Thermodynamic Consequences of Single-mutation on Association of an Antibody with Its Specific Antigen: The Case of HyHEL-10-hen Lysozyme Complex

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The interactions between protein antigen and its specific antibodies with one-point mutation have been investigated using isothermal titration calorimetry. Some mutation led to the increase in enthalpy change, and others to the decrease. Contributions of each amino acid to the interaction could be classified with two types, i.e., enthalpic and entropic.

Specific molecular interactions occur pervasively in biological systems, providing the fundamental mechanism for selectivity in every aspect of biological structure and function. Contribution of each amino acid residue in the interfaces to the interactions has usually been examined using site-specific mutated proteins.

Proteinaceous antigen–antibody interaction is one of the model cases for biomolecular interactions, since antibody binding site is formed by a small number of protein segments of highly variable structure (the CDRs) grafted onto a scaffolding of essentially invariant architecture (framework regions).[1] Here, we will report the thermodynamic analyses of the interactions between an antigen (hen egg-white lysozyme, HEL) and its antibody HyHEL-10 variable fragments (Fv) with one-point mutation into the CDR.[2]

Eleven sites (Ser31, Asp32, Tyr33, Tyr50, Tyr53, Tyr58, and Asp96 in the heavy chain; Asn31, Asn32, Gln53, and Tyr96 in the light chain) have been selected for the analyses. These sites selected have been shown to make electrostatic interactions (i.e., hydrogen bonds and salt bridges) with the antigen directly from X-ray crystallographic study.[2] Seven mutants of heavy chain (H-Ser31Ala, H-Asp32Ala, H-Tyr33Phe, H-Tyr50Phe, H-Tyr53Phe, H-Tyr58Ala, and H-Asp96Ala) and four mutants of light chain (L-Asn31Asp, L-Asn32Asp, L-Gln53Ala, and L-Tyr96Phe) have been constructed by Kunkel's method.[3] Each mutant was prepared using bacterial expression system as described previously.[4]

In order to estimate the thermodynamic parameters of the interactions between one-point mutated antibodies and antigen, HEL, isothermal calorimetric titration has been performed. The Fv fragment at a concentration of 5 μM in 50 mM phosphate buffer (pH 7.2) containing 200 mM NaCl in a calorimeter cell was titrated with a 125 μM solution of HEL in the same buffer at 303 K. The ligand solution was injected 16 times in portions of 7 μL during a period of 15 s. Thermogram data were analyzed using a computer program (Origin) supplied by MicroCal, Inc,[5] supposing the following equilibrium;

$$\text{Fv} + \text{antigen} \overset{K\text{a}}{\longleftrightarrow} \text{Complex}$$

The enthalpy change (ΔH) and binding constant (K_a) on antigen–antibody interaction are directly obtainable from the experimental titration curve. Gibbs energy change ($\Delta G = -RT \ln K_a$) and the entropy change ($\Delta S = (-\Delta G + \Delta H) / T$) on the association could be calculated from ΔH and K_a. The typical titration profile has been shown in Figure 1, and thermodynamic parameters at 303 K have been shown in Table 1.

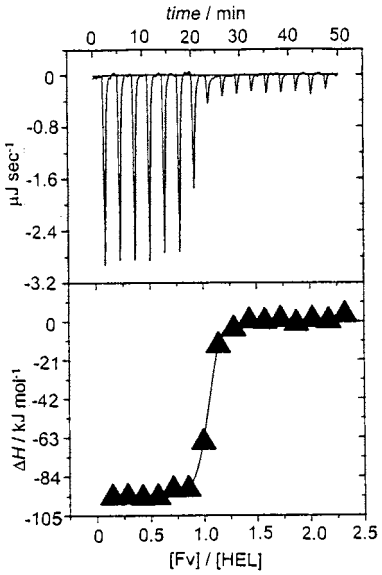

Figure 1. Isothermal titration calorimetry of HyHEL-10 Fv-HEL interaction. Top, typical calorimetric titration of HyHEL-10 Fv fragment (mutant L-N31A) with lysozyme; Bottom, integration plot of data calculated from raw data.

