Chapter 6

Perspectives and Future Works

6.1 Slow Motion Measurement in Catalytic Pathway

In our relaxation measurement only can indicate that TEP-I behaves in μs-ms time range in the MC state. The biological function processes usually are in this time regime. Thus, the detail of the slow motion in enzyme catalysis becomes a very important research topic. The study of motion in μs-ms time scale has already developed an experimental approach.[63; 64] Using the CPMG experiment to investigate the slow exchange rate of the molecule identifies which residues behave in this time range. This approach has already successfully applied at dihydrofolate reductase catalysis[65]. Thus, the next step of the relaxation measurement is to analyze the slow motion of TEP-I in the catalytic pathway.

6.2 Side-Chain Relaxation

The backbone relaxation can only investigate the backbone motion of molecule, while the detail motions of side-chain were not characterized. Especially, ligand binding is directly applied on the side chain of active site. Thus, the side-chain relaxation of catalytic triad and oxyanion holes becomes an important issue. Besides, some residues which have significant local motion measured by backbone relaxation are worth to do side-chain relaxation measurement to understand the detail dynamics undergoing the catalytic process.

6.3 Molecular Dynamics Simulation of Michealis Complex of TEP-I

To understand the atomic motion of TEP-I in catalytic pathway, we have applied the molecular dynamics simulation on *apo*- form and TC-form of TEP-I. But, if we would like to understand the molecular motion in catalytic process, the molecular dynamics simulation of the MC state will make this study more complete. In order to understand how the ligand binds to active site, we have conducted the potential of mean force to calculate the free energy landscape of the active pocket. Figure 6.1 shows that the free energy landscape of wild type and mutant, L109P, of TEP-I. This mutant has change the energy landscape of DENP binding. There are a lot of unknown of enzyme during the catalytic process. Hence, an atomic visualization of molecular motion in MC state will also help substantially for under standing the whole catalytic mechanism.

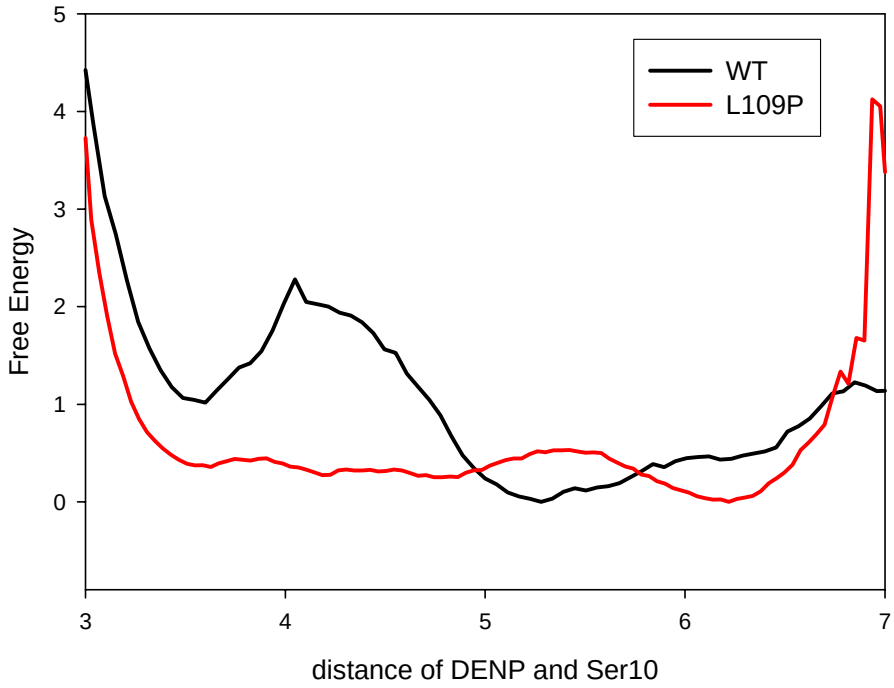

Figure 6.1

The free energy landscape of DENP binding was calculated by WAHM algorithm. And the MD trajectory is produced by AMBER force field. The restrain atoms were P of DENP and C_β of Ser10. The protein structures are started from X-ray structure of wild type and L109P of TEP-I.