<Advanced Energy Utilization Division> Structural Energy Bioscience Research Section

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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 and X-ray. 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 2021.

2. Synergistic effect of a lytic polysaccharide monooxygenase and commercial cellulase cocktail

Cellulose is the most abundant organic polymer on earth. The second-generation biofuels are produced from cellulose by saccharification and following fermentation processes. However, the high cost of the saccharification process remains an issue. Cellulose-active lytic polysaccharide monooxygenases (LPMOs) catalyze the cleavage of cellulose chain on the crystalline cellulose surface by utilizing electron and oxygen source. Since this cleavage produces new accessible chain-ends for cellulases, LPMOs accelerate the saccharification of cellulose. Previously we solved the crystal structure of an LPMO of a white-rot fungus, *Ceriporiopsis subvermispora* (*Cs*LPMO). Then, a high synergistic effect of *Cs*LPMO and commercial cellulase cocktail was demonstrated. This year, we varied the ratio of *Cs*LPMO to cellulase cocktail, and optimized the conditions of saccharification reaction. By treatment of 5 mg/mL microcrystalline cellulose (MCC) with 37.5 μg/mL *Cs*LPMO and 12.5 μg/mL cellulase cocktail, the yield of reducing sugar reached 8.5-fold of the sum of the yields obtaind by the treatment with the individual enzymes (Figure 1). The degree of synergy turned out to be the highst among the reported ones for other LPMOs.

We also investigated the role of Tyr residues on the substrate-binding surface of *Cs*LPMO for substrate binding and synergistic effect. The two of the three Tyr residues, Y27 and Y74, were not conserved among LPMOs and unique for *Cs*LPMO. Site-direct mutagenesis and pull-down assay with MCC revealed that Y27 and Y74 are involved in substrate binding. Unexpectedly but interestingly, the synergistic effect of *Cs*LPMO increased by substituting Y27 and Y74 to Ala. It is known that unbound LPMOs produce H_2O_2, which is an efficient oxygen source for LPMO activity. We assume that the decrease in substrate affinity by the Y27 and Y74 to Ala substitution led to an increase of the substrate-unbound *Cs*LPMO, by which H_2O_2 was produced and provided to the substrate-bound *Cs*LPMO. The synergistic effect of *Cs*LPMO with the commercial cellulase cocktail may be applicable to the improvement of the process for cellulosic biomass utilization.

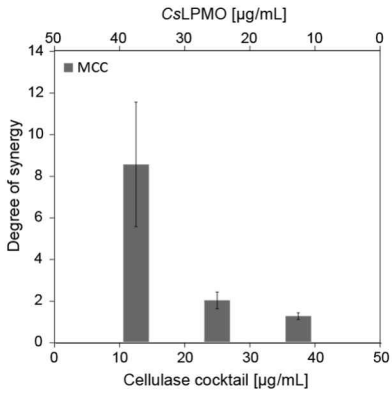

Figure 1. Degree of synergy (DS) at various ratios of *Cs*LPMO and cellulase cocktail on the degradation of 5 mg/mL MCC. DS was culculated using the following equition; $DS = Y_{CL}/(Y_C + Y_L)$, where Y_C, Y_L, and Y_{CL} are the yields of reducing sugars of the treatment with cellulase cocktail, *Cs*LPMO, and both, respectively.

3. Improving the degradation of lignin in beech wood by manganese peroxidase using a bioreactor system

Lignin, one of the major components of woody biomass, is a valuable aromatic polymer. For the utilization of lignin, efficient fragmentation of the lignin structure is required. Although ligninolytic enzymes such as manganese peroxidase (MnP)

catalyzes lignin degradation, the degradation is reportedly competed by undesirable repolymerization. To prevent repolymerization, we deployed a semi-continuous bioreactor system to separate the fragmented lignin compounds from the reaction solution. By using this system, the overall net lignin degradation of beech wood catalyzed by MnP was successfully improved.

4. Determination of the crystal structure of *a* feruloyl esterase

Ferulic acids decorate hemicellulose via ester-linkage, and bridge hemicellulose and lignin in herbaceous biomass. Feruloyl esterases (FAEs) hydrolyze the ester-linkage between hemicellulose and ferulic acid and enhance the efficiency of hemicellulose degradation. Here, we determined the crystal structure of an FAE, which is phylogenetically related to acetyl xylan esterase (AXE), at 1.5 Å resolution. Additionally, the binding pocket for a substrate, methyl ferulate (MFA), was predicted by molecular docking analysis. Cys39, Glu49, Pro158, and Val163, were close to MFA (< 4 Å) in the docking model and thereby suggested to be involved in direct binding. This is the first structural characterizaion carried out for AXE-related FAE.

