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Keywords: cambialistic, superoxide dismutase, Aeropyrum pernix

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"ON" and "OFF" States of Reversibly Phoswitchable Fluorescent Protein rsTagRFP

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Reversibly photoswitchable fluorescent proteins (rsFPs) are a new class of FPs changing their spectroscopic characteristics under illumination with the light of a specific wavelength. This unique property holds a great potential for rsFPs to be used as biomarkers to study protein migration and protein-protein interactions in living cells. rsTagRFP is the first red fluorescent protein with reversibly photoswitchable absorbance spectra. Irradiation of rsTagRFP with blue (440 nm) and yellow (567 nm) light switches it between the fluorescent (ON) and non-fluorescent (OFF) states, respectively. Transition of rsTagRFP from the OFF to the ON state is accompanied by 200-fold increase of fluorescence intensity at 585 nm, disappearance of absorbance band at 440 nm and appearance of absorbance band at 567 nm.

We have determined the structures of rsTagRFP in the fluorescent and the dark states at 2.2 and 1.8 Å resolution, respectively. The analysis of X-ray structures have shown that photoswitching of rsTagRFP is accompanied by *cis-trans* isomerization of the chromophore. *Cis*- and *trans*- chromophore isomers correspond to the ON and OFF states of rsTagRFP, respectively. Both *cis*- and *trans*- chromophore conformers are essentially coplanar. The nearest aminoacid environment of the *p*-hydrohyphenyl site of the chromophore is essentially hydrophobic providing no direct hydrogen bond between the chromophore and the protein scaffold. The lack of a distinct stabilization of either *cis*- or *trans*- conformation of the chromophore is a key factor responsible for the photoswitching phenomenon. Additionally, *cis-trans* isomerization of the chromophore is accompanied by a slight expansion/contraction motion of the β-barrel. This is especially noticeable for the loop comprised of residues 31-40 and 68-80, indicating that this area requires a certain freedom in order to provide the efficient chromophore isomerization.

Coplanarity of the chromophore in both ON and OFF states, its essentially hydrophobic environment, and the absence of direct hydrogen bonds with the protein scaffold are unique features of rsTagRFP that have not been previously observed in other rsFPs reported up today.

Keywords: photoswitchable fluorescent proteins, X-ray structure, cis-trans isomerization.

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Structural Basis of Different Substrate Preferences of Yeast Old Yellow Enzymes

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Old yellow enzyme (OYE) is an NAD(P)H-dependent reductase that can catalyze asymmetric reduction of C=C bond of enone substrates. Thus, OYE is applicable to the synthesis of chiral compounds. Although the molecular mechanism of the asymmetric reduction catalyzed by OYE has been proposed, the structural basis of different substrate preferences of different OYEs has not been fully understood. For example, the two OYEs from the two yeast strains, *Candida macedoniensis* (CYE) and *Torulopsis* sp. (TYE), show 46% sequence identity, but they show different substrate preferences to 3,5,5-trimethyl-2-cyclohexene-1,4-dione (ketoisophorone) and 4-hydroxy-2,6,6-trimethyl-2-cyclohexanone ((4*S*)-phorenol); CYE catalyzes the asymmetric reduction of ketoisophorone only, whereas TYE catalyzes the asymmetric reductions of both ketoisophorone and (4*S*)-phorenol. To reveal the structural basis of the different substrate preferences between CYE and TYE, we analyzed their crystal structures in the absence and presence of a substrate analogue, *p*-hydroxybenzaldehyde (*p*-HBA), and performed mutational analysis. As a result, we demonstrated that the two loop regions, Loops 5 and 6 located between β_5 and α_5 and between β_6 and α_6, respectively, function as the substrate filters which confer their different substrate preferences (Fig. 1). Detailed structural basis of their different substrate preferences will be presented and discussed.

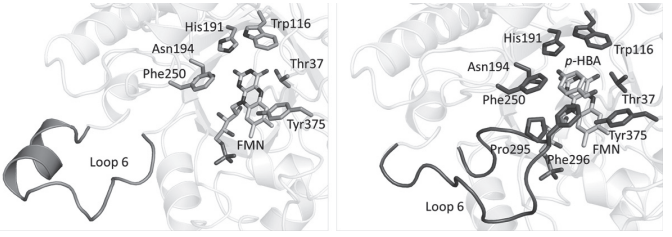

Fig. 1. Open (left) and closed (right) forms of CYE.
Mutation analysis indicates that Loop 6 plays significant roles in substrate preferences.

Keywords: old yellow enzyme, substrate preferences

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Stabilization of a full A-T hairpin oligonucleotide

