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論　文　要　旨　(博士)

論文題目	高精度分子シミュレーションによる転写制御タンパク質 ラクトースリプレッサーと DNA 間の特異的相互作用の解析

(要旨　1,200 字程度)

生体の主な構成要素であり、生体機能を制御しているタンパク質は、DNA に蓄えられた遺伝情報を基に、DNA から RNA への転写機構、及び RNA からタンパク質への翻訳機構を経て合成される。このタンパク質の合成機構、及びこの機構を制御するタンパク質の機能に関しては、これまでの実験により、その概要は明らかにされているが、転写や翻訳を制御するタンパク質と DNA 間の生体内での相互作用機構、あるいは、生体内での環境変化がこれらの機構に及ぼす影響については、未解明である。本研究の対象である転写制御タンパク質ラクトースリプレッサー(LacR)に関しても、生体内での様々なリガンドの有無に応じて、転写機構を巧みに制御することは明らかになっているが、その機構は原子・電子レベルでは未解明である。通常、LacR は DNA に結合し、転写を抑制しているが、周囲に存在するインデューサが LacR に結合すると、LacR と DNA 間の結合親和性が弱まり、LacR が DNA から分離する。その結果、それまで抑制されていた DNA の転写が開始される。一方、アンチインデューサが LacR に結合すると、インデューサとは逆に LacR と DNA 間の結合親和性が強まり、転写をさらに抑制することが分かっている。しかし、LacR のリガンド結合位置と DNA 結合位置は、約 30 Å 以上離れており、LacR にリガンドが結合した影響が、LacR と DNA 間の結合親和性にどのように伝わるかは、原子・電子レベルでは未解明である。この現象が解明できれば、生命現象の中で最も基本的な現象である転写機構に関して、新たしい知見を得ることができると考える。

本研究では、LacR+DNA+リガンド複合体の実験構造を基に、単量体及び二量体の複合体を作成し、さらに、リガンドが結合していない複合体、インデューサ IPTG (Isopropyl-β-D-thiogalactpyranose)、あるいは、アンチインデューサ ONPF (o-nitrophenyl-fucoside) が結合した複合体を作成した。それらの構造を、古典分子力学(MM)及び分子動力学(MD)法により、水中で最適化し、*ab initio* フラグメント分子軌道(FMO)法を用い、最適化構造の電子状態を解析し、LacR へのリガンド結合が LacR と DNA 間の特異的結合特性、結合親和性にどのような影響を与えるかを、電子レベルで解明した。

まず、長時間 30 ns の MD 計算を実行し、複合体の構造変化を広範囲に探索し、LacR に結合するリガンドの種類による、LacR と DNA 間の結合部位周辺の構造変化を明らかにした。さらに、FMO 計算により、LacR の各アミノ酸残基とリガンド及び DNA 間の特異的相互作用を解析し、実験で重要とされていた Arg22、Asp149、Asn246 が、LacR とリガンド間の結合に重要であることを明らかにした。さらに、LacR と DNA 間の相互作用には、LacR のアミノ酸残基、及び DNA の塩基 Thy14、Gua15 が重要であることを明らかにした。これらの結果を基に、LacR への様々なリガンドの結合が、LacR と DNA 間の特異的相互作用に与える影響に関する新たなモデルを提案した。

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Abstract

Title	Analysis of specific interactions between lactose repressor protein and DNA by molecular simulations based on classical MD and ab initio fragment MO methods

(800 words)

