

Structural Energy Bioscience Research Section

M. Katahira, Professor
 T. Nagata, Associate Professor
 Y. Yamaoki, Assistant Professor

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 2020.

2. Activity analysis of *PeGE* on the cleavage of ester-linkage in natural woody biomass

The major components of woody biomass, cellulose, hemicellulose, and lignin, are promising carbon-neutral resources to produce biofuels and various materials. These components form the rigid complex so-called lignin-carbohydrate complex (LCC) in natural biomass. One of the direct linkage contributing to LCC formation is ester-linkage between lignin and glucuronic acid residue in hemicellulose. Fungal glucuronoyl esterases (FGEs) catalyze the cleavage of the ester linkage and thereby facilitate the isolation of woody biomass components. Our previous study revealed that FGE from *Pleurotus eryngii* (*PeGE*) has the highest activity toward a model substrate, benzyl glucuronic acid. This year, we have investigated the activity of *PeGE* on the cleavage of the ester-linkage in natural LCC. NMR spectra of the LCC fraction that was extracted from natural woody biomass were recorded before and after the treatment by *PeGE*. Change in the signal intensity of the ester-linked lignin and glucuronic acid residue proved that *PeGE* can catalyze the cleavage of the ester-linkage in woody biomass.

3. Structural and functional analysis of lytic polysaccharide monooxygenase

Saccharification of cellulose by cellulolytic

enzymes is a crucial process in the production of 2nd-generation biofuels. Lytic polysaccharide monooxygenases (LPMOs), which catalyze oxidative cleavage of glycosidic linkage, are known to have a synergistic effect on the activity of cellulases on the degradation of crystalline cellulose. Previously we had obtained a crystal of LPMO of a white-rot fungus and solved the crystal structure. We further refined the structure, which revealed that this LPMO has a canonical structure of AA9 family, having a copper ion at the catalytic center (Figure 1). Some polar amino acid and three tyrosine residues around the catalytic center apparently participate in the binding with cellulose. Furthermore, the synergy of LPMO

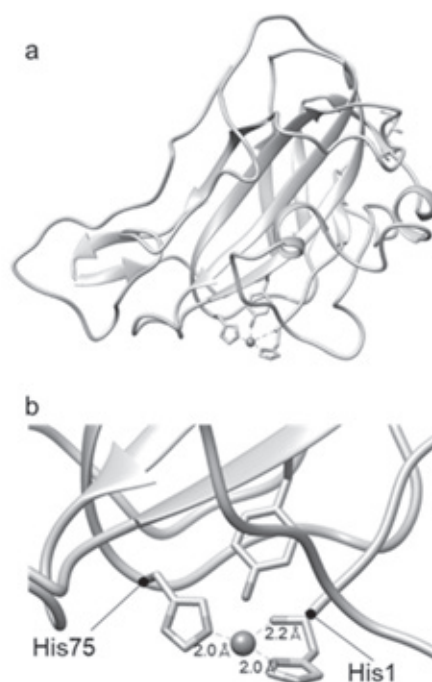


Figure 1. Over-all structure (a) and copper-coordination (b) of LPMO. The copper ion and its coordinating residues are connected with dashed lines and distances.

and cellulase cocktail was investigated. In our previous study, simultaneous use of LPMO and cellulase cocktail exerted a 1.3-fold higher glucose yield from crystalline cellulose than the individual use of LPMO and cellulase cocktail. This year we

optimized the conditions for the cellulose degradation, by which a 4.7-fold higher glucose yield was recorded.

4. Aggregation of FUS induced by mechanistic shear stress on pipetting and its suppression by non-coding RNA

Fused in sarcoma (FUS) has been considered as a molecular link between apparently different neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Although the neurodegenerative diseases have different manifestations, FUS aggregation is associated with all of them, which suggests a common pathway for their neuropathologies. We found that FUS transforms into the amorphous aggregation state as an instant response to the shear stress caused by usual pipetting, by means of fluorescence spectroscopy, fluorescence microscopy, and transmission electron microscopy (TEM) (Figure 2). The more the number of strokes of pipetting is, the more the number of aggregates is. It was also revealed that non-coding RNA can suppress the transformation of FUS into aggregates. There is the possibility that RNA bound to the C-terminal region of FUS masks the interface required for the formation of aggregates, resulting in the prevention of aggregate formation. It is also likely that RNA bound to the C-terminal region of FUS neutralizes the cations and reduces the cation- π interaction between the N- and C-terminal regions, resulting in the prevention of aggregate formation. The suppressive effect of RNA on FUS aggregation is sequence-dependent. These results suggested that the non-coding RNA could be a prospective suppressor of FUS aggregation caused by mechanistic stress in cells. Our finding might serve for the development of therapies for neurodegenerative diseases by using RNA as aggregation inhibitors.

