論文 / 著書情報
Article / Book Information

題目(和文)	PaCS-MD と MSM による p53-DBD/DNA 複合体の 解離過程と結合自由エネルギーの解析
Title(English)	Investigating dissociation process and binding free energy of p53-DBD/DNA complex by PaCS-MD and MSM
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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Science)
学生氏名： Student's Name	Mohamed Marzouk Sobeh	指導教員（主）： Academic Supervisor(main)	Akio Kitao
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要旨（和文 2000 字程度）
Thesis Summary (approx.2000 Japanese Characters)

Binding of p53 DNA binding domain (p53-DBD) as a transcriptional factor to DNA is essential for the defensive role of p53 as a "guardian of the genome" against cancer. As a transcription factor, p53 regulates cell responses to various stresses including DNA damage by binding to DNA promoters in a sequence-specific (consensus sequence) manner and initiating transcription of genes involved in cell cycle arrest, apoptosis, and DNA repair. The accumulation of mutations in p53-DBD may destabilize and unfold the structure, impairing p53's capability of detecting and attaching to its target sequence. Investigating how the key residues of binding surface stabilize the p53-DBD/DNA complex structure and the energy of binding is critical for understanding the recognition mechanisms of the specific DNA sequence by p53-DBD. These mechanisms remain unclear, and more research is needed for the critical residues that sustain sequence-specific p53-DBD DNA binding.

While molecular dynamics (MD) simulation is a widely used technique for deciphering the mechanisms underlying biological processes, most critical biological events, such as protein-ligand, protein-protein, and protein-DNA binding/unbinding, frequently occur over much longer timescales and thus cannot be observed using all-atom MD simulation. As a result, I adopted one of the newly established improved sampling approaches termed dissociation parallel cascade selection molecular dynamics (dPaCS-MD) to enable the detection of these slow processes and rare events in my work. Three major objectives may be outlined in this research. 1) Simulation of the dissociation of the p53-DBD/DNA complex using dPaCS-MD. 2) Identification of the p53-DBD/DNA binding interface's key residues. 3) Generation of preferred dissociation pathways and estimation of binding free energy, both of which remain computationally difficult.

In this dissertation, I chose the PDB ID: 1TSR structure because one of its monomers (Chain B) as the initial structure of the simulation because this structure is substantially bound to the consensus sequence. After solvating the complex in a box of water molecules of TIP3P model and neutralizing system charge with 150 mM potassium chloride (KCl) ions, the basic model of p53-DBD was created. The prepared model of p53-DBD was equilibrated in three successive steps; Energy minimization, and NVT and NPT equilibrations followed by 1 μs MD simulation without any positional restraints to sufficiently sample the conformational space. Then, to observe sufficient dissociation, I extended the simulation box of the most populated cluster in the 1 μs MD simulation and filled the gap with TIP3P water and KCl followed

by short energy minimization and equilibration.

The dissociation simulation was performed using dPaCS-MD starting from five initial structures chosen around the last snapshots of the equilibration extended box MD. Cycles of parallel short MD simulations without external force begin with the selection of starting conformations, each of which is utilized for a unique replica (10 replicas in my case). Short-time MD simulations are initiated for each replica using the Maxwell-Boltzmann distribution to regenerate the starting atomic velocities. These cycles are continued indefinitely until complete dissociation occurs at Inter-COM distance, d, = 70 Å.

Using the combined trajectories of 75 PaCS-MD trials, I constructed an MSM model based on three-dimensional (3D) Inter-COM coordinates between p53-DBD and DNA interface residues, termed 3D-MSM. 3D-MDM provides the stationary probability of the microstates that can used to estimate the binding free energy difference from the potential of mean force (PMF), ΔG_{PMF}, by

$$\Delta G_{PMF} = -k_B T \ln \frac{P_b}{P_u}$$

In this dissertation, 75 trials of dPaCS-MD were conducted with an average simulation time of 11.2 ± 2.2 ns. During the dissociation process, 93% of the trials dissociated along the +X and −Y directions (−Y directions), while 7% moved along the +X and +Y directions (+Y directions). Along the −Y directions, p53-DBD dissociated from the major groove first and then detached from the minor groove, while unbinding from the minor groove occurred first along the +Y directions, followed by dissociation from the major groove. The standard binding free energy calculated from the standard free energy landscape was ΔG° = − 10.9 ± 0.4 kcal/mol, which agrees with the value obtained by ITC, -11.1 kcal/mol.

The minor groove binding is stabilized mainly by R248 and R249. R248 is the most critical residue, tightly packed within the minor groove. In 75% of dPaCS-MD trials, R248 was the last residue detached from DNA. Thus, R248 mutations, one of the most common missense mutations in p53, affect p53 DNA binding and p53 functions, and ultimately cause human cancer. The binding of p53-DBD to the major groove is stabilized by R280 and R283. R280 was the last residue dissociated from the DNA in 20 % of trials while R283 tended to dissociate faster. These results show that the p53 key residues for the DNA binding are known as the cancer-related mutations

Therefore, the promising combination of dPaCS-MD/MSM can be used to investigate multiple pathways during the dissociation of two large molecules, as well as to identify critical residues for major dissociation pathways and to quantitatively calculate the complex's binding free energy.