

Theoretical Study on Nucleotide Dependent Transportation Mechanism of ABC Transporter

著者	裴 強
号	52
学位授与番号	3886
URL	http://hdl.handle.net/10097/37602

氏名	べい きょう 裴 强
授与学位	博士(工学)
学位授与年月日	平成19年9月12日
学位授与の根拠法規	学位規則第4条第1項
研究科, 専攻の名称	東北大学大学院工学研究科(博士課程) 応用化学専攻
学位論文題目	Theoretical Study on Nucleotide Dependent Transportation Mechanism of ABC Transporter (ABC トランスポーターのヌクレオチド依存性輸送機構に関する理論研究)
指導教員	東北大学教授 宮本 明
論文審査委員	主査 東北大学教授 宮本 明 東北大学教授 中山 亨 東北大学教授 魚住 信之

論文内容要旨

ATP binding cassette (ABC) transporters are one of the major classes of trans-membrane carriers found in all eukaryotic and some prokaryotic cells. Their main function is to translocate several kinds of substrates from and out of the cytoplasm through the cellular membrane. The energy necessary to perform this function is provided by interaction of these membrane proteins with ATP. ABC transporters are found related to a number of human genetic diseases and multi-drug resistance. Recent development in crystallographic techniques has provided a means to establish a general framework for unveiling the details of the substrates translocation process. ABC transporters consist of four core domains, two transmembrane domains (TMDs) and two nucleotide binding domains (NBDs). The latter, which are located in the cytoplasm, are the on/off switch for the translocation tunnel formed by the TMDs. The atomic mechanical details of these processes have not been studied at the atomic level, which prevent thus a detailed understanding of the reactions and conformational changes associated with the translocation cycle, which are of the utmost importance in elucidating the type of substrates that can or cannot be translocated, as well as the type of ABC transporters. I have focused the study from two standpoints, the first is the structural analysis of ABC transporters, and the second one is related with the analysis of the energy source for the translocation process to occur. The results of this study are of a great impact in the future development of new drugs and treatment for several diseases caused by disfunction of ABC transporters.

The protein-protein soft-docking module in "MIAX" is used to construct a model for the NBD dimer. A flexibility analysis is then performed on the constructed NBD dimer and is compared with the crystal NBD monomer and dimer employing the system "RIGIX". Density functional theory (DFT) method and classical molecular dynamics with chemical reaction extension coded in the program "New-Ryudo-CR" are used to study NBD promoted ATP hydrolysis and the subsequent conformational changes of the NBD dimer during ATP hydrolysis.

In order to construct a suitable model for the NBD dimer, a new methodology coded in the soft-docking module in the MIAX system was used. The main characteristic of the soft-docking module in MIAX is its ability to predict complexes taking into account possible rearrangement in the conformation of the interacting subunits. The "soft-docking" module was validated by 48 pairs of unbound proteins. The validation result prove that the

soft docking module in MIAX program is able to reproduce a protein-protein interface, with which a set of starting conformation of protein complexes can be constructed.

The soft docking module was applied in constructing the crystal monomer HisP into dimer conformation. Two monomers are connected upside down with each other mediated by two ATP molecules, as shown in Fig.1. The constructed dimer shares a high morphology similarity with crystal NBD dimer. Softened layer enables the interpenetration of p-loop of one subunit and signature motif (c-loop) of the other, and composing a complete ATP binding site. Although the soft docking module in MIAX is able to infer conformational changes, these are performed by decreasing atom clashes in the decoys by using molecular mechanics methods. The further analysis is carried out in subsequent chapters for seeking deeper insights of conformational changes within protein structure.

ATP binding mediated NBD dimerization process is accompanied with conformational changes of functional motifs of each subunits of NBD dimer. In this chapter, NBD dimerization process and function of conserved sequences are explained from the structural flexibility point of view. The constructed NBD dimer was used as one of the input structures to study the flexibility changes of NBD in different states. The other two structures are NBDs in monomer and co-crystallized dimer states, respectively. The flexibility predictions were performed by an algorithm from graph theory coded in the "RIGIX" program. The algorithm is applied to analyze the constrain network in protein structures.

