of 2.97 Å^3/Da and a solvent content of 59%. The crystal structure has been determined by molecular replacement. The current model was refined at 2.6 Å resolution to an *R*-factor of 23.8% (R_{free} = 31.6%).

In crystals, LsAspR adopts a homodimeric form. The subunit consists of two domains: the N-terminal domain (residues 1-104 and 216-234) and the C-terminal domain (residues 105-215). In each domain, a central four-stranded parallel β-sheet is flanked by six α-helices. The spatial arrangement of the strictly conserved residues Cys84 and Cys196 strongly indicates that the active site of LsAspR must be located in the cleft between the two domains.

Keywords: racemase, aspartate racemase, crystal structure

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Insights into the fumarase reaction mechanism from crystal structures of Rv1098c

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Fumarase (fumarate hydratase: EC 4.2.1.2) catalyzes the reversible conversion of L-malate to fumarate and H_2O during the TCA cycle. In Mycobacteria tuberculosis, this reaction is catalyzed by the essential enzyme Rv1098c encoded by the fum gene [1, 2].

Rv1098c belongs to a superfamily of enzymes that catalyze reactions involving fumarate molecules. This superfamily not only includes fumarases but also L-aspartases, δ-crystalline, 3-carboxy-cis,cis-muconate lactonizing enzymes, arginino-succinatases as members. Despite a low sequence identity (~25%), the crystal structures of several members of this superfamily revealed a conserved overall fold.

We determine the crystal structures of Rv1098c in its apo form as well as its complexes with the competitive inhibitor meso-tartrate and the substrate/product malate. These structures reveal that ligand binding promotes conformational changes at the enzyme C-terminal domain that close the active site. Inspection of the active site clefts allowed us to propose a catalytic mechanism through a detailed description of the interactions between substrate and catalytic residues.

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Keywords: mycobacteria, TCA cycle, fumarase

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Cap architecture conservation in monoglyceride lipases from bacteria to humans

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Monoglyceride lipases (MGL) catalyze the hydrolysis of monoglycerides into free fatty acid and glycerol. MGL have been identified in all kingdoms of life and have adopted different substrate specificities with respect to chain lengths, depending on their different roles in the host organisms. In humans, MGL play an integral part in lipid metabolism affecting energy homeostasis and signaling processes. It also hydrolyzes the endocannabinoid 2-arachidonoylglycerol, which regulates a range of physiological processes (e.g. pain sensation). In bacteria, especially short chain monoglycerides are highly toxic. Thus, MGL is crucial for the survival of the bacteria. Despite the biological, pharmacological and biotechnological interest in MGL, the 3D structure of human MGL was the first member of this enzyme family to be solved in 2010. We report the first crystal structures of MGL from a bacterial species (Bacillus sp. H257) in its free form at 1.2Å and covalently complexed to phenylmethanesulfonyl fluoride at 1.8Å resolution, molecular dynamics simulation of bacterial MGL (bMGL, 250 aa) and a comprehensive comparison of the resulting structural insights with the human homolog (303 aa): The core structure is composed of an α/β hydrolase fold. A cap region, which is often observed in α/β hydrolase, is inserted between α4 and β5 elements of the core structure. An ~22Å long tunnel leads from the surface of bMGL to the catalytic triad and thus keeps the active center unexposed to the polar environment. The rim at the entrance to the tunnel has a diameter that is ~12Å wide and is lined with hydrophobic side chains mostly from the cap domain. A hole is located in the cap region of the protein and corresponds to the proposed exit hole for glycerol to leave the active site in human MGL. Molecular dynamics simulation shows open and closed states of the entrance tunnel and the glycerol exit hole.

No electron density was observed for a stretch of 3 aa in the cap region. In human MGL, a corresponding region was also found to be highly flexible and was not visible in one chain of the dimeric structure. Comparison of the cap regions of hMGL and bMGL shows intriguing similarities, despite a sequence identity of only 10%. The 62-amino acid cap of hMGL has been described as U-shaped and comprises of three helices connected by loop regions. The 45-amino acid cap of bMGL harbors a short helical turn and a short two-stranded antiparallel β-sheet connected by linkers. It is very interesting that despite these differences in length and secondary structure composition, the overall shape and arrangement of the cap region of both the proteins superimposes almost precisely in the observed open conformation. It can be speculated that this specific lid architecture is conserved throughout evolution of MGLs.

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Keywords: lipase, crystallography, hydrolase

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A hybrid multifunctional antioxidant system: peroxiredoxin nitroreductase enzyme

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A natural fusion occurring between a peroxiredoxin (Prx) and a nitroreductase (Ntr) motif has been identified in *Thermotoga maritima*. The corresponding protein TmPrxNtr, the mutant TmPrxNtrC40S and its separate modules (TmPrx, TmNtr) have been overexpressed in *Escherichia coli,* then purified and their catalytic activity studied. TmPrxNtr and TmPrxNtrC40S display similar circular dichroism spectra showing that the mutation of cysteine 40 into serine does not

significantly modify the structural properties and organization of the protein. The crystal structure of TmPrxNtrC40S has been determined for the oxidized form and for the reduced form in presence of hydrogen sulfite, as well as two crystalline forms of the TmPrx module.