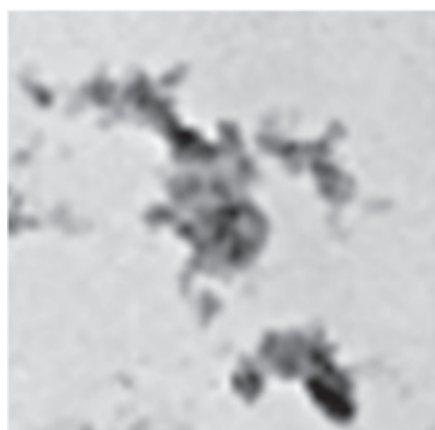


Figure 2. The TEM image showing FUS aggregates formed after 30 strokes of pipetting.

5. The detection of the DNA triplex structure in the living human cells

DNAs reportedly fold into not only a double-helix structure but also a triple-helix or triplex structure under *in vitro* conditions. However, the formation of the triplex structure of DNA in living human cells has not been proven. Here, the oligodeoxynucleotide (triplex ODN) that forms a triplex structure under *in vitro* conditions was introduced into HeLa cells by cell-resealing system utilizing a pore-forming toxin. The NMR spectrum of this sample was recorded, in which the imino proton signals of the triplex ODN inside the living cells were clearly detected. Most importantly in the living cells, all the imino proton signals that correspond to the triplex structure of the triplex ODN formed under *in vitro* conditions were commonly observed. This result indicates that the triplex ODN forms the triplex structure that is similar, if not the same, to that formed in *in vitro* even in the living human cells.

6. Interaction of human origin recognition complex subunit 1 with human G-quadruplex DNAs

DNA replication initiates from particular loci in genomes called replication origins. Origin recognition complex (ORC) binds to a replication origin and recruits other replication factors. ORC in some organisms reportedly bind to a replication origin sequence-specifically. However, the manners by which human ORC (hORC) recognizes a replication origin is elusive. Genome-wide studies revealed previously that guanine (G)-rich sequences are present in most replication origins in human genome. Our previous study implied that the amino acid region 413-511 of the first subunit of hORC, hORC1(413-511), binds preferentially to G-rich DNAs that form a G-quadruplex (G4) structure. Here, we investigated the interaction of hORC1(413-511) with various G-rich DNAs derived from human c-myc promoter and telomere regions. Fluorescence anisotropy revealed that hORC1(413-511) binds preferentially to DNAs that formed G4 structures over ones having double-stranded structures. CD and NMR clearly showed that the G4 structures of those G-rich DNAs were retained even after complex formation. Importantly, NMR chemical shift perturbation analyses revealed that hORC1(413-511) primarily binds to the external G-tetrad planes of the G4 structures. Our study propose that human ORC1 may recognize replication origins through the G4 structure.

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

片平正人, Nanyang Technological University (シンガポール) & University of Bordeaux (フランス), iモチーフ 4 重鎖 DNA と低分子化合物の相互作用様式の解明

片平正人, 永田崇, BIOTEC, NSTDA (タイ), LIPI (インドネシア), NUOL (ラオス), サトウキビ収穫廃棄物の統合バイオリファイナリー

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, 王立ブノンペン大学, アジア新興国産天然資源を由来とする機能性物質創生のための高度分析研究拠点の形成

永田崇, Institute of Biophysical Chemistry, Goethe-University, Frankfurt am Main, (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

永田崇, Institute of Biophysical Chemistry, Goethe-University, Frankfurt am Main (ドイツ), 有糸分裂から減数分裂への切り替えを担う因子 YTHDC2 の立体構造解析

永田崇, State University of New York at Albany, Albany, NY, United States (アメリカ), 核酸の in-cell NMR 測定方法の開発

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1. Grant-in-Aid for Scientific Research

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), 同一 RNA 分子によるプリオン蛋白質と A β 蛋白質の無毒化及び三者間のクロストーク (期間延長)

片平正人, 挑戦的研究(萌芽), A β 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

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永田崇, 基盤研究(C), 癌・幹細胞増殖性維持に関わる翻訳抑制複合体の形成原理と創薬に向けた分子基盤の構築 (期間延長)

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