学 位 論 文 の 要 約
Summary of Doctoral Dissertation

博士の専攻分野の名称	博士（生命科学）	氏 名	葉宇鑫
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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. X-ray crystallography deserves much of the credit as a major method to solve the atomic structure of biomacromolecules. Its application of analyze biomacromolecules has ushered in a new discipline. In this study, we focus on the biomacromolecules concerning two hotspot issues, renewable biofuels and live health, 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. The pro-treatment of biomass is an essential process for producing biofuels. In this step, multiple mannan-degrading enzymes efficiently conduct the bioconversion of original biomass into monomeric sugars and subsequent products. Glycoside hydrolase family 130 (GH130), containing hydrolases and phosphorylases that catalyze 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 (MGP-Type) and subfamily2 (MOP-Type), respectively, with significant different substrate specificity. *Ra*MP1 catalyzed the phosphorolytic and synthetic reactions of Man-Glc with high specificity, while *Ra*MP2 had higher phosphorolytic activity toward β-1,4-mannooligosaccharides longer than β-1,4-mannobiose. So far, the details of substrate recognition mechanisms of them are still unclear.

In this study, we determined the crystal structure of *Ra*MP1, *Ra*MP2, and their complex with substrates. Both enzymes are oligomer and the catalytic pockets of them are generated by two adjacent molecules of the homotrimer. The structural comparison and the calorimetry experiments of *Ra*MP1 and *Ra*MP2 indicate that the oligomeric structure of *Ra*MP1 should be more stable than that of *Ra*MP2. In addition, the structures provided evidence to illuminate the substrate recognition mechanisms of *Ra*MP1 and *Ra*MP2. The highly conserved five-bladed β-propeller fold core structure and the interactions with the Man residue at the −1 subsite and phosphate in

*Ra*MP1 and *Ra*MP2 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. Three loops are important in substrate recognition in the +1 subsite of two enzymes. Loop2 plays a role in restricting the derivatives at the C6 position of +1 substrate residue by precise interaction with substrate in *Ra*MP2 but not in *Ra*MP1. The conformational differences of Loop1 and Loop3 (Loop3 is contributed from the adjacent molecule) in *Ra*MP1 and *Ra*MP2 indicate that *Ra*MP1 has a structural restriction for substrate chain length, but *Ra*MP2 does not. Deletion of Loop1 from *Ra*MP1 did not significantly reduce the activity, and it allowed the accommodation of methyl β-D-glucoside as an acceptor, while deletion of Loop3 would almost completely abolish the phosphorolytic activity in *Ra*MP1. Moreover, we found that *Ra*MP1 forms a hydrogen-bond network in the catalytic center through the His245 of the Loop3 linking adjacent molecules. The substitution of His245 with Ala in *Ra*MP1 resulted in losing most of activity and lower affinities to the substrate. This result suggests that the hydrogen-bond network might be important for binding substrate.

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 in translation initiation. The eIF2/GTP/Met-tRNA$^{Met}_{i}$ ternary complex binds the eIF1, eIF3 and eIF5 to form a multifactor complex for high accuracy recognizing the start codon in eukaryotic initiation. After start codon recognition, GTPase activity in eIF2 is triggered and the GTP bound to eIF2 is converted to GDP and form eIF2/GDP complex. This eIF2/GDP complex binds a pentameric factor eIF2B (comprising α, β, γ, δ and ε subunits) for re-activation by guanine nucleotide exchange. Therefore, eIF2 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.