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 marcescens and shows a conserved Zn²⁺ 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 cellulose 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

P04.03.205

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-pi 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 peroxoide compound I. Theoretical QM/MM calculation of a catalytic intermediate (CPA-Fe(V)=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

P04.04.207

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 thermodrhuor experiments were performed to find a buffer where the protein is more theromally 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