3P025 リジン 2,3-アミノミューターゼにおける高反応性ラジカル反応機構の解明
Taming the Reactive 5'-Deoxyadenosyl Radical by Enforcing van der Waals Contact with Substrate in Lysine 2,3-Aminomutase

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Lysine 2,3-aminomutase (LAM) utilizes a [4Fe-4S] cluster, *S*-adenosyl-L-methionine (SAM) and pyridoxal 5'-phosphate to isomerize L- α -Lys to L- β -Lys. LAM is a member of the radical-SAM enzyme superfamily in which a [4Fe-4S]⁺ cluster reductively cleaves SAM to produce the 5'-deoxyadenosyl radical (5'-dAdo*), which abstracts an H-atom from Lys. 5'-dAdo* is so reactive that it has never been observed, thus this makes characterization of this step difficult. We utilize multinuclear ENDOR to characterize this radical mechanism in LAM by using SAM surrogate. We conclude that the active-site facilitates hydrogen atom transfer by enforcing van der Waals contact between radical and Lys. This constraint enables the enzyme to minimize and even eliminate side reactions.

3P026 糖結合モジュール Trp 導入変異体の基質結合能
Substrate binding ability of the Trp introduced mutant of carbohydrate-binding module

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The carbohydrate binding module attached to endo-1,3- β -glucanase from *Cellulosimicrobium cellulans* DK-1, CBM-DK, have putative imperfect tandem α -, β -, and γ -repeats. Among the three repeats, we recently showed that the α -repeat mainly contributes to the carbohydrate binding, in which Asp270 and Trp273 have the critical role [1]. While the residues corresponding to Asp 270 are conserved in both β - and γ -repeats, the residues corresponding to Trp273 are Asp314 and Gly358, respectively. In this study, we generated the Trp introduced mutants, D314W and G358W, and analyzed the interactions with laminarin and laminarioligosaccharides, using surface plasmon resonance biosensor and isothermal titration calorimetry.

[1] Tamashiro *et al.*, Glycoconj. J. 29, 77-85, 2012.

3P027 炭酸脱水酵素のある変異体の His64 の 2 つの配向の間の化学交換は NMR 時間軸上十分遅い
Chemical Exchange between Two Conformations within His64 in a Mutant of Carbonic Anhydrase Is Sufficiently Slow on the NMR Timescale

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Human carbonic anhydrase II (hCAII) has been extensively studied as a model system for investigating the finely tuned movement of protons in an efficient histidine-regulated hydrogen bond relay during enzymatic catalysis. Here, we report structural information of the histidine (His64), using site-directed mutagenesis and 2D ¹⁵N/¹H NMR experiments for determining the tautomeric constant of histidine residues. We found that the NMR resonance of ¹⁵N nucleus in the imidazole of His64 splits into two signals in a mutant enzyme. This shows that 1) His64 has two conformations and 2) the chemical exchange is sufficiently slow on the NMR timescale. This feature strongly supports that the proton transfer process does not require a change in orientation of His64 during catalysis.

3P028 Rhodococcus rhodochrous J1 由来ニトリラーゼの温度による構造変化の ¹H NMR による追跡。
Structural changes of the J1 nitrilase from Rhodococcus rhodochrous upon temperature increase tracked by ¹H NMR

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Nitrilase is an industrial enzyme that hydrolyzes nitrile compounds into ammonia and carboxylic acids. The nitrilase from *Rhodococcus rhodochrous* (J1-NTase) associates from an inactive dimer to an active oligomer upon heating. The C-terminus of J1-NTase is known to be easily degraded by proteases and its loss induces a helical complex with higher activity.

We, therefore, examined structural change of J1-NTase upon heating by ¹H NMR. Despite of its large molecular weight (80kDa in dimer), the methyl signal peak of J1-NTase was clearly resolved at low temperatures and gradually decreased with temperature increase. We are going to interpret these spectral changes in comparison with small-angle scattering data.

3P029 Analysis of unfolded structure of Staphylococcal nuclease mutants by using FRET

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Staphylococcal nuclease mutants, 33A34 and W140A, exhibit induced folding but their mechanisms have been shown to be different from each other. To understand the difference, we focused on the unfolded structures of these mutants under physiological conditions. We assume that the initial unfolded structure determines the induced-folding mechanism. The unfolded structures were characterized by the fluorescence resonance energy transfer (FRET) between a donor at D146 and an acceptor at K5. The FRET efficiency of W140A was higher than that of 33A34, suggesting that W140A contains more residual structures than 33A34. The results suggest that the induced folding depends on the local stiffness and flexibility.

3P030 高時間分解能で蛋白質の分子揺らぎと構造変化を計測するための X 線 1 分子動態計測法の開発
The Refinement of the Diffracted X-ray Tracking Method for Recording the Single-Molecule Motions of Proteins with Higher Time Resolution

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In the Diffracted X-ray Tracking method, synchrotron white x-rays are irradiated to samples to track the motions of the Laue diffraction spots from a gold nanocrystal attached to a protein molecule as a probe. Random fluctuations and global conformational changes of a single-molecule protein are readily measured with the high spatial resolution. In the unfocused white x-ray beamline, BL28B2, in SPring8, we have newly introduced the 1 m of toroidal mirror designed for focusing the beam effectively and the x-ray spectrum measurement system. The set of equipment enabled us to optimize the spectrum for tracking the protein motions with minimal radiation damages of proteins, leading to the recordings in sub-millisecond time resolutions.