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The interaction analysis between an antibody and its antigen by using a phage display: The case of an anti-lysozyme antibody, HyHEL-10

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Introduction

Phage display technology is widely used for *in vitro* selection of desired proteins and peptides on the basis of affinity for the targets. However, studies on the protein-protein interactions based on the phage display, correlated with the thermodynamic and structural analyses, are not sufficiently performed. In this study, we focused on the interaction between hen egg white lysozyme (HEL) and its monoclonal antibody, HyHEL-10 as a model. Using the information on the crystal structure of HEL-HyHEL-10 Fv complex, we prepared the phage antibody libraries of which specific amino acid residues at the antigen-antibody interface in VH were randomly mutated, and performed the selections on the basis of affinity for the antigen. The selected tendencies of residues at mutated sites suggested that it was possible to identify the residues which have high contribution to affinity, and this system is highly sensitive to a little change of affinity to elucidate the effect of mutation. On the basis of selection, we prepared HyHEL-10 Fv mutant (i.e. HD99S) and performed the thermodynamic analysis of HEL-HD99S interaction with isothermal titration calorimetry. Compared with the wild type, an unfavorable entropy change due to binding was compensated by a favorable enthalpy change, resulting that an affinity of HD99S for HEL was slightly higher than that of the wild type (Table 1). Here, we report the crystal structure of complex of HD99S mutant Fv with HEL, and discuss the antigen-antibody interaction from a structural viewpoint.

Experimental

The HD99S mutant Fv-HEL complex was crystallized as described previously [1]. The best crystal of the Fv-HEL complex was grown from 100 mM Hepes buffer (pH7.7), 12% (w/v) polyethylene glycol 6000, 15% glycerol, and 6% 2-methyl-2,4-pentanediol.

Diffraction data were collected at 100K using synchrotron radiation on beamline NW12A of the Photon Factory and an ADSC Quantum 210 CCD detector, and then were processed using the program HKL2000. The structure of HD99S mutant Fv-HEL complex was determined by a molecular replacement (MR) method with the program MOLREP in the CCP4 suite. Refinement of the structure of HD99S mutant Fv-HEL complex was carried out using the programs O and CNS.

Results and Discussion

The HD99S mutant Fv-HEL complex was superimposed onto the wild type Fv-HEL complex. The root mean square deviations between the C atoms of mutant Fv structure and those of the wild type structure were evaluated. The results demonstrated that the overall structure of the HD99S mutant Fv-HEL complex is almost identical to that of the wild type Fv-HEL complex. One water molecule (*W67*) was introduced into the mutation site, participating in hydrogen-bonding networks in the antigen-antibody interface (Figure 1). We have recently reported that hydrogen bond formation via interfacial water molecules makes an enthalpic contribution to the antigen-antibody interaction [2]. Taken together, the thermodynamic and structural analyses indicate that hydrogen binding via an additional water molecule, introduced by mutation, enthalpically contributes to the Fv-HEL interaction despite the partial offset because of entropy loss, resulting in a slight affinity enhancement for HEL.

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
HD99S	13.0	-53.0	-104.9	51.9

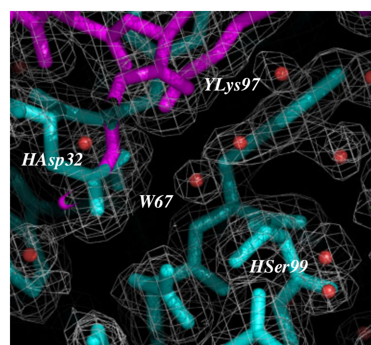

Figure 1. Electron density map around the mutated site in HEL-HD99S complex.

References

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