論文 / 著書情報
Article / Book Information

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Title(English)	Computational Study of Candida antarctica Lipase B: Lid Dynamics and Substrate Binding
著者(和文)	WIJAYATegar Nurwahyu
Author(English)	Tegar Nurwahyu Wijaya
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	Tegar Nurwahyu Wijaya	審査員主査： Chief Examiner	Akio KITAO

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Candida antarctica lipase B (CALB) is a popular lipase in industry and academia because of its versatility[1]. Important residues in the catalytic site of CALB are the catalytic triad (Ser105, Asp187, and His224) and the oxyanion hole (Thr40 and Gln106). The conformational dynamics of the lid region of CALB (α5 and α10 helices) plays an important role in substrate binding and can be influenced by the existence of a hydrophobic surface. In this research, the conformational dynamics of lid region of (CALB) in water (CALB/W) and on the water-tricaprylin interface (CALB/I) were studied using computational methods followed by prediction of CALB-tricaprylin complex structure. Molecular dynamics (MD) simulation, the Markov State Model (MSM), and molecular docking were employed to simulate molecular motions, analyze the simulation trajectories, and predict the complex structure respectively. Parallel cascade selection molecular dynamics (PaCS-MD)[2], an advanced MD method, was employed to expedite the exploration of conformational dynamics of CALB.

During the conventional MD simulation, CALB was adsorbed to tricaprylin surface, indicating strong interaction between the lid region and tricapylin. However, conformational space was not explored adequately by conventional MD simulation. On the other hand, PaCS-MD was successfully employed to simulate the conformational dynamics from the close to open and open to close states of the lid in both CALB/W and CALB/I systems. The free energy surface (FES) of both systems shows a shift in the global minimum from the close conformation in CALB/W to the semi-open conformation in CALB/I. Moreover, CALB is less flexible in CALB/I than in CALB/W indicated by a smaller area of the FES of CALB/I than that of FES in CALB/W and deeper minima on FES of CALB/I. Transition path theory was employed to identify the major transition pathway from the close to open conformation followed by residue-residue contact and hydrogen bond analysis between residues in the lid region and neighboring residues. The analysis indicates that the increasing interactions between Ala148 near α5 and Asn292/Gln291 near α10 contribute to the stabilization of semi-open conformation in CALB/I.

A set of 1613 representative structures were extracted from the PaCS-MD trajectories and were used as possible receptor conformations while tricaprylin was used as the ligand for molecular docking. A set of 16130 complex structures were predicted by molecular docking. A systematic selection process based on the formation of essential CALB-tricaprylin hydrogen bonds and docking score were carried out to obtain a set of 20 complex structures that will be used in subsequent MD simulations. The results of MD simulations show no dissociation of tricaprylin from CALB, however in most simulations, the essential hydrogen bonds were broken. Only in two simulations the essential hydrogen bonds were retained. Analysis of the essential hydrogen bond distances suggests the existence of three binding states of tricaprylin: full binding, transition, and initial binding states. Visual analysis of the MD results shows rotational motion during transitions from the full binding to initial binding state. The ester bonds of tricaprylin are facing the CALB catalytic triad in the full binding state. In contrast, the ester bonds are located in the opposite direction from CALB catalytic triad in initial binding state. Furthermore, the analysis also shows that binding with terminal ester bond is more preferable than binding with central ester bond of tricaprylin. Comparison with CALB-Tween 80 complex structure[3] shows that interaction between tricaprylin and the oxyanion hole is weaker in central ester bond binding than that in terminal ester bond binding. Further analysis of the correlation between the formation of essential hydrogen bonds and CALB conformation suggest that initial binding happens with the highest probability in the semi-open conformation, which agrees with a minimum in FES of CALB/I which is very close to the global minimum. On the other hand, the full binding can happen in various degrees of CALB conformations, from the close, semi-open, and open conformations, indicating that binding happens in the semi-open conformation first, followed by transitions to the full binding accompanied with a relaxation of

the CALB conformation.

While this research answered several questions regarding CALB conformational dynamics and tricaprylin binding mode and its mechanism, there are still many critical questions unanswered. The binding mechanism from the dissociated state to the full binding state has not been investigated. Furthermore, the reaction feasibility of the obtained tricaprylin-CALB complex structure and the subsequent product release need to be addressed. These questions should be the focus in the future works.

References:

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