Upon comparison with wild-type Fv, all mutants had decreased affinity for HEL. Enthalpy and entropy changes, however, have been varied between mutants. Namely, the interactions between HEL and five mutants (H-Tyr33Phe, H-Tyr50Phe, H-Tyr53Phe, H-Tyr58Ala, and L-Asn32Asp) showed the decrease in negative enthalpy change (Table 1). These changes in enthalpy have been in part compensated by the changes in entropy, leading to reduce the effect of the mutations on the interaction. Thus, it can be concluded that each residue makes enthalpic contribution to the interaction. On the other hand, six mutants (H-Ser31Ala, H-Asp32Ala, H-Asp96Ala, L-Asn31Asp, L-Gln53Ala, and L-Tyr96Phe) showed the increase in negative enthalpy change (Table 1), and favorable changes in enthalpy have been compensated by the changes in entropy, leading to decrease in affinity for the antigen. Then, it would be proper to conclude that these residues make entropic contribution to the interaction. From these observations, contributions of the residues in CDRs can be classified with two types from thermodynamic viewpoints, i.e., enthalpic and entropic, in comparison with the wild-type.

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Table 1. Thermodynamic parameters of the interactions between one-point mutant HyHEL-10 Fv fragments and HEL at 303 K

Fv	ΔG/kJ mol^{-1}	ΔH/kJ mol^{-1}	$T\Delta S$/kJ mol^{-1}
Wild-type	-50.2±2.5	-91.5±4.0	-41.3±2.0
H-Ser31Ala	-46.3±2.0	-97.9±4.5	-51.6±3.0
H-Asp32Ala	-46.4±2.5	-112.9±5.0	-66.5±3.5
H-Tyr33Phe	-45.6±2.2	-73.2±2.5	-27.6±1.5
H-Tyr50Phe	-43.1±2.0	-59.8±2.0	-16.7±1.0
H-Tyr53Phe	-47.2±2.4	-84.4±2.5	-37.2±2.0
H-Tyr58Ala	-43.1±1.8	-72.3±2.2	-29.2±1.7
H-Asp96Ala	-48.2±2.0	-113.7±5.0	-65.5±4.0
L-Asn31Asp	-46.1±2.1	-105.8±4.5	-59.7±3.5
L-Asn32Asp	-40.5±1.8	-53.6±2.0	-13.1±1.0
L-Gln53Ala	-45.7±2.2	-96.8±4.0	-51.1±2.5
L-Tyr96Phe	-46.8±2.5	-94.6±4.0	-47.8±2.2

Values are the averages of at least three experiments. ΔG, ΔH, and ΔS, changes in Gibbs energy, binding enthalpy, and entropy, respectively.

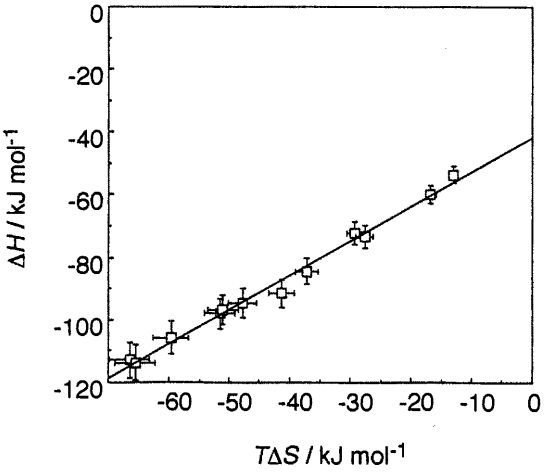

Figure 2. Enthalpy-entropy compensation plot of the interactions between one-point mutated HyHEL-10 Fv fragments and HEL. Enthalpy changes in Table 1 are plotted against the entropy changes.

It has been suggested that electrostatic interactions such as hydrogen bonds and salt bridges make favorable contribution (8–16 kJmol^{-1}) to the biomolecular interactions through enthalpic advantage.[6] The results reported here, however, indicate that the favorable enthalpy and entropy changes are almost completely compensated by the unfavorable entropy and enthalpy changes, respectively. The enthalpy change of the interaction between each mutant Fv and HEL has been plotted against the entropy change (Figure 2). The slope in Figure 2 is 1.09, indicating the almost complete compensation. This indicates that all single-mutations in this study are "tolerant" due to enthalpy–entropy compensation.[7–9] As has been proposed, shape complementarity in itself might be critical for binding of an antibody with the protein antigen.[10,11]

Although the antigen–antibody interaction reported here is enthalpy-driven, i.e., favorable enthalpy change (–92 kJ mol^{-1} at 308 K) is in part compensated by unfavorable entropy change (–42 kJ mol^{-1}), some residues in CDRs make entropic contribution. Investigation of structural evidence for the entropic contribution would be intriguing for further interpretation of thermodynamics of biomolecular interactions.

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