5. Finding of inhibitory effect of Vif on cytidine deamination of DNA by APOBEC3 proteins as revealed by biochemical and real-time NMR methods –new implication on the strategy for developing anti-HIV compounds-

APOBEC3 proteins (A3s), such as APOBEC3G (A3G) and APOBEC3F (A3F), convert cytidine residues to uracil residues through deamination of cytidine residues of minus strand DNA of HIV and thus destroy the genetic information of HIV. Thus, A3s function as guards against HIV. Vif protein of HIV forms a five-membered complex (VβBCC) which comprises a transcription factor, CBFβ, and the components of human E3 ubiquitin ligase, Elongin B, Elongin C, and Culin5 in infected cells. VβBCC ubiquitinates A3s and causes proteasomal degradation of A3s. Thus, Vif neutralizes A3s. In order to avoid the neutralization, compounds which interfere with the A3s-VβBCC interaction is being developed. Here, by means of biochemical and real-time NMR methods we found that VβBCC directly inhibits deamination by A3s independent of ubiquitination and resultant degradation. It was noted surprisingly that the inhibition is caused by the interaction between VβBCC and the C-terminal domain of A3G, which had been regarded not to interact directly with Vif. This finding implies that to develop anti-HIV-1 drugs that can avoid neutralization of A3G by Vif, it is necessary to consider the interference of the interaction of VβBCC with the C-terminal domain of A3G, in addition to the interference of the interaction of VβBCC with the N-terminal domain of A3G targeted for ubiquitination.

6. Proving the formation of parallel and antiparallel DNA triplex structures in living human cells

The parallel and antiparallel triplex structures comprise Watson-Crick duplex and an additional third strand that is oriented parallel and antiparallel with respect to the polypurine strand of the duplex. These triplex structures formed in human genomic DNA are believed to be involved in known diseases. However, there had been no direct evidence of the actual formation of these triplex structures in living human cells. To prove the formation of the triplx structures in living human cells, we used an advanced in-cell NMR technique incorporating bioreactor system that can supply fresh media to the living cells in NMR tube during spectral acquisition (Figure 2). The oligo DNAs, PT-ODN and APT-ODN, that form parallel and antiparallel triplex, respectively, in *in vitro* were introduced in living HeLa cells. The in-cell NMR spectra were acquired and compared with the *in vitro* NMR spectra. We identified the signals of all the imino protons belonging to the parallel and antiparallel triplex structures in in-cell NMR spectra. This is the first direct evidence of the formation of the parallel and antiparallel DNA triplex structures in living human cells. Additionaly, the imino proton signals derived from the duplex structures were also identified in in-cell NMR spectra. These duplexes were resultant of the triplex degradation. In-cell NMR spectra were also used to quantify the population of the triplex and duplex structures. Our in-cell NMR technique should be applicable for investigating the proteins and small compounds targeting the disease-related triplex structures in living human cells.

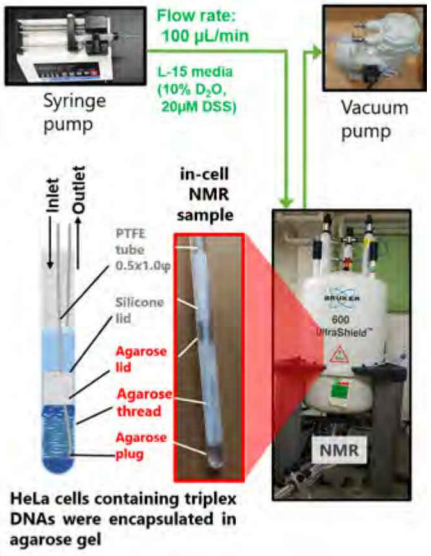

Figure 2. The bioreactor system for in-cell NMR experiment.

Collaboration Works

片平正人，Gyeongsang National University （韓国），プリオン蛋白質の悪性化を阻害する RNA アプタマーに関する構造機能相関

片平正人，University of Naples "Federico II" （イタリア），プリオン蛋白質の悪性化を阻害する RNA アプタマーへの化学修飾の導入による高性能化

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片平正人，永田崇，BIOTEC，NSTDA （タイ），LIPI（インドネシア），NUOL （ラオス），e-ASIA

大垣英明，森井孝，片平正人，野平俊之，モンゴル国立大学，インドネシア大学，フィリピン大学ディリマン校，ベトナム国家大学ハノイ校，ラオス国立大学，王立プノンペン大学，研究拠点形成事業 B. アジア・アフリカ学術基盤形成型

永田崇， Institute of Biophysical Chemistry, Goethe-University（ドイツ），深層学習の技術を取り入れた多次元 NMR 解析とタンパク質立体構造解析のシステム開発

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片平正人，基盤研究(B)，神経変性疾患に関連した反復配列 RNA 分子の反復回数に依存した液液相分離の構造基盤

片平正人，挑戦的研究（萌芽），Aβ 受容体であるプリオン蛋白質を RNA で阻害することによる抗アルツハイマー病効果

永田崇，基盤研究(C)，核酸とペプチドツールを用いたアルツハイマー病関連複合体の形成原理の解明

永田崇，基盤研究(C)，データサイエンスを導入した原子間力顕微鏡による四重鎖 DNA 検出法の開発（分担金）

山置佑大，若手研究，In-cell NMR 法を用いたヒト生細胞内核酸の構造安定性および相互作用の評価

近藤敬子，基盤研究(C)，リグニンと多糖を分離する酵素の実バイオスに対する活性および構造機能相関の解析

2. Others

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