Biological Science

NW12A/2006G144

Structural and thermodynamic analyses of anti-lysozyme antibody mutants selected by phage display

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Introduction

To dissect a protein-protein interaction, we have applied a phage display technology. Using the anti-hen egg lysozyme (HEL) antibody HyHEL-10 as a model, studies on the antigen-antibody interaction were performed. Based on the structural information, we have prepared the phage antibody libraries at the antigen-antibody interface in a variable domain of heavy chain (VH) and then performed the selections on the basis of affinity for the target. The statistical data for selected amino acid residues suggested that this system was highly sensitive to a little change of affinity to elucidate the effect of mutation. Furthermore, we have evaluated the mutations to a variable domain of light chain (VL). In the systematic analysis by phage display, we focused on the VL mutant (i.e. LS93D) and investigated the thermodynamics of HEL-LS93D interaction with isothermal titration calorimetry (ITC). Compared with the wild type, slight decrease of the negative enthalpy change and reduction of the entropy loss were observed (Table 1). As a result of mutation, an affinity of LS93D for HEL was slightly higher than that of the wild type. Now we report the crystal structure of the HEL-LS93D complex and discuss the mutational effects.

Experimental

The crystal of LS93D mutant Fv-HEL complex was obtained under similar condition as described previously [1]. The best crystal of the complex was grown from 100 mM Hepes buffer (pH7.9), 9% (w/v) polyethylene glycol 6000, 15% glycerol, and 7% 2-methyl-2,4-pentanediol.

Diffraction data for the complex were collected at Photon Factory beamline NW12A with an ADSC Quantum 210 CCD detector, and then were processed using the program HKL2000. The structure of LS93D mutant Fv-HEL complex was determined by a molecular replacement (MR) method with the program MOLREP in the CCP4 suite. The structure of LS93D mutant complex was refined using the programs O and CNS.

Results and Discussion

The crystal structure of complex of LS93D mutant with HEL demonstrated that the overall structure of the mutant Fv-HEL complex was almost identical to that of the wild type Fv-HEL complex. Substitution of Ser93 with Asp resulted in the rearrangement of one water molecule (*W164*) participating in hydrogen-bonding networks in the antigen-antibody interface (Figure 1). Recently, we have reported that hydrogen bonding via interfacial water molecules enthalpically contributes to the antigen-antibody interaction [2]. Thus, the structural and thermodynamic analyses suggested that deletion of one hydrogen bond by the mutation was enthalpically unfavorable and entropically favorable, leading to the slight increase in affinity.

Table 1: Thermodynamic parameters of the interaction

Mutant	K_a	ΔG	ΔH	$-T\Delta S$
	$x\ 10^8 M^{-1}$		$kJ\ mol^{-1}$	
Wild type	8.2	-51.7	-99.7	48.0
LS93D	13.5	-52.9	-98.3	45.4

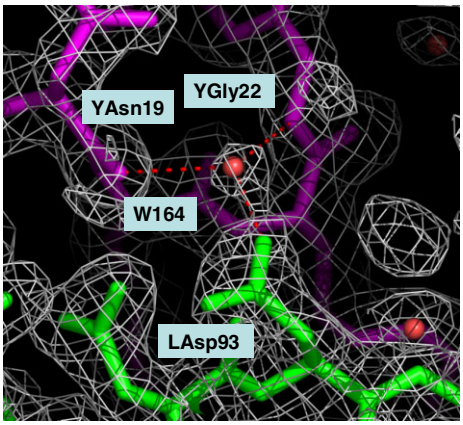

Figure 1. Electron density map around the mutated site in HEL-LS93D complex. VL, green; HEL, pink; water, red.

References

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