Copper amine oxidases catalyze oxidative deamination of various primary amines to the corresponding aldehydes with concomitant production of ammonia and hydrogen peroxide. The enzyme contains a quinone cofactor, 2,4,5-trihydroxyphenylalanine quinone (topaquinone or TPQ), produced by post-translational modification of the conserved precursor tyrosine residue in the presence of Cu²⁺ ion and O₂. In a previous study, three intermediary structures formed during the TPQ formation have been determined and the detailed reaction mechanism of TPQ biogenesis has been proposed on the basis of their structures [1]. On the other hand, the catalytic reaction of amine oxidase proceeds by a Ping-Pong bi-ter mechanism, consisting of reductive and oxidative half-reactions [2]. In the former reductive half-reaction, a substrate Schiff-base is formed between an amine substrate and the cofactor in the initial oxidized form (TPQ⁺), which is finally converted to a semiquinone radical (TPQ⁻), yielding an aldehyde product.

To elucidate the structure-based reaction mechanism of copper amine oxidase, the reductive half-reaction catalyzed by the recombinant enzyme from Arthrobacter globiformis (AGAO) was analyzed by time-resolved X-ray crystallography. The AAGO crystals were grown anaerobically in a nitrogen-filled glove box. The crystals were subjected to single-crystal microspectrometry for monitoring the absorption spectrum of TPQ that reflects its chemical structure. Diffraction data were collected at 100 K in the BL38B1 and BL44B2 at SPring-8, Japan. Diffraction images were processed by using HKL2000 and structure refinements were performed by using the program Refmac5. Crystal structures of four distinct intermediates formed during the reductive half-reaction have been determined at atomic resolution. Concerted conformational changes of TPQ, several active-site residues located in the substrate-binding pocket and gate residues at the entrance of the substrate channel are observed with the progress of reductive half-reaction.

Keywords: enzyme, catalytic reaction, structure

MS01.P31

The influence of nickel ions on the photoswitching of the GFP-like protein PDM1-4

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PDM1-4, a reversible photoswitchable fluorescent protein, has been generated by random mutation on Dronpa in order to be more effective in far-field fluorescent nanoscopy techniques. Compared to Dronpa, PDM1-4 exhibits slower switching properties, increasing the resolution in the photoactivated localization microscopy (PALM) technique [1]. We now have determined the crystal structure of both the on- and off-state of PDM1-4.

Plate-like crystals of PDM1-4 were obtained by the hanging-drop vapor-diffusion method. The on-state crystals strongly exhibit green fluorescent emission under ultraviolet light. The off-state crystals were

Keywords: enzyme, catalytic reaction, structure
obtained by irradiating the crystals for 10 min. with a blue laser (508 nm). Diffraction data were collected at the PXII beamline of the Swiss Light Source at 100K using a MarResearch CCDCC detector.

The structures were solved in space group P2_12_12, by molecular replacement using the monomer of Dropon (pdb code 2zl0) as search model. The asymmetric unit contains 12 monomers, associated in three tetramers. The structures were refined at an effective resolution of 3.0 Å for the on-state and 3.15 Å for the off-state. R/ R_red values converged at 21.1/25.7% for the on-state and 21.3/25.0% for the off-state.

PDMD-4 exhibits a β-barrel structure typical for GFP-like proteins. Furthermore, a light-driven cis-trans isomerization of the chromophore is observed. From the structures of the on- and off-states we elucidate that the presence of nickel ions, interacting with His 194 and His 212, decreases the flexibility of the β-strands, resulting in the slower switching kinetics of PDMD-4. The phototwitching mechanism of reversible photoswitchable fluorescent proteins not only arises from the flexibility of the chromophore accompanied by a rearrangement of the proximate residues, but is also influenced by the flexibility of the β-strands.


Keywords: fluorescence, nickel, protein

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Crystal Structures of Bacterial Peptidoglycan Amidase AmpD and an Unprecedented Activation Mechanism

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AmpD is a cytoplasmic peptidoglycan (PG) amidase involved in bacterial cell-wall recycling and in induction of β-lactamase, a key enzyme of β-lactam antibiotic resistance [1, 2]. AmpD belongs to the amidase_2 family that includes zinc-dependent amidases and the peptidoglycan-recognition proteins (PGPRPs), highly conserved pattern-recognition molecules of the immune system [3, 4]. Crystal structures of *Citrobacter freundii* AmpD were solved for the apoenzyme, for the holoenzyme at two different pH values, and for the complex with the reaction products, providing insights into the PG recognition and the catalytic process. These structures are significantly different compared to the previously reported NMR structure for the same protein [5]. The NMR structure does not possess an accessible active site and shows the protein in what is proposed herein as an inactive “closed” conformation. The transition of the protein from this inactive conformation to the active “open” conformation, as seen in the X-ray structures, was studied by targeted molecular dynamics simulations, which revealed large conformational rearrangements (as much as 17 Å) in four specific regions representing one third of the entire protein. It is proposed that the large conformational change that would take the inactive NMR structure to the active X-ray structure represents an unprecedented mechanism for activation of AmpD. Analysis is presented to argue that this activation mechanism might be representative of a regulatory process for other intracellular members of the bacterial amidase_2 family of enzymes.


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Non-catalytic glycogen-binding site and its functional role in glycogen synthase

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Glycogen is a polymer of α-1,4- and α-1,6-linked glucose residues