s8a.m1.p25 Structural characterisation of ActVA-orf6, a monooxygenase involved in the biosynthesis of polyketide actinorhodin. A.E. Miele, G. Sciara, S.G. Kendrew[#], B. Vallone[#] & A. Boffi*. *Institute Pasteur – Fondazione Cenci Bolognetti, *C.N.R. Centre of Molecular Biology and [#]Department of Biochemical Sciences, University of Rome "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy.*

Keywords: enzyme catalysis, protein engineering.

Active anti-microbial peptides are synthesised by a lot of bacterial strains in a non-ribosomal manner, which involves many different enzymes acting in a strictly temporal and spatial sequence. ActVA-orf6, discovered in *Streptomyces coelicolor* strain A3(2), is a small enzyme of 113 amino acids, which adds a ketone group to an anthracene-like precursor of actinorhodin (Kendrew et al., 1995). Its main characteristic is the absence of any cofactor or metal ion, which usually help the catalysis. Moreover analysis by multiple sequence alignment with different bioinformatic programs, revealed the uniqueness of this protein. In fact it shares features common to only other two members of the same pathway (called orf2 and orf5) and not with any other known protein. This fact highly implies that its fold could be unique and new.

The enzyme has been cloned in *E. coli* to scale up its production. Equilibrium sedimentation experiments demonstrate that the enzyme is dimeric at pH 7.0. The secondary structure content of recombinant ActVA-orf6 has been quantitatively determined by means of CD and FTIR spectroscopy. The overall line-shape of the far-UV CD spectrum show significant features typical of α/β proteins. The second derivative and Fourier self-deconvolved SATR spectra in the amide I region display a rich pattern that allows the identification of several secondary structure related peaks. Assignments, by means of a GOR constrained prediction method (Manavalan & Johnson, 1987), revealed the presence of three 8-10 residues long α–helices (27%) and of four β-sheets (31%). From a screening of substrate analogues, a high affinity binding capacity has been shown for mercurochrome ($K=6x10^6M^{-1}$), which has a pronounced emission fluorescence quenching and peak shift. The complex between the protein and analogue has been further demonstrated by the presence of a strong Cotton effect in the CD spectrum of mercurochrome in the visible region in the presence of an equimolar amount of protein.

Crystallisation trials have been set up and very good quality crystals have been obtained, using ammonium sulphate as precipitant agent at pH values in which the protein is active. The best native data collection was 99.7% complete at 1.4 Å resolution. Analysis of the self-rotation function revealed the presence of a dimer in the asymmetric unit, confirming data obtained in solution (Kendrew et al., 2000).

The small solvent content in the crystal implies a very compact fold of the numerous dimers in the crystal tetragonal lattice, limiting the solvent channels. Strategy actually employed to gain phase information, and hence solves the structure, is MIR with heavy metals, given the non-similarity with proteins of known structure.

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Manavalan, P. & Johnson, W.C. (1987). *Anal. Biochem.* **125**, 177-188.

s8a.m1.p26 Crystal structures of the cellulase Cel48F in complex with inhibitors and substrates give insights into the mechanism of processive cellulases. G.Parsiegla[1], C.Tardif[2], C.Reverbel-Leroy[2], H.Driguez[3] and R.Haser[1], *[1]Institut de Biologie et Chimie des Protéines, Laboratoire de Bio-Cristallographie, CNRS UPR 412, 7 passage du Vercors, 69367 Lyon cedex 07, France. [2]Laboratoire de Bioénergétique et Ingénierie des Protéines, Institut de Biologie Structurale et Microbiologie, CNRS, 31 Chemin Joseph-Aiguier, 13402 Marseille cedex 20 and Université de Provence, Place Victor Hugo, 13331 Marseille cedex 03, France. [3]CERMAV, CNRS, Domaine Universitaire de Grenoble, 601 rue de la Chimie, 38041 Grenoble, France.*

Keywords: processive, cellulases, stacking interactions.

The cellulase Cel48F from *C. cellulolyticum* is a member of the glycosyl hydrolase family 48 and belongs to the class of processive endo-cellulases which cleave the cellulose chain with inversion of the anomeric carbon. The active site is composed of a 25 Å long tunnel which is followed by an open cleft[1]. The architecture of active center resembles in its construction the tunnel of processive cellulase CBHI of *T. reesei* (GH-family 7) which is contrary to Cel48F a retaining enzyme[2]. During the processive action the cellulose has to slide through the tunnel to continuously supply the leaving group side with sugar residues after the catalytic cleavage.

To study this processive action in the tunnel, the native catalytic subunit of Cel48F and the inactive mutant E55Q, have been cocrystallized with thiooligosaccharide-inhibitors and cellooligo-saccharides. Seven subsites in the tunnel section of the active center could be identified and three of the four reported subsites in the open cleft section were reconfirmed. The subsites observed for the thioligosaccharide inhibitors and the cellooligosaccharides are located at two different positions in the tunnel corresponding to a shift in the chain direction of about one and a half sugar subunit. These two positions have a different pattern of stacking interactions with aromatic residues present in the tunnel. Multiple patterns are not observed in non-processive endo-cellulases, where only one sugar position is favored by aromatic stacking. It is therefore proposed, that the aromatic residues serve as lubricating agents to reduce the sliding barrier in the processive action as shown for sugar transporting molecules like malto-porins[3].

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