FA1-MS03-P06

Structural Basis for Natural Lactonase and Promiscuous Phosphotriesterase Activities. Mikael Elias[a], Luigia Merone[b], Claude Lecomte[c], Mosè Rossi[b], Patrick Masson[d], Guiseppe Manco[b], Eric Chabriere[a]. *[a]CNRS-Université de la Mediterranee, AFMB, Marseille, France. [b]Consiglio Nazionale delle Ricerche IBP, Napoli, Italy. [c] Nancy Universite, CRM2, Nancy, France. [d]CRSSA, dpt de toxicology, Grenoble, France.*
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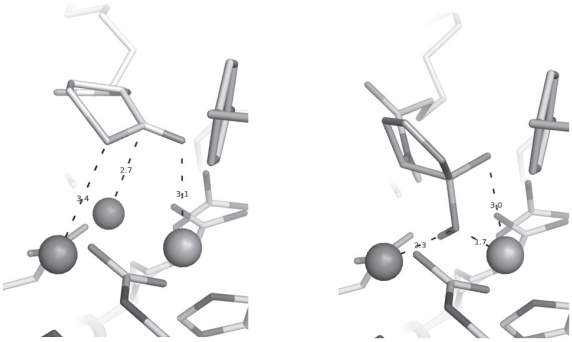
Organophosphates constitute the largest class of known insecticides and several of them are potent nerve agents. Consequently, organophosphate-degrading enzymes are of interest, as bioscavengers and biodecontaminants. Recently, a phosphotriesterase (known as *Sso*Pox) from the hyperthermophilic archeon Sulfolobus solfataricus was isolated and was found to possess high lactonase activity. Here, we report the three-dimensional structures of *Sso*Pox in its apo form (2.6 Å resolution) and in complex with a quorum sensing lactone mimic compound (2.0 Å resolution) [1]. The structures reveal a unique hydrophobic channel that perfectly accommodates the lactone analog. Analysis of the structure strongly hints that lactonase activity is the cognate function of *Sso*Pox. These findings illustrate how the promiscuous phosphotriesterase activity of lactonases like *Sso*Pox has served as a seed to develop optimized phosphotriesterases[2]. This example demonstrates that promiscuous activities probably constitute a large and efficient reservoir for creating novel catalytic activities.



Catalytic cycle of the "quorum sensing" lactone hydrolysis

[1] Elias et al., *Extremophiles*, **2009**, in press. [2] Elias et al., *J Mol Biol*, **2008**, 379, 1017-1028.

Keywords: phosphotriesterase; „quorum sensing" lactonase; enzyme evolution

FA1-MS03-P07

Getting What You Screen for But not Quite. John Cutfield[a], Sue Cutfield[a], Iain Lamont[a], Yoshio Nakatani[a]. *[a]Biochemistry Department, University of Otago, Dunedin, New Zealand.*
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Through a selection/screening process a marine bacterial glycosyl hydrolase with exo-1,3-β-glucanase activity was isolated and its gene subsequently over-expressed in *E.coli*. The sequence showed it to be a GH3 family member, closely related to barley exo-1,3-1,4-β-glucanase [1] in two of its three domains. This was confirmed by crystallographic analysis which also showed how the additional C-terminal domain interacts with the N-terminal domain. Activity studies demonstrated the extra domain was essential for enzyme activity.

[1] Hrmova, M., De Gori, R., Smith, B.J., Fairweather, J.K., Driguez, H., Varghese, J.N., Fincher, G.B. *The Plant Cell* **2002**, 14, 1033.

Keywords: GH3 enzyme; crystal structure; marine bacteria

FA1-MS03-P08

Coupling of Endonuclease and Translocase Functions in EcoR124I. Tatsiana Baikova[a], Mikalai Lapkouski[a], Igor Shevelev[b] , Pavel Janscak[b] , Ivana Kuta-Smatanova[a], Jannette Carey[c], Marie Weiserova[d], Rudiger Ettrich[a], Eva Csefalvay[a]. *[a]Department of Structure and Function of Proteins, Institute of Systems Biology and Ecology ASCR and Institute of Physical Biology USB, Nove Hrady, Czech Republic. [b]Institute of Molecular Genetics ASCR, Prague, Czech Republic. [c]Princeton University, NJ, USA. [d]Institute of Microbiology ASCR, Prague, Czech Republic.*
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The type I restriction-modification enzymes differ significantly from the type II enzymes commonly used as molecular biology reagents. On hemi-methylated DNAs type I enzymes act as conventional adenine methylases at their specific target sequences, but unmethylated targets induce them to pull thousands of base pairs through the enzyme before cleaving distant sites nonspecifically. Biochemical, biophysical, and molecular biological studies of their translocation and cleavage mechanisms offer a wealth of detail that has lacked a structural framework. The crystal structure of the motor subunit responsible for DNA translocation and cleavage by the type I enzyme EcoR124I, resolved at 2.6 A, shows a lysine residue on the endonuclease domain to contact N3 on the exposed edge of ATP bound at the helicase domains, potentially coupling endonuclease and translocase functions [1]. Site-directed mutagenesis in combination with protein crystallography leads to crystal structures of functionally altered enzymes. This structural information, *in vivo* testing of the mutants, and computational modeling, are our strategy to explain the coupling of endonuclease and translocase functions in *Eco*R124I and draw conclusions valid for type I restriction-modification complexes in general.

[1] Lapkouski M., Panjikar S., Janscak P., Kuta Smatanova I., Carey J., Ettrich R., Csefalvay E. *Nat. Struct. & Mol.Biol*, **2009**, 16, 94.

Keywords: dsDNA translocation; restriction enzymes; ATPase