m09.p03

Structure and function of human PPTase: implications from ternary complexes

Gábor Bunkóczi,[a] Xiaoqiu Wu,[a] Anil Joshi,[b] Stuart Smith,[b] Frank von Delft[a], Udo Oppermann[a]

[a]Structural Genomics Consortium, University of Oxford, Botnar Research Centre, OX3 7LD Oxford, UK, and [b]CHORI, 5700 Martin Luther King Jr Way, Oakland, CA 94609, USA. E-mail: gabor.bunkoczi@sgc.ox.ac.uk

Keywords: protein crystallography structures, acyl carrier protein, lipid biosynthesis

The modification of invariant Ser residues in acyl carrier proteins (ACPs) by transfer of a 4-phosphopantetheine moiety is essential to all living organisms. The reactions is carried out by a diverse group of enzymes collectively characterized as 4-phosphopantetheine transferases (PPTases), also known as holo-ACP synthases, yielding holo-carrier proteins by group transfer of the 4-phosphopantetheine moiety from coenzyme A (CoA) as donor substrate. Recent molecular cloning and biochemical characterization of human PPTase revealed that it is a cytosolic, monomeric enzyme with broad acceptor substrate specificity, and requires Mg^{2+} for catalysis.
In order to better understand its mode of action, human PPTase was crystallised and structures of the enzyme, its complex with CoA, and in complex with CoA and cytosolic fatty acid synthase (FAS) ACP were determined. The enzyme, which adopts the same fold observed in bacterial group II PPTases, consists of two nearly identical domains with the active site located in a deep cleft between the domains. ACP binds with its essential Ser pointing towards the bound CoA, interacts with the enzyme on a large surface and is positioned primarily by hydrophobic forces. CoA-binding is mediated by a Mg^{2+} ion complexed by Glu residues. Mutagenesis studies indicate that in addition to the residues involved in Mg^{2+}-binding, a base catalyst Lys and a His are essential for catalysis.
Although the shape of FAS ACP is complementary to the large basin on the enzyme near the active site, there are still features on the binding surface not explained by the ternary complex. It is tempting to speculate that these regions play a role in binding mitochondrial ACP, since it has been demonstrated that human PPTase can activate ACP from the mitochondrial fatty acid synthesis machinery as well. Progress on establishing the structure of this complex will also be reported.

m09.p04

Structure of Thermus thermophilus type 2 Isopentenyl Diphosphate Isomerase inferred from crystallography and molecular dynamics

Jérôme de Ruyck[a], Jenny Pouyez[a], Steven C. Rothman[b], C. Dale Poulter[b], Johan Wouters[a]

[a]Laboratoire de Chimie Biologique Structurale, University of Namur, B-5000 Namur, Belgium [b]Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, USA.

Keywords: protein crystallography, flavoproteins, molecular modelling

IDI-2 is an essential enzyme for those pathogenic microorganisms, such as some strains of *Streptococcus* and *Staphylococcus*.[1] Thus, IDI-2 is a logical target for the design of anti-infectious agents. The catalytic mechanism of the enzyme remains unknown. A crystal structure of the *B. subtilis* protein was solved in 2003.[2] However, electron density was not detected for several amino acids at the active site due to its conformational flexibility. In the present work, we obtained some small yellow crystals which belonged to orthorhombic $P2_12_12_1$ space group and diffracted to 2.8 Å at the ESRF, Grenoble, France. Crystal structures of *T. thermophilus* and *B. subtilis* type 2 IPP isomerases were, then, combined to generate a complete model of the FMN-bound structure of the enzyme. In contrast to previous studies, positions of flexible loops were obtained and carefully analyzed by molecular dynamics. Docking simulations find a unique putative binding site for the IPP substrate.[3]

[1] Kuzuyama, T., and Seto, H. (2003) *Nat Prod Rep* **20**, 171-183.
[2] Steinbacher, S., *et al.* (2003) *J Mol Biol* **329**, 973-982.
[3] de Ruyck, J., *et al.* (2005) *Biochem Biophys Res Commun* 338, 1515-1518.