Structural Energy Bioscience Research Section

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1. Introduction

We explore the way how biomolecules such as proteins (involving enzymes) and functional nucleic acids (DNA and RNA) work at atomic resolution based on structural biology with NMR. We determine both static and dynamical structures with the aid of our own development of the new methodology and elucidate the underlying mechanism of functions of these biomolecules. Structural biological approach is also applied to analyze enzymes involved in degradation of wood biomass at atomic resolution. The analysis is useful to develop the way to extract energy and valuable materials that can be used as starting materials of various products from the wood biomass. Thus, we pursue to contribute to the paradigm shift from oil refinery to biorefinery. Followings are main research achievements in the year of 2023.

2. Structural insights and mutagenesis of *Acremonium alcalophilum*'s feruloyl esterase unveil superior catalytic activity

This research provides groundbreaking insights into the enzymatic mechanisms and potential biotechnological applications of feruloyl esterases (FAEs) in the subfamily 5 (SF5), derived from *Acremonium alcalophilum* (*Aa*FaeD). By elucidating the crystal structure of *Aa*FaeD's catalytic domain (CD) for the first time, both in its free form and when complexed with ferulic acid (FA), the study illuminates the critical role of a hydrophobic cleft in substrate binding and catalysis. This structural analysis revealed that FA binds within a distinct hydrophobic cleft, leading to targeted mutagenesis experiments which demonstrated the significance of key hydrophobic residues for enzymatic activity. Remarkably, a specific mutant, F120Y, displayed a 1.5-fold increase in activity towards methyl ferulate compared to the wild type. The study's comparison of SF5 FAEs with those in subfamilies 2 and 3 highlighted a broader substrate specificity for SF5 FAEs, capable of processing both monomeric and dimeric phenolic substrates. This capability contrasts with the preference of SF2 and SF3 FAEs for monomeric substrates, showcasing the unique potential of SF5 FAEs in biotechnological applications for sustainable biomass degradation. These findings significantly advance our understanding of SF5 FAEs' structure-function relationships and pave the way for exploiting these enzymes in developing more efficient methods for biomass conversion, contributing to sustainable biofuel production and biorefining processes.

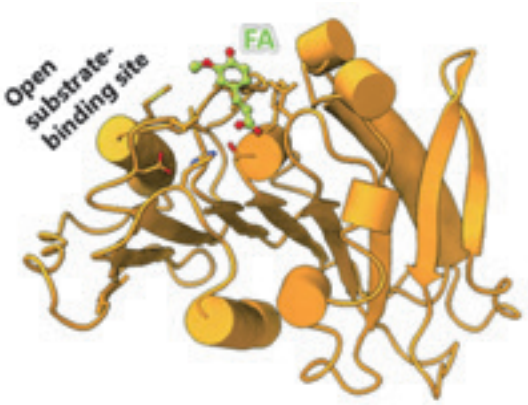

Figure 1. The determined crystal structure of *Aa*FaeD.

3. Synergistic effects of co-displayed xylanase and feruloyl esterase on *Pichia pastoris*

This study explores an innovative approach to enhancing lignocellulosic biomass degradation using yeast surface display (YSD) technology to co-display xylanase (XYN) and feruloyl esterase (FAE) on the surface of *Pichia pastoris* (*Komagataella phaffii*). By engineering three *P. pastoris* strains—X-Pichia with XYN, F-Pichia with FAE, and X/F-Pichia with both enzymes—the research examines the synergistic action and the impact of enzyme proximity on the decomposition of acid-pretreated sugarcane trash. The findings reveal a clear synergistic effect when XYN and FAE are co-displayed, with X/F-Pichia showing a 1.5-fold increase in reducing sugar yield compared to X-Pichia alone, and a 1.1-fold increase in ferulic acid production over F-Pichia or a mixture of X-Pichia and F-Pichia. These results highlight the potential of enzyme co-display on *P. pastoris* for significantly improving biomass degradation efficiency. The study demonstrates the enhanced bioconversion process made possible through the strategic co-localization of XYN and FAE on the yeast cell surface, facilitating effective substrate turnover and increased product

yields. This synergistic and proximity effect of co-displayed enzymes offers a promising avenue for efficient and sustainable utilization of lignocellulosic biomass, contributing to advancements in bioprocessing technologies for biofuel production and biorefining, thereby supporting more sustainable and environmentally friendly biotechnological applications.