Based on flexibility predictions, two highly conserved loops, Q-loop and pro-loop, are determined as flexible region, as shown in Fig. 2. These two loops are the linkers between α -helical subdomain and core domain. Their flexibility determines "rigid body" rotation of α -helical subdomain with respect to core domain, which plays the crucial role in the NBD dimerization process. Residues within TMD/NBD interface follow the Q-loop, in which the movement can be influenced by the flexibility of the Q-loop. An ATP binding mediated NBD dimerization mechanism was proposed based on flexibility predictions. The functions of highly conserved sequences, p-loop and catalytic loop, during the dimerization process were proposed based on flexibility changes of NBD in different states.

ATP binding mediated NBD dimerization process was followed by ATP hydrolysis promoted by NBDs. In

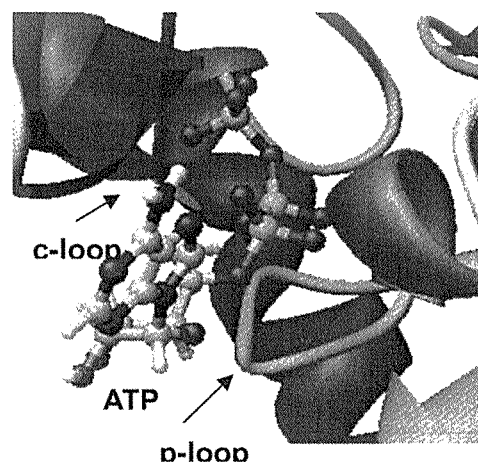


Fig. 1 One of the ATP binding sites of constructed NBD dimer using soft docking module within MIAX .

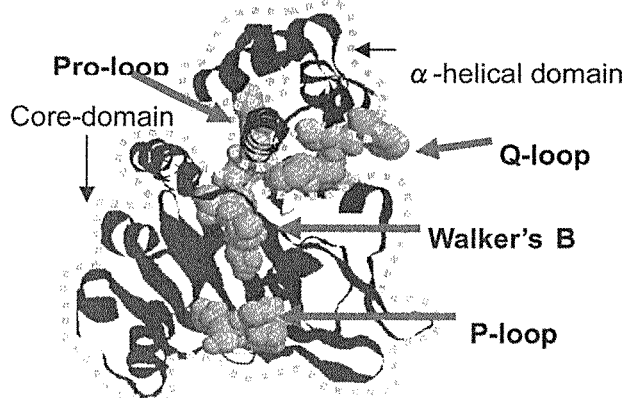


Fig. 2 Illustration of flexibility analysis result on ATP unbound NBD monomer, rigid bodies are shown in ribbon representation, high flexible regions are shown in grey and space-filled representation. Two subdomains, α -helical subdomain and core-domain, connected by Q-loop and pro-loop, are marked by dot line separately.

order to investigate the reaction mechanism of NBD promoted ATP hydrolysis, density functional theory (DFT) method was employed. The calculation model consists of ATP, surrounding water, and protein residues including catalytic Glu179. According to the literature, Wat407 was substituted by Mg^{2+} , which is necessary for ATP hydrolysis in ABC transporters.

An S_N2 -like nucleophilic attack mechanism was proposed based on DFT calculations. As shown in Fig. 3A, γ -phosphate group of ATP molecule exhibits a planar conformation; The value of dihedral angle $O_{\gamma1}-O_{\gamma2}-O_{\gamma3}-P_{\gamma}$ is 32.8° for the initial state, while is -36.8° for the product, which suggest a reversal conformation. The interaction between reactants in TS is stabilized by interaction between the highest occupied molecular orbital (HOMO) of Wat437 and the lowest unoccupied molecular orbital (LUMO) of ATP, as shown in Fig. 3B. In the proposed mechanism, Mg^{2+} plays an important role to mediate a complex formation among ATP, water molecules and catalytic Glu179. By decreasing the distance between ATP, H_2O and residues, Glu179 promotes the nucleophilic attack of water on ATP molecule by accepting a proton.

