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The first structure of a protein classified in family 61 of glycoside hydrolases is presented. The crystal structure of Thielavia terrestris GH61 isoform E was determined at a resolution of 1.9 Å by Multiple Isomorphous Replacement. The fold is a β-sandwich consisting of two sheets and is a variation of the fibronectin type III fold. The structure shows significant structural similarity to the chitin-binding protein CBP21 of Serratia marscecens and shows a conserved Zn-binding site which is likely to be functionally relevant. No hydrolytic activity has been reported for this protein, although it potentiates the activity of enzyme cocktails used for the degradation of cellulosic wastes. Together with the information available in the literature, serious doubts can be cast on the nature of family 61 proteins as true glycoside hydrolases, we propose instead that they have an accessory function in cellulose degradation, in analogy to the one shown for chitin binding protein.

Keywords: biotechnology, plant cell wall, chitin binding protein

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Crystal structure and mechanism of cytochrome P450 StaP that constructs the indolocarbazole core

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Staurosporine isolated from Streptomyces sp. TP-A0274 is a member of the family of indolocarbazole alkaloids that exhibit strong antitumor activity. A key step in staurosporine biosynthesis is the formation of the indolocarbazole core by intramolecular C-C bond formation and oxidative decarboxylation of chromopyrrolic acid (CPA) catalyzed by cytochrome P450 StaP (StaP). We have solved x-ray crystal structures of CPA-bound StaP. Hydrogen-bonding interactions of two carboxyl groups and CH-pai interactions with indole rings hold the substrate in the substrate-binding cavity with a conformation perpendicular to the heme plane. Based on the crystal structure of StaP-CPA complex, we propose that C-C bond formation occurs through an indole cation radical intermediate that is equivalent to cytochrome c peroxidase compound I. Theoretical QM/MM calculation of a catalytic intermediate (CPA-Fe(IV)=O pai cation radical) suggests that the indole cation radical can be formed in the catalytic process and that the spin density of the indole cation radical is controlled by the surrounding H-bonding network. Our crystallographic and theoretical studies provide valuable insights into the process of staurosporine biosynthesis, combinatorial biosynthesis of indolocarbazoles, and the diversity of cytochrome P450 chemistry.

Keywords: indolocarbazole, heme, P450

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Bacsu PerR : Metal binding sites and unambiguous highlights of 2-oxo-His in the oxidized protein

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In Bacillus subtilis, the PerR protein is a metal-dependent sensor of hydrogen peroxide that regulates the adaptive response to H_2O_2. PerR is a dimeric zinc protein with a regulatory metal-binding site that coordinates either Fe^{2+} (PerR-Zn-Fe) or Mn^{2+} (PerR-Zn-Mn). Here we present the structural studies of both active forms: the crystal structure of the PerR-Zn-Mn protein and X-ray absorption spectroscopy experiments of PerR-Zn-Fe. While most of the peroxide sensors use redox-active cysteines to detect H_2O_2, it has been shown that reaction of PerR-Zn-Fe with H_2O_2 leads to the oxidation of one histidine (H) residue that binds the Fe^{2+} ion. This metal-catalyzed oxidation of PerR leads to the incorporation of one oxygen atom into H37 or H91. However the exact position of the added oxygen is still unknown. This study presents the crystal structure of the oxidized PerR protein (PerR-Zn-ox) that clearly shows a 2-oxo-histidine residue in position 37.

Keywords: 2-oxo-histidine, metalloproteins, protein-DNA complex

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Crystallization and SAXS of α -Actinin 2

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α-Actinin is involved in cytoskeletal network and it is composed of an N-terminal actin binding domain (ABD) connected by a neck to a central rod domain composed of 4 spectrin-like repeats (SR) and a C-terminal calmodulin like domain (CaM). Its functional unit is an anti-parallel homodimer allowing the protein to crosslink actin filaments. Different isoforms are present in human cells: isoforms 1 and 4 are found in non-muscle cells, while the isoforms 2 and 3 are present in muscle cells. The described work is related to the muscle isoform 2. To try to understand the α-actinin structural mechanisms underlying its regulation, the full length α-actinin 2 was expressed, purified and submitted to crystallization experiments. No crystals grew with the wild type protein in any standard conditions; therefore thermofluor experiments were performed to find a buffer where the protein is more thermally stable. Mutations in the primary structure were then designed to decrease the surface entropy of the protein and lysine methylation assays were performed. Both strategies helped enhancing the propensity of the protein to crystallize and several hits were identified. Crystal optimization is ongoing with success. In an attempt to understand α-actinin molecular architecture a small angle X-ray solution scattering (SAXS) experiment was carried out on the full length and on the half dimer construct (ABD-SR1-SR2// SR3-SR4-CaM). The low-resolution data of the molecular envelope presented the expected protein shape and dimensions, if compared to the solved structures of the individual domains. Reported biochemically information on the interaction points between CaM