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 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 CAI), 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 CO2 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.


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

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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 Galp into the cell wall or capsular polysaccharide. UMG is essential for viability in many pathogenic organisms due to the broad distribution of Galp in crucial structures, making the biosynthetic pathway of Galp a desirable therapeutic target. Several UGMs have been crystalized and structurally characterized in Escherichia coli, Klebsiella pneumoniae, and Mycobacterium tuberculosis[1],2 Prokaryotic UMGs catalyze the reversible ring contraction of UDP-Galp to UDP-Galp 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 cja1439 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 Guillain-Barre syndrome, an autoimmune neurological disorder [4]. C. jejuni does not incorporate Galp into glycoconjugates, instead 2-acetamido-2-deoxy-D-galactofuranose (GalpNAc) 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-Galp and UDP-GalpNAc in vitro. UNGM is the first example of an enzyme able to catalyze the interconversion of UDP-GalpNAc and UDP-GalpNAc.

Structural analysis of UNGM was initiated by crystallization trials, which yielded crystals suitable for diffraction using synchrotron radiation. UNGM crystals were diffracted to 2.1Å with unit cell parameters a = 73.97, b = 48.01, c = 120.77, β = 96.46°. A solution was found by molecular replacement using the structure of UGM from E. coli (PDB entry 1801), with which UNGM shares 59% identity. Several active site residues and a putative mechanism for catalysis have been identified.


Keywords: carbohydrate, flavoprotein, structure.