学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

| 博士の専攻分野の名称 Degree requested | 博士（生命科学） Doctor of Life Science | 氏 名 Applicant name | 葉 宇鑫 Yuxin Ye |
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| 審査担当者 Examiners | | | |
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学 位 論 文 題 名
Title of Doctoral Dissertation

Study on biomacromolecules concerning biofuel and illness by X-ray crystallography
(生物燃料，疾患に関するタンパク質の X 線構造生物学の研究)

Biomacromolecules, including nucleic acids (polynucleotides), proteins (polypeptides), and glycans (polysaccharides), have always been the central topics of biochemical literature and at the very core interest of scientist for their important roles for human life. To better understand the active mechanism of these biomacromolecules, their detail structure information at the atomic level are essential. In this study, we focus on the biomacromolecules concerning two hotspot issues, renewable biofuels and live health (protein synthesis), and try to use X-ray crystallography to reveal the related mechanisms of molecular biology.

I. Study on phosphorylases from GH130 concerning renewable bioenergy

In recent decades, how to generate the sustainable biofuels energy by effectively utilizing renewable biomass has become the very core interest of global world. Mannan, as an important component of biomass, is an abundant resource for the production of biofuel. Glycoside hydrolase family 130 (GH130), catalyzing the hydrolysis and phosphorolysis of β-mannosidic linkages at the non-reducing end of substrates, was identified recently. *Ruminococcus albus* 4-O-β-D-mannosyl-D-glucose phosphorylase (*Ra*MP1) and β-(1-4)-mannooligosaccharide phosphorylase (*Ra*MP2) are classified into GH130 subfamily1 and subfamily2 respectively, with significant different substrate specificity. So far, the details of substrate recognition mechanisms of them are still unclear. In this study, we determined the crystal structures of *Ra*MP1, *Ra*MP2, and their complex with substrates. The structures provided evidence to illuminate the substrate recognition mechanisms of *Ra*MP1 and *Ra*MP2. Both enzymes are oligomer and the catalytic pockets of them are generated by two adjacent molecules of the homotrimer. The highly conserved core structure and the interactions with the Man residue at the −1 subsite and phosphate suggest that these two enzymes share a recognition mechanism toward the Man residue at the non-reducing end. In contrast to the −1 subsite, recognition at the +1 subsites of these two

enzymes is very different. *Ra*MP1 shows a compact +1 subsite with a narrow substrate entrance pathway, while the +1 subsite of *Ra*MP2 is spacious and has a wide-open entrance. In addition, three loops are proposed to determine the different substrate specificities. One of these loops is contributed from the adjacent molecule of the oligomer structure. In *Ra*MP1, His245 of loop 3 forms a hydrogen-bond network with the substrate through a water molecule, and is indispensible for substrate binding.

II. Study on translation initiation factors involved in illness

Translation initiation is the most complicated process for mRNA translation on the ribosome in all cells. Deregulation of translation initiation results in abnormal gene expression, and thus leads to uncontrolled cell growth potentially resulting in several illnesses such as cancer and leukoencephalopathy. In eukaryotic cells, eukaryotic initiation factors 2 (eIF2), a trimeric factor containing a typical G protein subunit, tRNA-binding subunit, and β subunit, plays an essential role and binds different partners, eIF5 and eIF2B, during translation initiation. Both eIF5-CTD and eIF2Bε-CTD possess two conserved eIF2β subunit binding site, AA-box, while of eIF2β binds eIF5-CTD/eIF2Bε-CTD through three Lysine-rich motifs (K-box) in N-terminal domain. Structural comparison showed that eIF5-CTD and eIF2Bε-CTD have very similar secondary structures and topologies but with different orientations in tertiary structure. It is suggested that eIF5-CTD and eIF2Bε-CTD may bind to the same binding site of eIF2β-NTD in different manners. However, it still remains unclear how eIF2β binds to different target proteins eIF5 and eIF2Bε with the same binding site. In this part, we tried to address this problem by solving the crystal structures of (eIF2β-NTD)-(eIF2Bε-CTD) complex and (eIF2β-NTD)-(eIF5-CTD) complex. The attempts of crystallization of two complexes from *Candida albicans* were performed. Only crystals contain eIF5-CTD was obtained. Interestingly, the AA-box2 motif was firstly observed in helix form by the structural analysis of (*Ca*eIF2β-NTD)-(*Ca*eIF2Bε-CTD) complex. Considering the disorder probability result and previous SAXS data, we proposed that the structural conformation change might be occurred in the AA box2 of eIF5CTD coupled with the interaction with eIF2β-NTD, which transfer from a flexible form to a rigid helix form.

In conclusion, the author has new findings on substrate recognition mechanisms of *Ra*MP1 and *Ra*MP2, and these will provide molecular bases for designing new enzymes which are able to hydrolyze specific oligosaccharides. The author also has new findings on the interactions of translation initial factors eIF5, eIF2Bε, and eIF2β, and these will contribute important information to life science.

Therefore, we acknowledge that the author is qualified to be granted the Doctorate of Life Science from Hokkaido University.