The proposed S_N2 -like nucleophilic attack mechanism for ATP hydrolysis in NBD was then introduced into a classical molecular dynamics (MD) simulation. A function of chemical reaction extension was implemented in the classical MD simulator, “New-Ryudo-CR”, to study the conformational changes of NBDs during ATP hydrolysis reaction. The key features of S_N2 mechanism, that is reversal conformation and planar transition state, were reproduced from “New-Ryudo-CR” calculation. An extremely movement of hydrolyzed products in two binding sites was obtained. The movement of ADP and P_i indicates that, after ATP hydrolysis, the produced ADP and P_i are no longer able to stabilize the NBD dimer conformation, which leads to a dis-dimerization process. As shown in Fig. 4, after hydrolysis, the highly conserved sequences p-loop and catalytic loop undergo a movement. This motion of the catalytic loop enables the approaching of the catalytic glutamate towards the reaction core, which is confirmed by the reaction mechanism deduced in

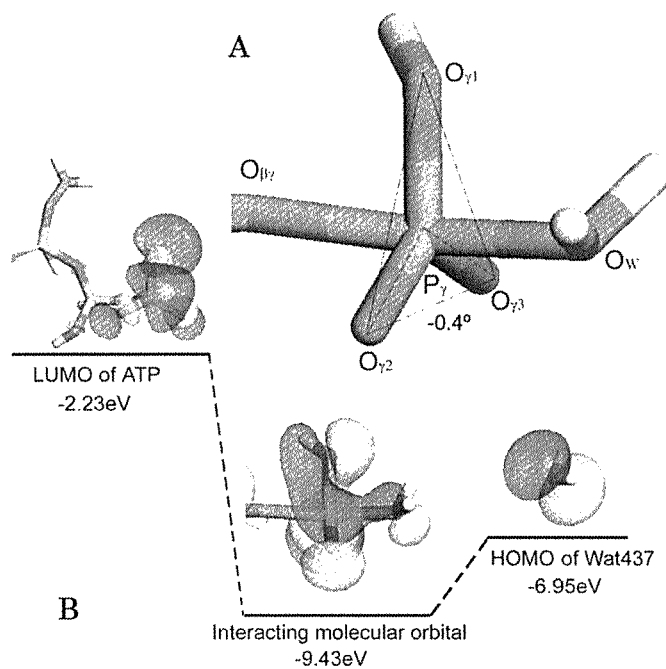


Fig. 3. A: γ -phosphate group in TS, where the dihedral angle $\angle O_{\gamma1}-O_{\gamma2}-O_{\gamma3}-P_{\gamma}$ is -0.4° . B: Analysis of molecular orbital interaction between LUMO of ATP and HOMO of Wat437.

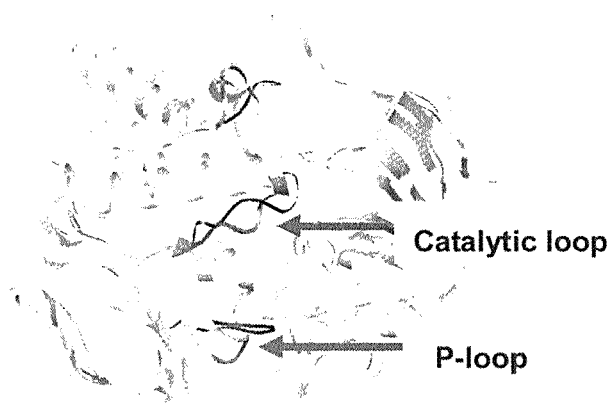


Fig. 4 Superimposition of initial and final conformations of NBDs dimer after classical MD simulation with chemical reaction extension. In one of the identical subunits, sequences, which have an extremely movement, are colored. Initial structure is shown in dim gray, and final structure is shown in black.

previous chapters. The motion of the p-loop indicates that the p-loop is restored to a flexible state, which implies the dissociation of NBD dimer.

