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Crystal structure of a dimeric fungal α -type carbonic anhydrase

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Carbonic Anhydrase (CA) catalyses the reversible conversion of CO₂ into bicarbonate ions. It is one of the fastest and better studied enzymes, found in all domains of life. Besides its biological and pharmaceutical importance, CA has gained attention from an industrial perspective.

CAs are classified in five classes (α , β , γ , δ and ϵ) which are unrelated in sequence and structure but have all converged to a metal dependent mechanism, most usually using Zn. The α -class is the only one found in mammals and the best studied mechanistically. In fungi, the β -class predominates, but some examples of the α -class are also found.

Here we present the 2.7 Å crystal structure of the α -Carbonic Anhydrase from *Aspergillus oryzae* (AoaCA), the first protein structure of a fungal α -CA. The structure superficially resembles those of most mammalian α -CAs, but has unexpected features of biological and potentially industrial importance.

AoaCA is a dimer in the crystal and was later found to be a dimer also in solution [1], an unusual feature in the α -class only found in two membrane anchored and cancer related mammalian CAs (human CAs IX and XII) [2], [3], but the dimerization interface is different and more extensive than in those.

Most CAs feature what is called the proton shuttle (His64 in the best studied human CAII), which accelerates the rate limiting step, removal of a proton from a zinc bound water to generate the nucleophile hydroxyl that will react with CO₂. In AoaCA this proton shuttle is missing, substituted by a phenylalanine which is structurally involved in the dimer interface and physically locked away from the active site channel. As a result CA activity is diminished raising interesting questions about the preferred biological substrate of this and other fungal α -CAs.

CAs have already shown benefits in applications for CO₂ capture and biofuel production. AoaCA is a very stable and very soluble protein that can be overexpressed and secreted. This makes AoaCA a promising candidate for industrial applications compared to its mammalian counterparts.

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Structural analysis of UDP-N-acetylgalactopyranose mutase from *campylobacter jejuni*

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UDP-galactopyranose mutases (UGM) are enzymes found in bacteria, parasites and fungi, and are not present in higher eukaryotes. UGMs are flavoproteins, which require the co-factor flavin adenine dinucleotide (FAD) to catalyze the conversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf). Expression of UGM, encoded for by the *glf* gene, is observed in organisms that incorporate Galf into the cell wall or capsular polysaccharide. UGM is essential for viability in many pathogenic organisms due to the broad distribution of Galf in crucial structures, making the biosynthetic pathway of Galf a desirable therapeutic target. Several UGMs have been crystallized and structurally characterized in *Escherichia coli*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis* [1], [2]. Prokaryotic UGMs catalyze the reversible ring contraction of UDP-Galp to UDP-Galf via a conserved mechanism, although many of the residues involved substrate binding remain to be elucidated.

A UGM homolog was recently identified in *Campylobacter jejuni* 11168 which is encoded for by the *cj1439* gene. The bacterial pathogen *C. jejuni* is the leading cause of inflammatory enterocolitis worldwide [3] as well as a causative antecedent in the development of Guillian-Barre syndrome, an autoimmune neurological disorder [4]. *C. jejuni* does not incorporate Galf into glycoconjugates, instead 2-acetamido-2-deoxy-D-galactofuranose (GalfNAc) is incorporated into the capsular polysaccharide. This homolog, UDP-N-acetylgalactopyranose mutase (UNGM), has been found to have a relaxed specificity in comparison to other known UGMs [5]. Kinetic analyses have demonstrated a bifunctional enzymatic activity for UNGM, allowing for the recognition of both UDP-Gal and UDP-GalNAc *in vitro*. UNGM is the first example of an enzyme able to catalyze the interconversion of UDP-GalpNAc and UDP-GalfNAc.

Structural analysis of UNGM was initiated by crystallization trials, which yielded crystals suitable for diffraction using synchrotron radiation. UNGM crystals were diffracted to 2.1 Å at the Canadian Light Source. The crystal belonged to the monoclinic space group P2₁ with unit cell parameters a = 73.97, b = 48.01, c = 120.77, β = 96.464. A solution was found by molecular replacement using the structure of UGM from *E. coli* (PDB entry 18it), with which UNGM shares 59% identity. Several active site residues and a putative mechanism for catalysis have been identified.

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***Brucella abortus* DRL structure: new antibiotics target against animal pathogens**

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The synthesis of essential terpenoid natural products depends on two unrelated biosynthetic routes: the mevalonate (MVA) and the

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 (*BaDRL*) 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 *BaDRL* structure were obtained by the single-wavelength anomalous dispersion method of an osmium-derivative crystal belonging to the primitive orthorhombic space group P2₁2₁2. 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

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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

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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-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