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マルチカノニカル法による RNA ポリメラーゼ C 末端領域の研究

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Study on the C-terminal Domain of RNA Polymerase II by using
Multicanonical Molecular Dynamics Simulation.

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In eukaryotic cells, the C-terminal domain (CTD) of the RNA polymerase II plays an important roles to regulate the transcription of genes. The CTD consists of 26-52 tandem heptapeptide repeats with the consensus sequence YSPTSPS. The structure of the CTD in the RNA polymerase has not been observed by X-ray crystal diffraction experiment, suggesting the CTD has intrinsically undefined structure. The serine residues at second and fifth position in the CTD consensus are known to be major phosphorylation sites. Thus, the regulated phosphorylation and de-phosphorylation of the CTD switch the conformation states and carry out a vital role in the recruitment and assembly of transcription complexes. However, so far, the nature of conformational states of the CTD is never elucidated in experimentally and theoretically. Knowledge of the conformation states corresponding to the different phosphorylation must be useful for understanding the CTD mechanisms.

Here, we have tackled the issue by using an advanced computational methodology. Recently, molecular simulations are considered to be a useful tool for supporting experiments even in molecular biology. Indeed, molecular simulations provide atomic detail of protein and peptide under a variety of physiological conditions. However, proteins and peptides have the huge number of degree of freedoms. Then, normal standard molecular simulation hardly searches the conformation space sufficiently. To address this difficulty, we employed an extended ensemble method, multi canonical molecular dynamics simulation (McMD). McMD is an umbrella sampling method using the temperature space as a reaction coordinate, significantly increasing the conformational search ability.

To investigate phosphorylation effects on the CTD conformation, we studied four systems: unit-repeat-heptapeptide (h-peptide) without phosphorylation, h-peptide

phosphorylated on 2Ser, h-peptide phosphorylated on 5Ser, and h-peptide phosphorylated on simultaneously 2 and 5Ser. The phosphorylated Ser residue which is absent in the standard force field parameter was created according to the amber general procedure.

We then conducted the McMD simulations for the unphosphorylated and phosphorylated h-peptides centered at cubic box in the periodic boundary conditions with explicit waters and added ions for neutralizing the systems as seen in Fig. 1. All of the systems were potential energy minimized for reducing hard contacts among atoms and then constant atom-number, temperature, and volume simulations were conducted until reaching the equilibrium condition, followed by constant atom-number, temperature, pressure simulations.

After fixed the volumes to their averaged values, the upper limit temperature of the McMD simulations was set to 600K which is found to ensure efficient conformation searches for those systems. McMD simulation needs an accurate density-of-state to perform a random walk in the temperature space. To build the density-of-state accurately, it is necessary to iteratively carry out preparation molecular dynamics-runs until the density-of-state converged. After over 20-times preparation-runs, we obtained the accurate density-of-state for all systems. Snap-shots of atomic trajectory of the system were taken at every 10psec interval for analysis. All of the simulations are performed by using myPresto program package.

We then analyzed the h-peptide system from the McMD simulations. First of all, the McMD data was re-weighted to obtain ensemble data at room-temperature. Using the re-weighted data, we study the conformation space of the h-peptides. Backbone dihedral angles, ϕ and ψ are well suitable to characterize a peptide conformation, so we analyzed the thermodynamically conformational properties dependence on the phosphorylation with respect to the dihedral angles space. The details of procedures of the analysis results would be explained and the biological significance including cis-trans isomerase enzyme Pin1 would be also discussed.