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Contribution of asparagine residues to the stabilization of an antigen-antibody complex, HyHEL-10-hen egg white lysozyme.

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

Many germ line antibodies have asparagine residues at specific sites to achieve specific antigen recognition. To study the role of asparagine residues in the stabilization of antigen-antibody complexes, we examined the interaction between hen egg white lysozyme (HEL) and anti-HEL antibody HyHEL-10 variable domain fragment (Fv). We introduced Ala and Asp substitutions into the Fv side chains of L-Asn-32, and L-Asn-92, which interact directly with residues in HEL via hydrogen bonding in the wild-type Fv-HEL complex, and we investigated the interactions between these mutant antibodies and HEL by thermodynamic analysis and x-ray structural analysis [1].

Experimental Procedures

Fv fragment-HEL complexes for the HyHEL-10 mutants LN32D and LN92D were crystallized under conditions similar to those used for the wild-type Fv-HEL complex [2]. The best crystals 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. For the LN32A-HEL and LN92A-HEL complexes, however, the samples of mutant Fv fragments were too poor to crystallize.

Diffraction data were 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 mutant Fv-HEL complexes were determined by a molecular replacement method and refined with the program CNS and O.

Results and Discussion

For the overall structures of LN32D-HEL and LN92D-HEL, the relative orientation of Fv (VL and VH) and HEL were notably altered by the mutations, resulting in the local conformational changes around the mutated site and interfacial regions from the wild-type Fv-HEL (Figure 1). The structural changes observed in LN32D-HEL and LN92D-HEL were about the same, except for the existence (in LN92D-HEL) or nonexistence (in LN32D-HEL) of the hydrogen bond between N-δ2 of L-Asn-32 and O of HEL-Gly-16.

Isothermal titration calorimetric analysis showed that in LN92D-HEL interaction, a small loss in binding enthalpy led to a minor decrease in the affinity compared with the wild-type-HEL interaction (Table 1). By comparison, for LN32A- and LN32D-HEL interaction, there was a large decrease in binding enthalpy gain and in binding entropy loss, which notably decreased the binding Gibbs energy.

These results suggest that hydrogen bonds buried at the interfacial area, such as L-Asn-32, had large enthalpic advantage, despite entropic loss, and were crucial to the strength of the interaction. Deletion of these strong hydrogen bonds could not be compensated for by other structural changes. Consequently, asparagine might be an appropriate residue for the antigen recognition through providing the two functional groups for strong hydrogen bond formation, and their contribution to the antigen-antibody interaction can be attributed to their limited flexibility and accessibility at the complex interface.

Table 1: Thermodynamic parameters of the interaction

Mutant	K_a	G	H	$-T\,S$
	$x\ 10^7 M^{-1}$	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$
Wild type	82.1	-51.7	-99.7	48.0
LN32A	0.17	-36.1	-74.0	37.9
LN32D	0.93	-40.3	-47.2	6.9
LN92D	14.0	-47.2	-94.6	47.4

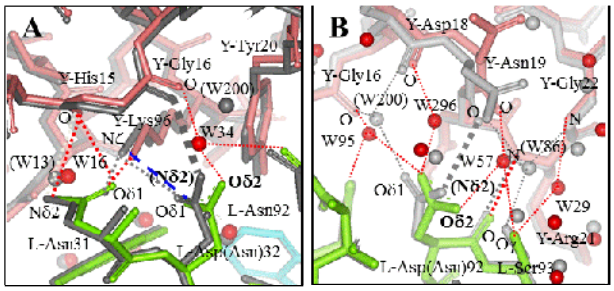

Figure 1. Comparison of local structures at the mutation site between mutant Fv-HEL (VL, green; VH, blue; HEL, pink; water, red) and wild-type Fv-HEL (grey) complexes. A, LN32D-HEL; B, LN92D-HEL

References

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