Biological Science

6A/2000G139

The role of hydrogen bond formation in antigen-antibody complexation: The case of an anti-lysozyme antibody, HyHEL-10, and its target antigen

Akiko YOKOTA[1], Kouhei TSUMOTO[1], Mitsunori SHIROISHI[1], Takeshi NAKANISHI[1], Hidemasa KONDO[2] and Izumi KUMAGAI*[1]

[1] Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Aoba-yama 6-6-11, Aoba-ku, Sendai 980-8579, Japan

[2] Research Institute of Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology (AIST), 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan

Introduction

There are various forces determining the affinity and specificity of antigen-antibody interfacial complementarities, including noncovalent bonds, such as hydrogen bonds, salt bridges, and van der Waals interactions. Among those noncovalent bonds, hydrogen bonds play a unique and functionally important role in molecular associations.

Previously, we examined the role of indirect hydrogen bonds via interfacial water molecules in the interaction between hen egg white lysozyme (HEL) and anti-HEL antibody HyHEL-10 variable domain fragment (Fv) [1]. We discovered that hydrogen bonds made a minor contribution by providing an enthalpic advantage to the interaction, despite the partial offset caused by entropy loss. Here, to elucidate the role of direct hydrogen bonding on complex formation, we focused on the site of Asn-31 in the light chain, of which side-chain interacts with residues in HEL via direct hydrogen bonds. We constructed mutant Fvs, LN31A and LN31D, and examined the Fv mutants-HEL interaction by thermodynamic analysis and x-ray structural analysis [2]

Experimental Procedures

The LN31D Fv-HEL complex was crystallized under conditions similar to those used for the wild-type Fv-HEL complex [3]. The best crystal were grown in 0.1 m Hepes buffer, pH 7.6–7.8, 9–11% w/v polyethylene glycol 6000, 7–9% (w/v) 2-methyl-2,4-pentanediol, and 15% glycerol as a cryoprotectant. However, the crystal of LN31A Fv-HEL complex was too small for obtaining data sets. Diffraction data was collected using synchrotron x-ray source at beamline BL6A of the Photon Factory and processed by the interactive data processing package DPS/MOSFLM/CCP4. Integration and scaling were carried out using the MOSFLM software and SCALA software, respectively. The final file of structural factors was obtained by using TRUNCATE and MTZ2VARIOUS in the CCP4 program suite. The structure was determined by a molecular replacement method and refined with the program CNS and O.

Results and Discussion

The overall structure and the local structure around the site of mutation, of LN31D Fv-HEL complex, were similar to those of the wild-type Fv-HEL complex (Figure 1). These results suggested that the effects of the removal of the hydrogen bond between N-δ2 in l-Asn-31 and O in His-15 of HEL are directly reflected in the changes to the thermodynamic parameters (Table 1). For the LN31D Fv-HEL interaction, a small loss in binding enthalpy and a smaller gain in binding entropy led to a small decrease in the binding affinity compared with the wild type Fv-HEL interaction. For the LN31A Fv-HEL interaction, large decreases in binding enthalpy gain and in binding entropy loss were observed, resulting in remarkable decrease in Gibbs energy of binding. The hydrogen bond between the O-δ1 atom of l-Asn-31 and the N-ζ atom of HEL-Lys-96 and/or the deleted van der Waals contacts might confer an enthalpy advantage despite entropy disadvantage. Thus, the direct hydrogen bonds formed through interfacial l-Asn-31 appeared to make an enthalpic contribution to the HyHEL-10 Fv-HEL interaction similar to indirect hydrogen bonds via interfacial water molecules.

Table 1: Thermodynamic parameters of the interaction

Mutant	K_a	ΔG	ΔH	$-T\Delta S$
	$x\ 10^7\ M^{-1}$	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$
Wild type	82.1	-51.7	-99.7	48.0
LN31A	0.13	-35.4	-48.9	13.5
LN31D	17.8	-47.9	-92.8	44.9

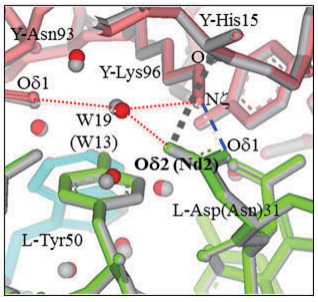

Figure 1. Comparison of local structures at the mutated site between mutant LN31D Fv-HEL (VL, green; VH, blue; HEL, pink; water, red) and wild-type Fv-HEL (grey) complexes.

References

[1] A. Yokota et al., J. Biol. Chem. 278, 5410 (2003).
[2] A. Yokota et al., J. Biol. Chem. 285, 7686 (2010).
[3] H. Kondo et al., J. Biol. Chem. 274, 27623 (1999).

* kmiz@kuma.che.tohoku.ac.jp