In this thesis, computational approaches were applied to investigate structure and functional mechanisms of ABC transporters. Each step of “ATP switch” mechanism, which was proposed on substrate translocation of ABC transporters, was investigated in detail on atomic/molecular level. The study was focused on two main steps, which are ATP binding mediated NBD dimerization and the ATP hydrolysis mediated disdimerization. The function of highly conserved sequences during “ATP switch” mechanism was clarified from the viewpoints of conformational changes, and by considering the functions of protein and environments involved in the ABC transporters related chemical reactions. The theoretical study presented in this thesis provided the possibility of constructing protein complexes using computational approach, and identified the targets for new drugs design to inhibit/enhance ABC transporters. By changing the flexibilities of conserved sequences or changing binding affinities of binding site, ABC transporters can be restrained in open or closed states. On the other hand, inhibition or enhancement of ABC transporters can also be realized by competitive occupation of new designed compounds.

論文審査結果の要旨

本研究は ATP Binding Cassette(ABC)トランスポーターの構造特性および輸送機能にかかわる反応機構を理論的に明らかにすることを目的として行われた。本論文は「Theoretical Study on Nucleotide Dependent Transportation Mechanism of ABC Transporter (ABC トランスポーターのヌクレオチド依存性輸送機構に関する理論研究)」と題し、以下の7章からなる。

第1章では、ABC トランスポーターについて概説した後に、ABC トランスポーター構成する各構造領域および輸送機能について述べている。トランスポーターの輸送のためのエネルギーを供給するための反応機構の解明の重要性について具体的に説明し、本論文の目的を明確にしている。

第2章では、本論文で用いた手法について説明している。はじめに相互作用構造を構築するために用いたタンパクタンパクソフトドッキングプログラム MIAx および構築された複合体構造のフレキシビリティ解析のための RIGIX プログラムについて述べている。続いて、反応機構の解析に用いた密度汎関数法および大規模複雑系の反応ダイナミクス解析のための新規化学反応対応型分子動力学法について詳説している。

第3章では、ABC トランスポーターのヌクレオチド相互作用領域のダイマー構造を MIAx のソフトドッキング機能を用いて予測している。結晶構造データにあるモノマーの構造に基づき複合体構造を構築し、以降の章における検討の基礎となる構造を得ることに成功している。

第4章では、ヌクレオチド相互作用領域のダイマー構造の形成に伴う構造変化に関して、タンパク質のフレキシビリティの観点から RIGIX プログラムを用いて解析している。RIGIX プログラムを活用することでフレキシブルな領域を予測することに成功しており、予測に基づきダイマー化過程に関する知見が得られた。得られた結果に基づき、ダイマー化機構に関する示唆を得ることに成功している。

第5章では、ABC トランスポーターのヌクレオチド相互作用領域により促進される ATP の加水分解反応機構に関して密度汎関数法を用いて解析している。ATP、Mg、水分子、タンパク質残基の一部を考慮したモデルに対して得られた計算結果から、SN₂ 型の求核反応機構で反応が進行することが解明された。さらに、遷移状態構造においては水分子が ATP 分子と相互作用することで反応を促進していることを示すとともに、Mg イオンが複合体形成に大きな役割を果たしていることを示すことに成功している。

第6章では、新規化学反応対応型分子動力学法を用いてヌクレオチド相互作用領域における ATP 加水分解反応ダイナミクスを調べている。第5章で解明された反応機構に基づきヌクレオチド相互作用領域全体を考慮した大規模モデルを用いた加水分解反応ダイナミクスにより、反応前は ATP により安定化されていたダイマー構造は、反応後には ADP により安定化されることなく脱ダイマー化が進行するとの示唆を得ることに成功している。

第7章は、本論文の総括である。

以上、本論文は、新規計算化学手法を活用し、異なるスケールでの解析を実現することで、従来解明が不十分であった ABC トランスポーターの輸送機能に関する新規知見を得ることに成功している。

よって、本論文は博士(工学)の学位論文として合格と認める。