The transcription of genetic information from DNA to mRNA is the first and essential process in a series of mechanisms for gene expression. In all living organisms, the transcription is precisely regulated by many types of regulatory proteins as well as small ligand molecules to respond to the perpetual change of biological conditions in a cell. Lactose repressor (LacR) is one of the regulatory proteins and controls the transcriptional mechanism in a ligand-dependent manner. Although the ligand-binding to LacR was found to change the mechanism drastically, the effect of ligand-binding on the conformation of LacR-DNA complex has not been clarified at atomic and electronic levels. LacR generally regulates the transcription mechanism by binding to the specific site of DNA. On the other hand, the binding of inducer such as lactose and IPTG (Isopropyl-β-D-thiogalactopyranoside) to DNA weakens the binding affinity between LacR and DNA, leading to the separation of LacR from DNA. As a result, the transcription is started, and some lactose metabolizing enzymes are synthesized to metabolize lactose. In contrast, when anti-inducer such as ONPF (*o*-nitrophenylfucoside) binds to LacR, the binding affinity between LacR and DNA is enhanced and the transcription is more regulated by LacR. The transcriptional regulation mechanism of LacR is well known as mentioned above. However, it is difficult to explain the mechanism by the change in interactions between LacR and ligand, because the distance between the ligand binding site and the DNA binding site of LacR is about 30 Å. Hence we considered that it is needed to investigate drastically structural change of LacR for explaining the mechanism.

In this study, we first obtained the structure of the complex with LacR, DNA, anti-inducer ONPF from PDB (PDB code: 1EFA) and constructed the monomer and the dimer of the LacR-ONPF+DNA complex. The structures of the monomer and the dimer of the LacR+DNA complex, which is the complex without ligand, were constructed by deleting ONPF from the monomer and the dimer of LacR-ONPF+DNA. And then the monomer and the dimer of the LacR-IPTG+DNA complex were constructed by adding IPTG to the monomer and the dimer of the LacR+DNA complex. The position of IPTG was determined based on the experimental structure of the complex with the dimer LacR and IPTG (PDB code: 2P9H), which do not include DNA and the DNA binding domain of LacR. Solvating water molecules were added to these six complexes, and the solvated structures were optimized by the classical molecular mechanics (MM) method based on AMBER99SB-ILDN and TIP3P force fields, and the 30 ns molecular dynamics (MD) simulations were performed at 300 K under the periodic boundary condition to elucidate the conformational change of the complex. Furthermore, *Ab initio* fragment molecular orbital (FMO) calculations performed to investigate

their electronic states and specific interactions between LacR and DNA.

After the MM and MD simulations, the structures of the ligand pocket and the DNA binding domain of LacR are changed by the ligand-binding. And we investigated the conformational change and the interaction energies between ligand, amino acid residues of LacR, and solvating water molecules to elucidate the important amino acid residues for binding the ligand. Both of the IPTG and ONPF ligands form some hydrogen bonds to Asp149 and Asn246 of LacR. These residues were also indicated as important amino acid residues by the previous experiments. Especially Asp149 is important because it does not form hydrogen bond to the ligand, but interacts with the ligand electrostatically (the interaction energy is about -40 kcal/mol). And our simulations also clarified that some water molecules are important for binding between the ligand and LacR. For example, Asp274 of LacR interacts repulsively with ONPF (18.1 kcal/mol). However, Asp274 and ONPF interact with a common water molecule by -27.4 kcal/mol and -6.4 kcal/mol energies, respectively. As a result, Asp274 and ONPF interact attractively to each other through the common water molecule. The analysis for the DNA binding domain of LacR clarifies that Arg22 of LacR is important for the binding between LacR and DNA. Arg22 interacts electrostatically with Thymine14 and Guanine15 of DNA. Some experiments also reported that Arg22 is important for the specific recognition between LacR and DNA. Furthermore, we elucidated that the side-chain of Arg22 is also important because the interaction energies between Arg22 and these DNA bases are dependent significantly on the direction of Arg22 side-chain.

The structural analysis of the dimer complex elucidates a new mechanism explaining the effect of ligand-binding on the conformation of LacR+DNA complex. We investigated the change in hydrogen bonds around the ligand-binding to the DNA binding domains of LacR. As a result, both ligands form some hydrogen bonds with the amino acid residues of LacR which exist on DNA side. These amino acid residues links DNA binding domain of other side monomer LacR in some hydrogen bonds. Therefore, we expected that these links shift with changing shape of ligand pocket. And difference of both the ligands is its volume and the position of interaction with amino acid residues of LacR. Thus this difference is the cause of turning the transcriptional mechanism.