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Theoretical elucidations on the activity modulation mechanism of vitamin D receptor and the DNA religation mechanism of DNA topoisomerase II

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Abstract

In this thesis two biomolecular processes are analyzed theoretically. The first subject is about the ligand dependent modulation of vitamin D receptor (VDR) activity. VDR is a member of nuclear receptors (NRs), which are important drug targets. Crystal strictures of NRs have been showed that the NR-targeted drugs control the NR mediated cell responses by turning the helix 12 of NRs into active or inactive conformations. Experiments indicated the relation between the receptor activity and solution conformational ensemble of the helix 12, ensemble of the major and minor conformations in solution. However, actual solution conformation of the helix 12 is unclear. It has been suggested there exists receptor conformations distinct from crystal structures. Because of the exceptional importance of NRs in drug design, mechanisms of NR activity modulation by various ligands have been heavily investigated by computational approaches. However, there is no theoretical description of the solution conformational ensemble of NRs, because the timescales of the conformational exchange of proteins are often significantly longer than the typical timescale of all atom molecular dynamics simulations.

In order to reveal the solution conformational ensemble of the VDR bound to a natural agonist, a compound that induces the active conformation of helix 12, a massive conformational sampling was performed by a replica exchange molecular dynamics simulation (REMD). REMD is an enhanced sampling technique to evaluate a correct free energy profile and has been widely used for protein folding studies. Calculated major conformation was in good agreements with a crystallographic active conformation, and minor conformations existed with very small excitation energies of 2~3 kcal. In the minor conformations, helix 12 was disordered or shifted, and one conformation was similar to a crystallographic inactive conformation of another NR, glucocorticoid nuclear receptor.

A non-crystallographic ligand binding pocket, inactive pocket, was found and relations between helix 12 conformations and the ligand bonding modes were revealed. In the crystallographic pocket, the active pocket, helix 12 maintained the crystallographic/active conformation, on the other hand, in the inactive pocket, helix 12 exhibited large conformational changes, which impair the receptor activity. The binding mode of the natural agonist in the inactive pocket was very similar to that of an antagonist, a ligand that induces inactive conformations of helix 12, in a crystal structure of the antagonist bound VDR. This

indicates that the antagonist mimics the binding mode of the natural agonist in the inactive pocket to induce the inactive conformation of helix 12. Based on this result, a mechanism of ligand specific activity can be proposed that ligands modulate the VDR activity by selecting the pre-existing active and/or inactive pockets. A novel strategy of VDR-targeted drug design can be derived that ligands can be controlled by tuning the relative binding free energies for the active and inactive pockets.

The second bimolecular process analyzed in this thesis is a DNA religation reaction catalyzed by DNA topoisomerase II (topo II). DNA topoisomerase is an essential enzyme that solves the topological problems of double-stranded DNA during various DNA-related events, such as replication, transcription, and chromosome segregation, by directing the cleavage and religation of DNA strands. Topoisomerases are the targets of some of the most successful anticancer and antibacterial drugs. Most of these drugs inhibit the religation step, which induces the accumulation of topoisomerase–DNA covalent complexes, toxic intermediates. Therefore, considerable attention has been paid to the mechanism underlying the cleavage/religation reaction. It is believed that the cleavage and religation reaction proceeds through a general acid–base catalysis. Despite numerous experimental studies, it is still unclear for the proton transfer pathway in the general acid/base catalysis. In this study, the DNA religation reaction of topo II was investigated by using quantum mechanical/molecular mechanical (QM/MM) calculations and possible proton transfer pathways were examined. In the QM/MM calculations, important atoms in the active site were included in the QM region, and the remaining atoms were handled by MM.

Calculated activation energy of the most favorable pathway was $\Delta E^{\#}$ = 19 kcal/mol, which can be comparable to the experimental value of $\Delta G^{\#}$ = 20 kcal/mol. The highest energy barrier was attributed to a nucleophilic attack of the 3’ O atom of DNA terminus on the cleaved phosphate. A characteristic feature of the reaction pathway is a proton relay pathway formed by the terminal 3’O, reactive phosphate, water, Arg781, and catalytic Tyr782. This novel pathway is named as substrate mediated proton relay (SMPR) pathway. The SMPR pathway is consistent with experiments on a series of Arg781 mutations. The proton transfer pathway elucidated in this study will assist for novel antibiotic/anticancer drug designs with high selectivity and efficiency.

As shown in the conformational analysis on VDR, many important biomolecular processes are accompanied by the conformational changes in a range from several angstroms to several tens of angstroms. Therefore descriptions of the conformational changes are necessary to understand the bimolecular processes, and one of the important roles of bimolecular simulation is elucidation of the biomolecular conformational changes that are difficult to be demonstrated from experimental approaches. However, a sufficient sampling of the biomolecular conformation is currently quite difficult except for conformational sampling of some small proteins, because there are large gaps between the actual timescales of the biomolecular conformational changes and the timescales of the biomolecular simulations. Therefore conformational analysis of an biologically and medically important protein is one of the most challenging topics. In this thesis, first description of the conformational space of VDR was realized by 1) using GPU accelerations, which speeded up by 2-3 times, 2) large scale parallel calculations and 3) the REMD algorism, which has a high parallel performance. As results, experimentally relevant minor conformations of VDR were obtained, and the molecular mechanism of the ligand dependent regulation of the conformation of helix 12 can be revealed. These results not only proceed VDR biology and VDR-targeted drug design, but also demonstrate the potency of the massively parallel REMD to sample the conformational space of the protein of typical size (~250 residues). This extends the scope of the biomolecular simulations. On the other hand, the classical MD

cannot handle the enzyme catalysis, and the QM calculations are used to analyze the enzyme catalysis. Because of huge computational costs of QM calculations, only the movements of the atoms involved in the reaction were analyzed in this thesis. However recent studies showed relations between the catalysis and dynamics of some enzymes. And elucidation of molecular basis of such phenomenon requires both the high accuracy calculations by QM/MM method and large-scale conformational sampling by MD method. Proceeding of the application of these two approach provided by this thesis will be increasingly important for future developments in biomolecular simulations.