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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

and neck of the opposing subunit was used as restraints in the process of fitting the ABD and CaM in the molecular envelope.

Keywords: actin-binding protein, crystallization strategies, SAXS

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Structural and biochemical characterization of actin binding by dystrophin and utrophin

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Duchenne and Becker muscular dystrophies (DMD & BMD) are muscle-wasting disorders caused by mutations in the X-linked dystrophin gene. Utrophin is an autosomal homolog to dystrophin that has been shown to functionally compensate for dystrophin in cultured muscle cells and in vivo in the muscular dystrophy (mdx) mice model and hence may prove to be useful as a therapeutic replacement for dystrophin in DMD and BMD. Both proteins belongs to the spectrin superfamily of proteins, which also includes spectrin and α-actinin. These proteins are characterized by N-terminal actin binding domains and C-terminal variable domains separated by numerous spectrin like repeats. The spectrin repeats are triple-helical coiled structures that can bind a variety of ligands, including F-actin, as is the case for some repeats found in dystrophin. The aim of this research is to examine the interaction of both dystrophin and utrophin with actin by structural and biochemical methods and compare their actin binding properties. Studies have shown that the N-terminal spectrin repeats of utrophin are required for a high affinity interaction of the actin-binding domain with F-actin. It is unclear whether these repeats have an intrinsic affinity for F-actin and in the current study we are determining the actin-binding properties of the two N-terminal spectrin repeats using X-ray crystallography and co-sedimentation assays. The crystal structure of first spectrin repeat of utrophin has been determined to 1.8 Å and exhibits the characteristic three helix structure as observed for spectrin, α-actinin, plakin.

Keywords: actin binding, Duchenne muscular dystrophy, becker muscular dystrophy

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Crystallization and crystal analysis of ATP synthase

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ATP synthase (FoF1) is responsible for ATP production in living cells, and is a membrane protein located in the energy conversion membrane. ATP synthase consists of a channel Fo portion (100,000 dalton, ab2c8-12) and a large soluble catalytic F1 portion (380,000 dalton, $\alpha 3\beta 3\ \gamma\delta\varepsilon$). The unique rotational catalysis mechanism of F1 includes rotation of the rod-like γ subunit, which is thought to control the conformations of the three catalytic β-subunits in a cyclic manner. We have purified and crystallized ATP synthase from a thermophilic bacterium PS3. Among detergents tried, dodecyl-maltoside was the best, though decyl-maltoside was the close second. Used columns were those of an ion-exchange and gel-filtration types. Initial tiny crystals from PEG6000 are now replaced by crystals that allowed diffraction analysis, after extensive and systematic crystallization condition search. ATP synthase in our crystals contains all the subunits. Those crystals diffracted to a 7 Å resolution at synchrotron. Our initial analysis had been hampered by incorrect beam position parameters supplied and a high mosaicity of crystals (nearly 4 degree). Though the first problem was got rid of with relative ease, the second problem was very difficult. Our current refined procedures for processing the data made us to think that the problem can be overcome if we could get accurate cell parameters since other possible problems have been ruled out in the analyses. As we realized that PS3 ATP synthase was difficult for x-ray study, we have searched for better thermophilic bacterial sources from hot springs. We have got some bacterial strains that may be suited to structural study of ATP synthase but other proteins as well.

Keywords: ATP synthase, crystallization, crystal analysis

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Structural view of the ATPase cycle of a myosin that moves backward

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Myosins are molecular motors that use the energy obtained from the hydrolysis of ATP to move along actin filaments. Among myosin family, class VI myosins are very intriguing because of their atypical motility properties. First, dimeric myosin VI is capable of taking multiple steps (processive movement) of 30-36 nm along actin filament. These steps are surprisingly large considering that myosin VI has a rather small lever arm and they cannot be explain by the structural transitions that occur within the myosin motor of other classes. Secondly, this motor produces force towards the minus end of actin filaments, which is the opposite direction of all other characterized myosins. In order to understand the molecular basis of these features, we would like to describe the structure of this myosin in different states of its ATPase cycle. During the ATPase cycle, myosin goes through states of strong and weak affinity for the actin filament. To this day, we solved the structure of three states of the cycle, one state that mimics the state of strong affinity for actin (at the end of the movement on the filament) and two states before the force production. One of them represents the starting point for movement on actin, the pre powerstroke state. The analysis of the structures from the beginning and the end of the powerstroke allows us to understand how myosin VI moves in the opposite direction (toward the minus-end of actin filaments) due to a unique insertion between the motor domain and the lever arm. These structures also allowed us to understand the origin of the large size of the myosin VI lever arm swing (powerstroke). Unexpectedly, we found that a conformational change occurs in the converter which allows an optimized movement of the lever arm during the stroke.

Keywords: myosin, conformational change, motility