change of EF1 play a key role of regulation.

Keywords: plants, EF-hand proteins, GTP-binding proteins

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Crystal structure and functional study of wild type and mutated *Bacillus cereus* NCTU2 chitinase

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Chitinases which hydrolyze chitin as carbon and nitrogen nutrient, occur in a wide range of organisms include in viruses, bacteria, fungi, insects, higher plants, and animals. Agene of family 18 chitinase from *Bacillus cereus* NCTU2 encodes a signal peptide (27amino acids) and a mature protein (333 amino acids), The gene of family 18 chitinase from *Bacillus cereus* NCTU2 was overexpressed by *E. coli* BL21 (DE3) strain. ChiNCTU2 and mutant E145Q of MW 36 kDa have been crystallized using the hanging-drop vapor diffusion method with solution consisted of polyethene glycerol 8000, sodium cacodylate and zinc acetate dihydrate. According to diffraction of ChiNCTU2 crystals at resolution 1.20 Å, the unit cell belongs to space group $P2_1$ and has parameters a = 50.789 Å, b = 48.788 Å and c = 66.867 Å. And E145Q crystal at resolution 1.49, the unit cell belongs to space group $P1$ and has parameters a = 61.306 50.820 Å, b =72.888 Å and c = 76.343 Å. The protein structure of ChiNCTU2 is monomer by using multiwavelength anomalous dispersion method and the crystal packing of E145Q is tetramer by using molecular replacement method. Four residues Asp143, Glu145, Glu190 and Gln225 bind with zinc atoms in the catalytic domain of ChiNCTU2 protein structure. We proved that zinc atoms decline activity of ChiNCTU2 by detecting the amount of chitobioside using DNS (3,5-Dinitrosalicylic acid). According to structure and mutagenesis we found that E145, Q225 and Y227 are the most important redidues for its function.

Keywords: chitin, chitinase, structure

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The structure of human diamine oxidase

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The crystal structure of human diamine oxidase (hDAO), the first reported structure of a diamine oxidase (DAO), has been determined to 2.9 Å resolution. DAO, a copper-containing amine oxidase (CuAO), contains a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor derived by post-translational modification of a tyrosine residue [1]. DAO is distinguishable among members of the CuAO enzyme family in its ability to oxidize diamines, such as putrescine and cadaverine, as well as monoamines. DAO is involved in many biological processes. In mammals DAO is found in several tissues, with the highest reported expression levels found in the placenta, small intestine and kidneys. In particular, hDAO may play an important role in histamine metabolism (1). We have grown orthorhombic crystals of hDAO belonging to the space group $C222_1$, with unit-cell dimensions a=95.0, b=97.2, c=179.2 Å. These crystals diffracted to 2.9 Å in-house at 100 K. Data were integrated and scaled with the HKL suite of programs, DENZO and SCALEPACK. The data is 98.3% complete in the range 50-2.9 Å with an overall R_{merge} of 8.4%. The most reasonable Matthews' coefficient suggests there is one molecule in the asymmetric unit with 40% solvent content using 100 kDa as the molecular mass. The structure was solved by molecular replacement, PHASER v1.3 giving a Z-score of 26.2 with a search model created using CHAINSAW, with human vascular adhesion protein-1 (hVAP-1, PDB code 1US1) as the target. Initial rigid-body and restrained refinement has been carried out using REFMAC v5.2. 2*Fo-Fc* and *Fo-Fc* electron-density maps were inspected with, and modeled using COOT.

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Keywords: diamine oxidase, amine oxidase, topaquinone

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A study of protocatechuate 3,4-dioxygenase muntants and substrate interactions

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Protocatechuate 3,4-dioxygenase is a nonheme, iron containing enzyme that catalyzes the intradiol oxidative cleavage of 3,4-dihydroxybenzoic acid to ß-carboxy-*cis*,*cis*-muconic acid via incorporation of molecular oxygen into the aromatic ring of the substrate. In an attempt to further understand the factors involved in substrate turnover and mechanism, a series of second sphere residue mutants has been created and structurally and kinetically examined. These crystals diffract to high resolution and show clearly that alterations of these second sphere residues can dramatically affect the interactions with substrate and substrate analogs. A detailed structural and kinetic comparison of these mutants will be presented.

Keywords: structure and function, structural enzymology, metalloenzymes

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Structural study of H_2O_2 reductase, rubperoxin

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Rubperoxin (Rpr) was identified as an O_2-induced protein in