methylerythritol 4-phosphate (MEP) pathways. However, some steps of these pathways are catalysed by completely different enzymes in different strains. For example, the NADPH-dependent production of MEP from deoxyxylulose 5-phosphate (DXP) in the first committed step of the MEP pathway is catalysed by DXP reductoisomerase (DXR) in most bacteria, whereas an unrelated DXR-like (DRL) protein was recently found to catalyze the same reaction in some organisms. Because DRL is found instead of DXR in animal and human pathogens such as *Brucella* and *Bartonella*, DRL might be a new target for highly specific antibiotics against these DRL-harboring bacteria that would not affect beneficial bacteria that use DXR (like those present in the gut). Despite their catalytic similarity, DRL and DXR only show some sequence identity at the level of the NADPH-binding domain.

We have determined two X-ray crystal structures of the cattle pathogen *Brucella abortus* DRL (*Ba*DRL) enzyme: the apo state and a complex with the broad spectrum antibiotic fosmidomycin (FSM); solved to 1.5 and 1.8 Å resolution, respectively. Initial phases to solve the *Ba*DRL structure were obtained by the single-wavelength anomalous dispersion method of an osmium-derivative crystal belonging to the primitive orthorhombic space group $P2_12_12$. To improve the electron density a crystal averaging protocol was followed, combining the initial phases with data from a selenomethionine-labelled DRL crystal belonging to space group C2 and native data from a primitive triclinic (P1) crystal.

DRLs are dimers, with each polypeptide folding into three distinct domains starting with the NADPH-binding domain in resemblance to the structure of bacterial DXRs. Other than that, DRLs and DXRs show a very low structural relationship with a different disposition of the domains and topologically unrelated C-terminal domains that in DRL presents an unsuspected structural similarity with antifreeze proteins. The DRL active site, containing a catalytic magnesium ion, shows a unique arrangement, suggesting that the design of drugs that would selectively inhibit the DRL or the DXR enzymes should be feasible. DRL catalytic residues were assigned and found to be strictly conserved among the active DRL sequences and, most interestingly, structurally non-equivalent to the catalytic residues in DXRs. Single-residue mutations of some of these amino acids confirmed their importance in catalysis. The structure of DRL in complex with FSM explains in particular why, despite the structural differences, the inhibition mechanism remains essentially identical in the DRL and DXR family of enzymes.

Keywords: terpenoid, inhibition

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Structural study of enzymatic anti-baldwin ring closure in polyether formation

Kinya Hotta,[a] Xi Chen,[a] Robert S. Paton,[b] Atsushi Minami,[c] Hao Li,[a] Kunchithapadam Swaminathan,[a] Irimpan Mathews,[d] Kenji Watanabe,[e] Hideaki Oikawa,[c] Kendall N. Houk,[b] Chu-Young Kim[a] *[a]Department of Biological Sciences, National University of Singapore, 117543, Singapore. [b]Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA, 90095-1569, (USA). [c]Division of Chemistry, Hokkaido University, Hokkaido, 060-0810, (Japan). [d]Stanford Synchrotron Radiation Laboratory, SLAC National Accelerator Laboratory, Menlo Park, CA, 95124, (USA). [e]Division of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, 422-8526, (Japan).* E-mail: dbshk@nus.edu.sg.

Despite recent advances in the understanding of polyether natural product biosynthesis, the enzymological mechanism of how these stereochemically complex compounds are generated remains elusive. In particular, many polyethers carry cyclic ether structures that are energetically disfavored to form, making them a chemically intriguing target of study. For example, it has been postulated [1] that the formation of a ladder polyether natural product brevetoxin B requires a series of 10 energetically disfavored, anti-Baldwin-type epoxide-opening ring closures to take place sequentially under a mild, aqueous condition. Recently, Jamison *et al*. demonstrated [2] that templated epoxide substrates in the presence of hydrogen-bonding water molecules undergo an epoxide ring opening cascade that leads to the formation of desired *trans-syn-trans* cyclic ether ladders with high stereoselectivity [3]. However, the question on the genesis of the initial templating cyclic ether units still remains unanswered.

Here, we report the crystal structure of Lsd19, an epoxide hydrolase responsible for the biosynthesis of ionophore polyether lasalocid A, in complex with substrate and product analogs. This is the first atomic structure of a natural enzyme capable of catalyzing the disfavored epoxide-opening cyclic ether formation. The unique catalytic ability of Lsd19 was analyzed through a combination of sequence analysis, crystal structure determination, and computational study. Our structural and computational studies indicate that an intricate pre-organization of the binding pocket which favorably stabilizes the transition state structure for the disfavored cyclization allows Lsd19 to violate Baldwin's rule for ring closure without the use of additional cofactors or modifications to the epoxidated substrate to steer the course of reaction pathway. Furthermore, amino acid sequence alignment and homology-based structure modeling of different ionophore polyether epoxide hydrolases has provided insight into the general mode of cyclic ether formation in polyether biosynthesis. Our study has significantly advanced the efforts toward understanding the chemical principle behind nature's solution for synthesizing highly complex polyether natural products, and provided a fundamental understanding of how an enzyme can overturn the natural course of a chemical transformation to deliver a reaction product that is otherwise difficult to obtain.

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A pentavalent G-actin:RPEL assembly required for regulation of MRTF-A subcellular localisation

Stéphane Mouilleron,[a] Carola A. Langer,[b] Sebastian Guettler,[b] Neil Q. McDonald,[a] Richard Treisman,[b] *[a]Structural Biology and [b]Transcription groups, Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London (UK). E-mail:stephane.mouilleron@cancer.org.uk*

Serum response factor transcriptional activity is controlled through interactions with regulatory cofactors such as the coactivator MRTF-A (Myocardin-Related Transcription Factor A). MRTF-A transcriptional activity and subcellular localization are regulated in vivo by changes in cellular actin dynamics, which alter its interaction with G-actin. The G-actin-sensing mechanism of MRTF-A resides in its N-terminal domain, which consists of three tandem RPEL repeats. We previously described the first molecular insights into RPEL function obtained from structures of two independent $RPEL^{MRTF\text{-}A}$ peptide:G-actin complexes [1]. Regulation of MRTF-A requires all three actin-binding RPEL motifs in the MRTF-A regulatory domain, but multimeric G-actin•MRTF-A complexes have not been characterized. We describe here a pentavalent and trivalent G-actin•RPEL domain assemblies. In the pentavalent complex actins bind each RPEL motif, and the spacer sequences between them; in contrast, the trivalent complex lacks the C-terminal