4. *Cs*MnP-catalyzed polymerization and condensation for high-value material synthesis

This study delves into the catalytic potential of manganese peroxidase (MnP) derived from *Ceriporiopsis subvermispora* (*Cs*MnP) for lignin modification and its application in Kraft lignin (KL) condensation to create high-value lignin-based materials. Using guaiacylglycerol-β-guaiacyl ether (GGE) as a model compound, *Cs*MnP's ability to polymerize the phenolic β-O-4′ lignin substructure was explored, revealing the formation of compounds with higher molecular weights indicative of polymerization. Specifically, NMR analysis of the products highlighted the formation of dimeric structures through 5-5′ linkages. Further experiments with KL demonstrated *Cs*MnP's efficacy in increasing the molecular weight of KL by 360% within 24 hours, with NMR spectroscopy suggesting condensation through α-5′, 5-5′, and 4-O-5′ linkages and a notable decrease in phenolic content by 37%. These findings underscore the capability of *Cs*MnP to not only degrade lignin but also to engineer its structure, offering a biotechnological route to valorize lignin into more complex and functional materials. The successful demonstration of *Cs*MnP-mediated condensation reactions provides a promising approach for the synthesis of novel lignin-based polymers, paving the way for their application in various industries, including materials science and sustainable chemistry. This research highlights the versatility of MnP enzymes as tools for lignin valorization, contributing to the development of environmentally friendly and resource-efficient bioprocesses.

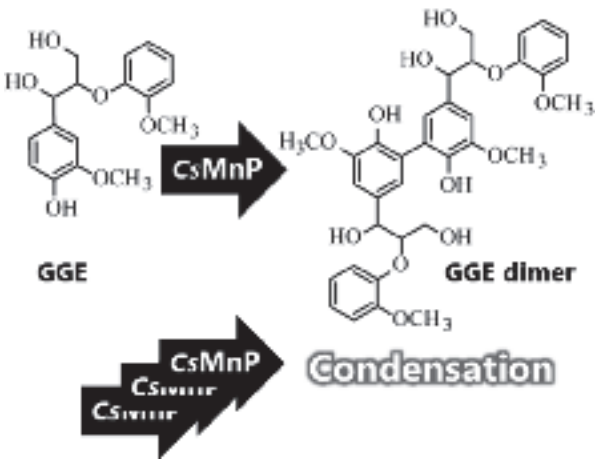

Figure 2. The conversion of GGE catalyzed by *Cs*MnP.

5. The structure and interaction with ligands of an RNA aptamer targeting HIV-1 Tat in living human cells analyzed by in-cell NMR

An RNA aptamer, which exhibits strong binding to the trans-activator of transcription (Tat) protein of HIV-1 within living human cells, holds potential as a therapeutic drug for HIV. To explore the structure and interactions of this RNA aptamer in living cells, in-cell NMR serves as a potent method. In this study, we investigated the interaction between the RNA aptamer and a peptide derived from Tat, which is crucial for the aptamer's binding, in living human cells using in-cell NMR experiments. The aptamer and the peptide were introduced into living HeLa cells, and we obtained in-cell NMR spectra of the aptamer-peptide complex. Through comparison of *in vitro* and in-cell NMR spectra, we discovered that the aptamer forms two U-A-U base triples upon binding to the peptide, even within the cellular environment. These base triples expand the originally narrow major groove of the aptamer, creating a space to accommodate two critical arginine residues of Tat during the binding process. Our research rationally explains the high binding affinity of the aptamer for the peptide in the cellular environment, leveraging both *in vitro* and in-cell NMR analyses.

6. Direct inhibition of human APOBEC3 deaminases by HIV-1 Vif

HIV-1 Vif is known to counteract the antiviral activity of human APOBEC3 (A3), a cytidine deaminase. Vif forms a complex called VβBCC, comprising CBFβ and the components of E3 ubiquitin ligase, Elongin B, Elongin C, and Cullin5. Together with the ubiquitin-conjugating enzyme, VβBCC induces ubiquitination-mediated proteasomal degradation of A3. Here, we elucidated that VβBCC inhibits deamination by A3G, A3F, and A3B, independently of proteasomal degradation. Surprisingly, we discovered that this inhibition for A3G is directly attributed to the interaction between VβBCC and the C-terminal domain of A3G which was not supposed to interact with Vif. Our findings suggest that inhibiting the interaction between VβBCC and the C-terminal domain, as well as the N-terminal domain known to be targeted for ubiquitination, of A3G may be needed to prevent counteraction by Vif.

Collaboration Works

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片平正人, 基盤研究(B), 核酸アプタマーによる細胞内認識の構造化学と脳関門透過戦略による神経変性疾患治療（分担金）

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