MS01.P01

Acta Cryst. (2011) A**67,** C215

Crystal structure of a dimeric fungal α-type carbonic anhydrase

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Carbonic Anhydrase (CA) catalyses the reversible conversion of CO_2 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 2.7Å crystal structure of the α-Carbonic Anhydrase from *Aspergillus oryzae* (*Ao*αCA), 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.

*Ao*αCA 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 *Ao*αCA 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 CO_2 capture and biofuel production. *Ao*αCA is a very stable and very soluble protein that can be overexpressed and secreted. This makes *Ao*αCA a promising candidate for industrial applications compared to its mammalian counterparts.

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Keywords: fungal alpha carbonic anhydrase, dimeric, proton Shuttle.

MS01.P02

Acta Cryst. (2011) A**67,** C215

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-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*). Expression of UGM, encoded for by the *glf* gene, is observed in organisms that incorporate Gal*f* into the cell wall or capsular polysaccharide. UGM is essential for viability in many pathogenic organisms due to the broad distribution of Gal*f* in crucial structures, making the biosynthetic pathway of Gal*f* a desirable therapeutic target. Several UGMs have been crystallized and structurally characterized in *Escherichia coli, Klebsiella pneumoiae*, and *Mycobacterium tuberculosis*[1], [2]. Prokaryotic UGMs catalyze the reversible ring contraction of UDP-Gal*p* to UDP-Gal*f* 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 Gal*f* into glycoconjugates, instead 2-acetamido-2-deoxy-D-galactofuranose (Gal*f*NAc) 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-Gal*p*NAc and UDP-Gal*f*NAc.

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 $P2_1$ 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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Keywords: carbohydrate, flavoprotein, structure

MS01.P03

Acta Cryst. (2011) A**67,** C215-C216

***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