Binding free energy calculation and structural analysis for antigen-antibody complex

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Antibodies have functions and structures are still the best understood. As a rule, they bind specifically to molecules from the pathogens and play a principle role in adaptive immune system. An important mechanism of antibody to recognize antigen depends on loop structures in complementarity determining regions (CDRs) that is from variable regions(i.e. HV1-3, LV1-3) which locate on Fab fragment. Some reversible non-bond binding forces between various amino acids contribute to the antigen-antibody interaction. Therefore, amino acid sequences on variable regions are the important factor for recognition to their high favorable antigen. Furthermore, genetic engineering by site-directed mutagenesis can further tailor an antibody's binding sequences to its complementary epitope. It suggests a possibility to design antibody drug more efficiently if antibody engineering collaborated with thorough understanding antigen recognition mechanism by simulation.

The aim of this study is to analyze solvated antigen-antibody structure and calculate its binding free energy by simulation. We investigated Influenza Hemagglutinin (wild type HA), mutated HA, and their neutralize antibody complex (their initial atomic coordinates were derived from Protein Data Bank, 2VIR, 2VIS, respectively) by molecular dynamics using AMBER03 force field.

According to the result of experiment[1] studied structural differences between wild type HA and mutated HA, the mutation caused structural distortion and loss of hydrogen bonding, and it resulted that affinity to antibody became 4000-fold lower than wild type HA's.

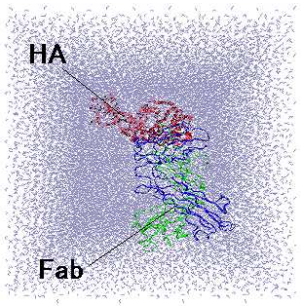

Figure 1: Solvated state of wild type HA and Fab fragment complex

To dynamically verify the above static result, we calculated root-mean-square fluctuations (B-factor) of loop structures of CDRs on Fab fragments in wild type HA, and made comparison the results to those of mutated HA's. The B-factor values correspond to stability of binding site[2]. At the same time, we calculated binding free energy of the complexes on equilibration phase during molecular dynamics, then compared the calculation results to disassociation constants which is derived from the experiment above. Some parts of calculation results are shown below(Table 1). B-factor is defined as eqs(1):

$$B = \frac{8}{3N}\pi^2 \sum_i^N \left\langle |R_i - \langle R_i \rangle|^2 \right\rangle. \quad (1)$$

Binding free energy can be calculated by eqs(2)[3].

$$\Delta G_{bind} = \langle E_{mm} \rangle + \Delta G_{solv} - T\Delta S. \quad (2)$$

Table 1: B-factor during 950-1000ps MD

	2VIR (Å^2)
HA127-132 residues	14.702857
HA155-161 residues	15.827036
HV1	12.504957
HV2	15.779722
HV3	17.820541
LV1	32.363053
LV2	29.986717
LV3	16.756486

References

[1] Damien Fleury. *Nature Structural Biology.* **5**, 119(1998).

[2] Tomoki Kinoshita, Masayuki Hata, Saburou Neya, Tyuji Hoshino and Chihiro Obinata *Symposium on Chemical Information and Computer Sciences.* **Vol. Tokyo No. SPACE** pp.JP12-(2003).

[3] Chong, L.T. *Proceedings of the National Academy of Sciences of the United States of America.* **96**, 14330(1999).