m11.o02

Promiscuity in biosynthetic enzymes: structural basis for antibiotic diversity

Gunter Schneider

Karolinska Institutet, Stockholm, Sweden, E-mail: gunter.schneider@ki.se

Keywords: antibiotics, enzymology, protein crystallography

Aromatic polyketide antibiotics are of particular medical importance, because some of the clinically most potent anti-tumor drugs are recruited from this class of compounds. A striking feature of many of the enzymes in aromatic polyketide biosynthesis is their relative broad substrate specificity, i.e. they accept intermediates from several related biosynthetic pathways as substrates. This feature is the basis of present-day combinatorial biosynthesis efforts to generate antibiotic diversity, where genes from the biosynthetic cluster of one species are transferred to another species, the hybrid antibiotic approach. Our laboratory is engaged in a small-scale structural genomics project aiming at the structural and functional characterisation of enzymes in the biosynthesis of aromatic polyketide antibiotics, in particular anthracyclines. Out of approximately 50 target genes, we have at present cloned twenty-two genes and produced sixteen enzymes in soluble form. Twelve of these have been crystallized and the crystal structures of ten enzymes have been determined so far. Several of these enzymes show novel mechanisms, for instance the polyketide cyclase SnoaL or the S-adenosyl-L-methionine dependent hydroxylase RdmB. The latter case is the first observation of S-adenosyl L-methionine as a cofactor in an enzymatic hydroxylation reaction. These studies have also provided insights into the structural basis of substrate recognition and specificity of these enzymes. Binding and recognition of the polyketide substrates is dominated by hydrophobic interactions. Specificity is controlled by the shape of the binding pocket rather than through specific hydrogen bonds. Re-design of the substrate binding pockets could possibly expand the substrate spectrum of these enzymes, and thus provide a larger repertoire of biosynthetic enzymes for combinatorial biosynthesis. This approach could therefore potentially facilitate the development of novel aromatic polyketide antibiotics with improved toxicity profiles.

m11.o03

Pathogenesis-related plant protein in complex with multiple hormone molecules

Humberto Fernandes[1], Wojciech Czyrek[2], Oliwia Pasternak[1], Luiza Handschuh[1], Grzegorz Bujacz[1,2], Michal Sikorski[1], Mariusz Jaskólski[1,3]

[1]Center for Biocrystallographic Research, Inst. of Bioorg. Chem., Pol. Acad. Sci.,Poznan, Poland; [2]Technical Univ. of Lodz, Poland; [3]Faculty of Chemistry, A.Mickiewicz Univ., Poznan, Poland, E-mail: humberto@man.poznan.pl

Keywords: plant pathogenesis-related proteins, plant hormones, zeatin

PR (pathogenesis-related) proteins of class 10 are small (17 KDa), slightly acidic (pI 5), and cytosolic. The main feature of their three-dimentional structure is a seven-stranded antiparallel β-sheet, surrounding a long C-terminal helix α3. Between these two structural elements, a large cavity is created. Although PR-10 proteins are very abundant in plants, their physiological role remains unknown. However, recent data indicate ligand binding as their possible biological function. It has been shown that PR-10 protein from white birch (Betv1) can bind various compounds (including fatty acids, flavonoids and cytokinins) and NMR data indicated that binding occurs in the internal cavity. Crystallographic studies have also shown that Betv1 can bind two deoxycholate molecules in the cavity. The present study of the yellow lupine LlPR-10.2B protein provides the first structural evidence of cytokinin binding by classic PR-10 proteins. Previously, zeatin binding has been reported in a related cytokinin-specific binding protein (CSBP). The present crystal structure of an LlPR-10.2B-zeatin complex has been solved by molecular replacement using X-ray diffraction data extending to 1.35 Å resolution. The structure reveals that a single LlPR-10.2B molecule is capable of binding four zeatin ligands. Three of the zeatin molecules are located in the binding cavity and one is shared by two symmetry-related protein chains. The ligand molecules could be unambiguously modeled since the corresponding electron density is of excellent quality.

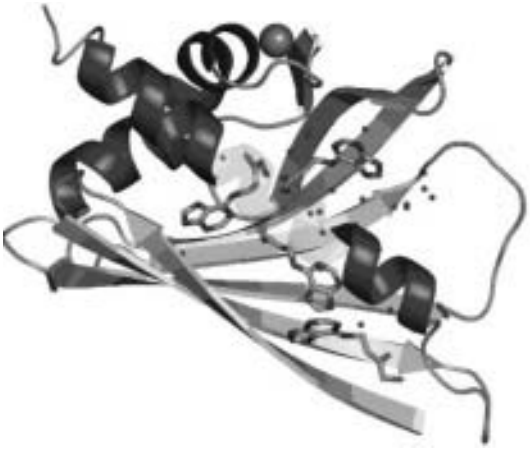

Fig. 1. The overall fold of the LlPR-10.2B protein, with zeatin and water molecules bound in the internal cavity.