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Structural and thermodynamic analysis of a pH-sensitive protein G mutant

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Introduction

Molecular interactions govern a number of biological processes including metabolism, signal transduction and immunoreaction. Interactions between biomolecules are generally characterized by their affinity, specificity, and environmental responsiveness such as sensitivity to pH. Such pH-dependent ligand binding enables biological processes to function in an "on and off" manner in response to environmental conditions, resulting in sophisticated systems of regulation e.g., pheromone production, immune systems and virus survival.

To better understand the design methodology for a pH-sensitive protein-protein interaction, we generated a pH-sensitive *Streptococcal* protein G B1 (GB) mutant GB0919 by rationally introducing histidine residues onto the binding surface to cause electrostatic repulsion under acidic conditions. We analyzed the molecular basis of the histidine-mediated interaction from a thermodynamic and structural perspective. The observed data revealed functional and structural consequences for the introduction of histidine residues.

Experimental procedures

A stabilized protein G, GB09, and a histidine-introduced mutant, GB0919, were dialyzed against 10 mM Tris-HCl (pH 7.4) buffer. GB09 crystal was obtained by using hanging drop vapor diffusion under 40% PEG8000, 0.2 M ammonium sulfate, 10 mM Tris-HCl (pH 8.0). GB0919 was crystallized under 70% MPD, 20 mM HEPES (pH 7.4). Before collecting diffraction data, the crystal was soaked into 70% MPD, 20 mM sodium acetate (pH 4.5) for 1 hour. The diffraction data was collected at Photon Factory BL-6A, and processed by HKL-2000. The crystal structures were determined by molecular replacement (Molrep) using a wild-type protein G B1 coordinate, refined by using CNS, REFMAC5 and Coot.

Results and Discussion

Crystal structures of GB09 and GB0919 were determined at 1.4 and 1.6 Å resolution, respectively. The slightly acidic conditions did not induce considerable conformational change and the rigidity of the domain was maintained. Therefore, the introduced histidine residues must participate in electrostatic repulsion between protein G and Fc as the rigid body model implies. The three-dimensional configuration of functionally important residues supports the evidence that the rigid body model retained the innate binding ability.

Examination of the histidine-introduced site illustrated that the histidine residues held clustered water molecules. Comparison of GB0919 to GB09 suggests an increase in the number of solvated water molecules particularly in the histidine-introduced area (Figure 1). The space formed by His40 and Trp43 accommodated a cluster of water molecules supported by hydrogen bonding via nitrogen atoms of the histidine imidazole ring and the tryptophan indole ring. Binding of GB0919 to Fc probably desolvates some of these water molecules, which is highly likely to contribute to the entropy gain in the interaction. Thermodynamic analysis showed that histidine-mediated electrostatic repulsion resulted in significant loss of exothermic heat of the binding, thereby improving the pH sensitivity. The sensitivity was partly suppressed by "enthalpy-entropy compensation" possibly because of exclusion of the solvated water molecules. Thus, the thermodynamic and structural data were entirely consistent.

Table 1: Thermodynamic parameters of the interaction

	ΔH (kJ/mol)	-TΔS (kJ/mol)
WT	-31 ± 3.1	-4.6 ± 2.2
GB0919	-2.1 ± 1.1	-32 ± 0.6

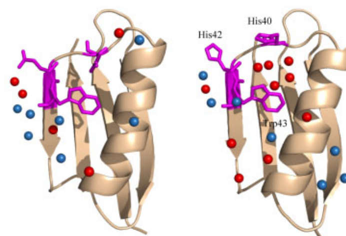

Figure 1. Possibly desolvated water molecules of GB09 (left) and GB0919 (right) upon GB-Fc binding. Crystal structure of GB09 or GB0919 was superposed on Fc-complexed protein G. Then, the water molecules located within 2.0 Å (red) and 3.2 Å (blue) from the Fc surface in the superposed structure were selected.

References

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