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論　文　要　旨　(博士)

論文題目	第一原理分子シミュレーションによる 芳香族炭化水素受容体とリガンド間の特異的相互作用の解析

(要旨　1,200字程度)

芳香族炭化水素受容体（AhR：Aryl hydrocarbon Receptor）は、異物が生体内に取り込まれた時に、これらを特異的に結合・認識し、その情報を核に伝え、代謝酵素の発現を誘導する転写活性因子である。この異物受容体が細胞分化機構を制御するハブとして機能しており、様々な疾患の発病に関係することが明らかになっている。しかし、現在、AhRの立体構造は未解明であり、AhRと様々なリガンド間の特異的相互作用の機構は、原子レベルでは明らかになっていない。更に、免疫機構に関与する細胞分化の方向性が、AhRに結合する物質により変化することは明らかになっているが、その原因は解明されていない。本研究では高精度な構造予測手法を用い、AhRのリガンド結合ドメインの構造を予測し、*Ab initio* 分子軌道(MO)法を用いた電子状態計算により、AhRとリガンド間の特異的相互作用を電子レベルで初めて明らかにした。更に、AhRの転写活性機構に重要と考えられているAhRとco-factorタンパク質ARNT(AhR Nuclear Translocator)とのヘテロ二量体構造を予測し、二量体形成機構解明への重要な知見を得た。

まず、本研究では、タンパク質立体構造予測プログラムを用い、Protein Data Bankに登録された立体構造既知のタンパク質を鋳型とし、ラットAhR（rAhR）のリガンド結合ドメインの立体構造を作成した。次に、タンパク質-リガンドドッキングプログラムを用い、細胞分化の方向性を変化させるリガンドをrAhRに結合させ、複合体の構造を古典分子力場計算用い、水中で最適化した。さらに、フラグメント分子軌道(FMO)法を用い、rAhR中の各アミノ酸とリガンド間の特異的相互作用を明らかにし、従来の実験結果を原子・電子レベルで説明できる結果を得た。

次に、リガンド結合ドメインの立体構造予測と同様の手法で、ヒトAhR (hAhR)とARNTの二量体の立体構造を作成した。更に、そこにリガンド付加した複合体構造を作成し、古典力場法を用い、水中で構造を最適化し、室温における構造変化を古典分子動力学計算により解析した。最後に、hAhRとARNT間の特異的相互作用をFMO計算により解析した。その結果、hAhRの二量体形成ドメインに存在する荷電アミノ酸が、ARNTの荷電アミノ酸と強く相互作用し、二量体の形成・分離に大きく影響することが明らかになった。

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Abstract

Title	Specific interactions between Aryl hydrocarbon receptor and various ligands: molecular simulations combined with classical MD and *ab initio* FMO methods

(800 words)

Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, mediates toxic and biological effects of a diverse spectrum of chemicals including environmental contaminants such as dioxin family. AhR binds extraneous substances as a ligand, and the information of the binding is transferred to the nucleus, resulting in the induction of metabolic enzymes. In the cell differentiation of various organism species, AhR plays a prominent role in the development of immune systems depending on the intracellular environment. Recent biochemical studies elucidated that some ligands bind specifically to AhR to have a significant effect on the development of immune systems. However, it has not been elucidated how the ligands binding to AhR affects the development of immune systems. Moreover, the three-dimensional structures of AhR itself and its complex with ligand have not been determined by experimental structural biology.

In our study, we first searched stable structures of the complexes with rat AhR (rAhR) and the ligands (TCDD, β-NF, FICZ, ITE) by protein-ligand docking and classical MM methods, and the binding affinity and the specific interactions between rAhR and ligands were investigated at an electronic level by the *ab initio* fragment molecular orbital (FMO) calculations. The results simulated were compared with the experimental results obtained by our collaborators.

In addition, we constructed the candidate structures of the complex of human AhR (hAhR) with co-factor protein ARNT, in order to elucidate the dimerization mechanism of hAhR and ARNT controlled by ligand binding. By the FMO calculations, the specific interactions between hAhR and ARNT were elucidated for the first time.

The binding energies between rAhR and the ligand evaluated by FMO are 30.8 (TCDD), 38.0 (β-NF), 51.0 (FICZ) and 55.8 kcal/mol (ITE), respectively. In addition, the FMO results elucidate that the endogenous ligands (FICZ and ITE) bind strongly to some specific amino acid residues (Gln381, Tyr320, Phe293) of rAhR, while the exogenous ligands (TCDD and β-NF) bind weakly to many residues of rAhR. In particular, the side chain of Gln381 is flexible and its amino group forms a strong hydrogen bond with the oxygen atom located at the center of the ligand in all the rAhR+ligand complexes. Therefore, it is expected that the amino group of Gln381 plays an important rule as an anchor in binding these ligands and that ligands forming a hydrogen bond with the amino group of Gln381 can be a potent agonist to rAhR.

To check the validity of the calculated results, we compared the results with the mutagenic experiments by Motto *et al.* They focused their attention specifically on the 26 residues contained within the 5 Å distance from the ligand-binding pocket of the mouse AhR (mAhR). These residues were mutated by the other amino acids, and the change in induction factor by the mutations was investigated by experiment. The results elucidated the 17 residues (Thr287, His289, Phe293, Pro295, Leu306, Leu313, Tyr320, Phe322, Ile323, Cys331, Met338, Phe349, Leu351, Ser363, Ala365, Ala379 and Gln381) are important for the binding between mAhR and TCDD. Our FMO calculations for rAhR+TCDD elucidate that the seven residues among the ten residues having the largest attractive interaction to TCDD are included in the above mentioned 17 residues. Consequently, it is elucidated that our computed results on the specific interactions between rAhR and TCDD are comparable to the results for mAhR and TCDD obtained by the experiment, although some residues are different between rAhR and mAhR.

In addition, we constructed several model structures for the hAhR+ARNT complex including a ligand by the homology modeling and the protein-ligand docking programs. Solvating water molecules were added around the complex, and their positions were fully optimized by a classical MM method. Futhermore, to search for various conformations of the solvated complex, classical MD simulations were performed by MM/MD program GROMACS. For the most stable conformation determined by the *ab initio* FMO calculations, the specific interactions between hAhR and ligand and between hAhR and ARNT were investigated to reveal the effect of ligand-binding on the specific interactions between hAhR and ARNT. Consequently, we elucidated that the dimerization and separation of the dimer are significantly affected by the electrostatic interactions between the charged residues included in hAhR and ARNT. In particular, Glu279, Ile280 and Arg281 of hAhR and Ser451, Asp452 and Glu453 of ARNT contribute to the dimerization between hAhR and ARNT. On the other hand, the Arg288, Lys290, Lys292 and Lys372 of hAhR, Arg362, Arg366, Hip378 and Arg379 of ARNT are important for the separation.