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Intramolecular H-bonds facilitate the recognition of a flexible peptide by an antibody.

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We have shown that intramolecular H-bonds transiently stabilize the conformation of a flexible peptide in solution. The stabilized conformation of the peptide in solution is consistent with the conformation of the peptide bound to its specific antibody as shown here by X-ray crystallography. Ablation of these intramolecular H-bonds modulates the affinity of the peptide for the antibody by up to 2 kcal mol^{-1} of free energy. These results reveal an important role of intramolecular H-bonds in antibody binding and have implications for designing efficient vaccines from short peptides.

1 Introduction

Short peptides are employed to generate antibodies by immunization procedures, such as in vaccines. Since short peptides do not possess stable secondary structure in solution, it is unclear how antibodies mature and recognize such flexible antigens. In particular, we are interested to understand the role of intramolecular H-bonds in antibody recognition, and in particular their possible role in the transient stabilization of conformations of the peptide in the unbound state (in solution) and their thermodynamic consequences.

X-ray crystallography, MD simulations and calorimetric techniques, all high resolution techniques, were employed to address this question. In this report we describe the preparation and determination of high-resolution crystal structures of the peptide pep1 (and mutations) derived from the therapeutically relevant human protein CCR5 (co-receptor of HIV-1) with its specific antibody 4B08.

2 Experiment

Recombinant scFv antibody 4B08 and mutants were obtained from inclusion bodies produced in *Escherichia coli* [1]. The refolded protein was purified by Ni^{2+}-affinity chromatography and by size exclusion chromatography. Antibody 4B08 (100 μM) dialyzed in MES buffer (10 mM MES, 30 mM NaCl, pH 5.9) was mixed with peptide (200 μM). Crystals of the complex were obtained in 1.8–2.4 M $(NH_4)_2SO_4$, 0.1 M Bis-Tris, pH 5.9–6.5 at 20 °C by the hanging-drop vapor diffusion method. The crystals were briefly transferred to mother liquor solution supplemented with 30% glycerol prior to their storage in liquid N_2 until data collection.

Diffraction data were collected at beamlines BL5A and AR-NE3A of the Photon Factory (Tsukuba, Japan) under cryogenic conditions (100 K). The resolution achieved for wild type, mutant N3A and T6A was 1.35, 1.96 and 1.35 Å, respectively. The coordinates and structure factors have been deposited in the protein data bank (PDB) under entry numbers 5YD3 (wild type), 5YD5 (N3A), and 5YD4 (T6A).

3 Results and Discussion

The crystal structure of the complex between scFv antibody 4B08 and wild-type peptide was determined at 1.35 Å resolution by the method of molecular replacement using the coordinates of a previously determined scFv [2]. The complex between antigen and antibody is stabilized by nine intermolecular and four water-mediated H-bonds, and an interaction surface of 995 ± 31 $Å^2$ is reported. Residues Asn3 and Thr6 engage in four intramolecular H-bonds with other residues of the peptide in the complex (Fig. 1). Since these two residues barely interact with the antibody, we considered them a good model to investigate the contribution of intramolecular H-bonds in protein-peptide interactions.

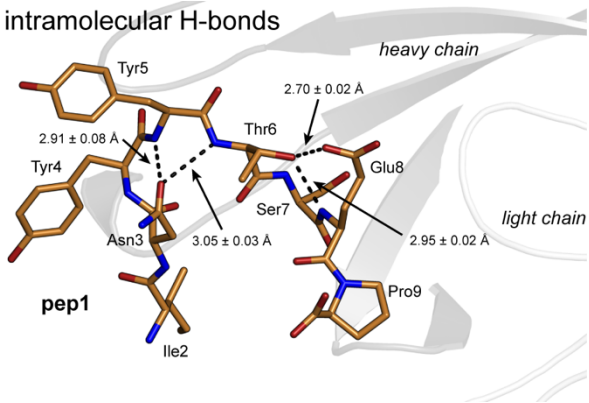

Fig. 1: Intramolecular H-bonds within the peptide (wild-type) when bound to the scFv antibody 4B08 [1].

Crystal structures of 4B08 in complex with peptides containing mutations N3A or T6A were determined at 1.96 and 1.35 Å resolution, respectively (not shown). Each mutation individually ablate half of the intramolecular H-bonds observed above. The coordinates and binding mode of these two peptides were essentially identical to those of wild-type peptide. As expected the mutations eliminate the intramolecular H-bonds between their side chains (Asn3 or Thr6) and other atoms in the peptide. Although these mutations have a significant

effect in affinity (2.7 and 27-fold decrease for N3A and T6A, respectively) the structure of the bound peptide is not altered in the crystal [1].

Structural data combined with isothermal titration calorimetry and MD simulations lead us to propose the model of interaction shown in Figure 2. Intramolecular H-bonds modulate the ensemble and dynamics of the peptide in solution, bringing significant changes to the affinity for the antibody.

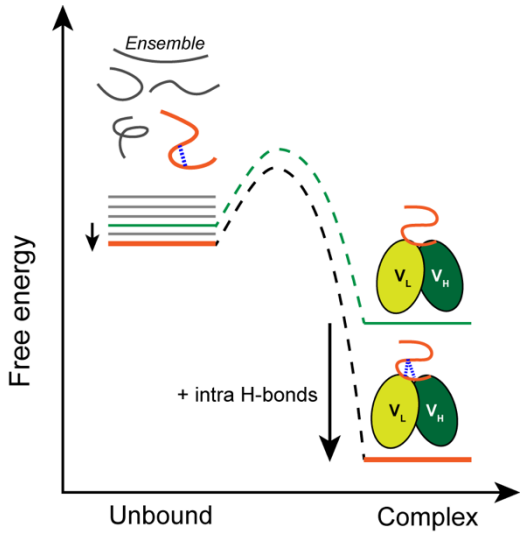

Fig. 2: Model showing the influence of intramolecular H-bonds in the recognition of a peptide by an antibody [1].

In summary, our study has established a role for intramolecular H-bonds in antibody-antigen interactions.

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