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The human genome contains many non-coding DNA regions which are A-T rich. Since the function of those regions is still unclear it appears of interest to study A-T rich oligonucleotides at the atomic structural level. The formation of stable DNA hairpins could be involved in the condensation of mitotic chromosomes and the recognition of homologous chromosomes in meiosis. They could also be involved in the regulation of DNA function. For all these reasons, we have undertaken the study of a DNA hairpin which only contains A-T bases. With a representative non-coding sequence of 16 bases d(ATATATTTAATATAT) it has been predicted the formation of a DNA hairpin with a 6 base pairs stem and a tetra-loop (T_3A) [1]. We have simulated its formation by kinetic folding. Followed by crystallization

of the oligonucleotide in the presence of a variety of metal ions [2] (Mg^{2+}, Mn^{2+}, Ca^{2+}, Ni^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, $Co(NH_3)_6^{3+}$, Zn^{2+} y Li^{+}) to stabilize the structure of the crystal. By X-ray diffraction we obtained patterns with low resolution from which in a first stage, it was only possible to determine the cell parameters and space group. The best crystals were obtained in presence of cadmium which has a high affinity for adenine bases. Contrary to common behavior of A-T rich oligonucleotides, when hairpin has been stabilized with cadmium the best crystals are obtained at 21°C, while using the other metal ions low temperatures is needed to obtain crystals; usually between 4 and 11°C. On the other hand, by performing DLS analysis was detected the influence of cadmium in the structure stabilization and in the intermolecular contacts stabilization, which compared to the magnesium interaction and the reference without metal ion is higher. Also by DLS analysis we confirmed the formation of the DNA hairpin by monitoring the diffusion coefficient and size of monomeric species. We constructed hairpin and duplex DNA theoretical structures and performed a simulation of the diffraction obtaining a model of molecular packing. We may assume the formation of a DNA hairpin from 100% A-T oligonucleotides and highlight the role of metal ions on the hairpin stabilization.

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Keywords: hairpin, oligonucleotide, crystallization

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Crystal structures of two CBSX proteins from *Arabidopsis thaliana*

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CBS (cystathionine-β-synthase) domain is a small intracellular module, mostly found in two or four copies within a protein, which has been identified in many proteins in all kingdoms of life. Tandem pairs of CBS domains can act as binding domains for adenosine derivatives and may regulate the activity of attached enzymes or other domains. Many proteins containing CBS domain are easily identified in plant genome. However, their exact physiological functions remain elusive. Two of them, AtCBSX1 and AtCBSX2 (CBS pair protein) from Arabidopsis thaliana have been cloned and analyzed. These encode 236 and 238 amino acid residues which contain two tandem CBS domains, respectively. Both proteins were over-expressed heterologously in E. coli and purified them as homogeneity. The structure of AtCBSX1 and AtCBSX2 determined at 2.2 and 1.9 Å resolution, respectively, reveals an unique architecture and a positively-charged pocket for AMP. The structure of AtCBSXs show that it is an anti-parallel dimer on its central two-fold axis and show a uniquely ~120º bent at the side whereas all the other parallel CBS domain proteins are approximately flat ~180º. However, the structure of dimeric AtCBSX2 with bound AMP is show approximately flat, which is significantly different from the apo form of that. This orientation, shape, molecular symmetry of AtCBSX protein and more importantly conformational change induced by ligand-binding might determine the interacting surface for binding molecules, which is related to its function.

Keywords: X-ray, structure, conformational change

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Structural and functional studies of the active-sites in four glycoside hydrolase β-jellyroll families

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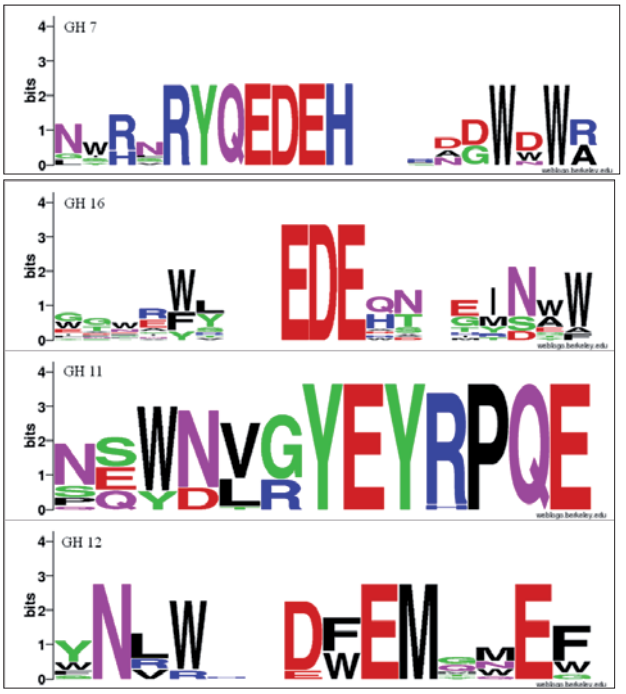
The three-dimensional (3D) structure of glycoside hydrolase family (GHF) 7, 11, 12 and 16 are collectively referred to as a jellyroll β-sandwich fold with a similar cleft catalytic active site, although the amino acid sequences of these four families are diverse. Based on the results of primary sequence alignment and 3D structural comparison, GHF 7 and 16 possess a conserved catalytic motif of RYQExDxEHWW and ExDxE/ExDxxE, whereas GHF11 and 12 share a general active site motif of $YE_nYPQE_{n+(88\sim94)}$ and $NE_nME_{n+(83\sim97)}$, respectively. The first and last glutamyl residues found in the catalytic motifs have been clearly identified as catalytic nucleophile and general acid/base for retention hydrolytic mechanism, respectively. A detailed structural comparison among the known structures reveals that they share a low level of amino acid sequence identity 5~20%, but the enzymes have a high degree of structural conservation at the active sites.



Keywords: glycoside hydrolase β-jellyroll family, conserved amino acid, structural and primary sequence comparison

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Crystal structures of symbiosis related lectin and its saccharide bound form

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