The full-length protein TmPrxNtr, which contains a FMN prosthetic group, displays nitroreductase, peroxidase and quinone, flavine, chromate and iron reductase activities using either NADH or NADPH as a donor. The mutation of the catalytic cysteine of the Prx module (TmPrxNtrC40S) does not alter the catalytic efficiency of the protein suggesting that the activity measured does not require the Prx module. Both modules TmPrx and TmNtr have peroxidase activity but the oxidized enzyme is not regenerated in the same way. NAD(P)H is the direct reductant for TmNtr while TmPrx is regenerated *in vitro* via glutaredoxins or thioredoxins. In addition TmPrx is able to reduce H_2O_2 and COOH whereas TMPrxNtr only reduces H_2O_2.

The TmPrxNtrC40S enzyme crystallizes as a dimer, the active form of the protein. The Ntr module of one monomer is in contact with the Ntr and Prx modules of the other one but no contact is observed between the two Prx modules. On the contrary, in the crystalline forms of the isolated domain, two TmPrx molecules are linked by an intermolecular disulfide bond. In TmPrxNtrC40S, the FMN prosthetic groups bind in deep pockets at the dimer interface and interact with elements of both monomers. An ion sulphate is positioned by hydrogen bonding to the catalytic site of the Prx modules. In the reduced TmPrxNtrC40S crystal, one sulphate is replaced by a bisulfite introducing structural modifications to the associated Prx module.

Altogether, the results indicate that the TmPrx and TmNtr modules apparently function independently, each possessing its own peroxidase activity, with the TmNtr module having diaphorase activity linked to the FMN moiety versus a broad range of substrates. This raises the question of their fusion which is specifically found in thermotogales.

Keywords: hybrid-flavoenzyme, reductase, X-ray

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Hpy188I-DNA structures - snapshots of the GIY-YIG nuclease mediated catalysis

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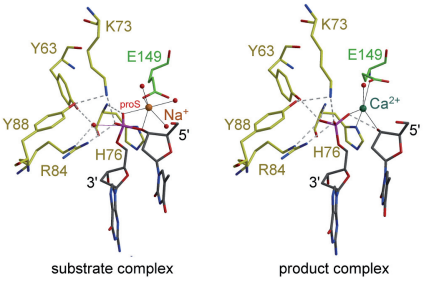
GIY-YIG nucleases are present in all kingdoms of life and have very diverse roles. Well characterized functions include transposon migration, flap cutting and Holliday junction resolution in eukaryotes and nucleotide excision repair in prokaryotes. Despite many roles of GIY-YIG nucleases, until very recently no GIY-YIG domain has ever been crystallized in complex with target DNA. Therefore, many questions about the catalytic mechanism of the GIY-YIG module were unresolved.

We have obtained Hpy188I crystal structures in ternary substrate and product complexes with DNA and metal ion in the active site [1]. Our structures suggest that GIY-YIG nucleases catalyze DNA hydrolysis by a single substitution reaction. They are consistent with a previous proposal that a tyrosine residue (which we expect to occur in the phenolate form) acts as a general base for the attacking water molecule. In contrast to the earlier hypotheses, our data identify the general base with the GIY and not the YIG tyrosine. A conserved glutamate residue (Glu149 provided in trans in Hpy188I) anchors a single metal cation in the active site. This metal ion contacts the phosphate proS oxygen atom and the leaving group 3'-oxygen atom, presumably to facilitate its departure. Taken together, our data reveal striking analogy in the absence of homology between GIY-YIG and ββα-Me nucleases. Our mechanistic conclusions agree with those for Eco29kI restriction endonuclease [2].



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Keywords: GIY-YIG nuclease, mechanism, restriction enzyme

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Structural variability of type II restriction endonucleases

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Type II restriction endonucleases are very diverse in sequence and have therefore long been considered as phylogenetically unrelated. This picture changed when crystal structures revealed that the frequently used enzymes all belong to the PD-(D/E)XK class of nucleases, which use a two metal ion mechanism to catalyze DNA hydrolysis. We have systematically studied restriction endonucleases that cleave DNA with unusual staggers. This property turns out to "select" for structurally novel enzymes. Almost simultaneously with Prof. Barry Stoddard, we have solved the first structures of ββα-Me and GIY-YIG restriction endonucleases, in our case with Hpy99I and Hpy188I as the model enzymes [1], [2], [3]. The structures reveal interesting mechanistic analogies between the new groups of restriction endonucleases and "classic" PD-(D/E)XK enzymes. Surprisingly, the metal ion B, which promotes the departure of the 3'-OH leaving group, and is "optional" in at least some of the PD-(D/E)XK nucleases is conserved in both ββα-Me and GIY-YIG nucleases. In contrast, the metal ion A, which is obligatory in PD-(D/E)XK enzymes and activates the nucleophilic water molecule is absent from both new nuclease families. The attacking water molecule is instead activated by a histidine or a tyrosine residue (we presume the latter to be present in the phenolate form).

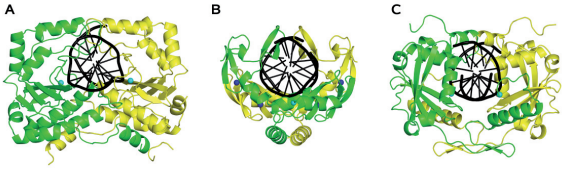

Fig. 1: PD-(D/E)XK endonuclease ThaI (**A**), ββα-Me endonuclease Hpy99I (without N-terminal domain) (**B**) and GIY-YIG nuclease Hpy188I (**C**). All three enzymes are functional dimers and recognize palindromic (symmetric) or pseudopalindromic (approximately symmetric) target sites.