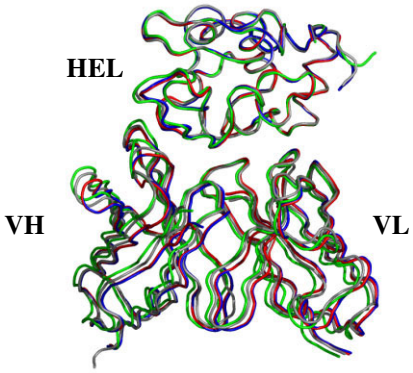

Fig. 1 Overall structure of the hHyHEL-10 Fv-HEL and mutant-HEL complexes.
Gray, murine; red, humanized; blue, HW47Y; green, HQ39KW47Y.

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