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論 文 要 旨

THESIS SUMMARY

専攻： Department of	生体分子機能工学　専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　工学　）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

ATP-binding cassette (ABC) transporters are found in all phyla and constitute one of the largest protein superfamilies. Due to its ability to translocate various substrates, this type of proteins is considered clinically important. For example, the overexpressed ABCB1 on bacteria or cancer cells would cause the multidrug resistance (MDR) to cytotoxic pharmaceuticals and cause the failure of many chemotherapies. On the contrary, several mutations that found on CFTR would cause the malfunction of chloride ion translocation across cell membrane and thus lead to the cystic fibrosis disease. Despite the importance of ABC transporters, the detailed translocation mechanism still remains elusive. Therefore, the purpose of this study is to elucidate the unsolved questions of ABC transporters, and I used the maltose transporter, an ABC importer from *E. coli*, as the target to explore this question.

In chapter 2, I focused on $MalK_2$, the subunits that mainly include the nucleotide-binding domains (NBDs) of the maltose transporter, to investigate the relationship between the conformational change and the binding of Mg^{2+}-ATP by performing both conventional all-atom molecular dynamics (cMD) and accelerated molecular dynamics (aMD) simulations. The results revealed that the binding of Mg^{2+}-ATP would modify the free energy surface of $MalK_2$, and thus induce the conformational change. The resultant free energy surface indicated that the structural transformation would follow an induced-fit model rather than a conformational selection model. Moreover, I further confirmed that the opening-closing of $MalK_2$ undergoes an asymmetric pathway due to the fact that the asymmetric pathway has lower energy barriers.

In chapter 3, I performed cMD simulations to examine the full transporter's ($MalFGK_2$ and $MalFGK_2E$) fluctuations and investigated the motional correlation between different subdomains. The results indicated that only binding Mg^{2+}-ATP to the NBDs is not sufficient to facilitate the maltose transporter from the resting (open) state to closed state. On the other hand, the maltose binding protein (MBP, also known as MalE) may be the key subdomain to mediate the closure of this transporter. With binding of MalE, the motion of $MalK_2$ showed obvious negative correlations with the MalF-P_2 region; however, without MalE, the correlations between MalF-P_2 region and $MalK_2$ decreased significantly. This observation was also confirmed by the principal component analysis (PCA), and thus gave me a new point of view to understand how a binding protein mediates the conformational change of an

ABC importer.

In chapter 4, I focused on the ATP hydrolysis reaction in the maltose transporter. In chapter 2, I found out that some water molecules remained at the closed ATP-binding pocket (ABP) sites during the simulation, and some of those water molecules formed several hydrogen bonds with ATP and its neighboring residues. As a result, I considered that those water molecules might be able to trigger ATP hydrolysis reaction. Based on the observation, I performed QM/MM metadynamics, another enhanced sampling method, to address this issue. The QM/MM calculation enabled me to observe bond formations and cleavages directly, and the metadynamics enabled me to overcome a high energy barrier in a limited simulation time as well as to rebuild the free energy surface along an assigned reaction coordinate. The results revealed that the ATP hydrolysis is a one-step exothermic reaction, and the overall reaction only included by one water molecule. The dissociation of γ-phosphate occurred prior to the bond formation between the lytic water and the γ-phosphate. Glu159 in the D-loop played as a general base to abstract the proton form the lytic water. As a result, the ATP hydrolysis reaction in the maltose transporter can be described as a one water (1W), general base catalysis (GBC), and dissociative-like model. Moreover, I have also observed two more interesting proton transfers during the post-hydrolysis state, in which one transferred from His192 ε-position nitrogen to the Pi, and the other one transferred from Lys42 side chain to the β-phosphate. These two proton transfers are considered to be able to stabilize the product state.

Above all, I have performed an intensive investigation for the *E. coli* maltose transporter and proposed an overall model to describe its mechanism. I have found that the binding of Mg^{2+}-ATP could modify the free energy surface and thus lead to the conformational change in a way similar to the so-called induced-fit model. Moreover, I also pointed out that Mg^{2+}-ATP binding alone is not sufficient to trigger the conformational change from resting (open) state to closed state, but the maltose binding protein (MalE) may be the key subdomain that mediate the whole structural transformation. Finally, I further studied the ATP hydrolysis in the maltose transporter and made an interesting picture for the reaction from different aspects. The results not only had good agreements with many previous studies but also offered several new viewpoints for this ABC importer, and I am expecting that this study would be applicable to other ABC transporters.