MS03 P01

Three Structural Snapshots Of The FPPS Catalytic Cycle Revealed By X-Ray Analyses

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Farnesyl diphosphate synthase (FPPS) is now well established as the molecular target of nitrogen-containing bisphosphonate (N-BP) drugs [1-3]. We have determined the X-ray structures of human FPPS in its unliganded state, in complex with the N-BP drug zoledronic acid, and in the ternary complex with zoledronic acid and the substrate isopentenyl diphosphate (IPP). By revealing three structural snapshots of the enzyme catalytic cycle, each associated with a distinct conformational state, these structures provide a novel understanding of the mechanism of FPPS catalysis and inhibition. In particular, the accumulating substrate, IPP, was found to bind to and stabilize the FPPS/N-BP complex, rather than competing with and displacing the N-BP inhibitor. Among other factors such as efficient bone targeting, this particular mode of FPPS inhibition contributes to the exceptional in vivo efficacy of N-BP drugs.

[1] van Beek E., Pieterman E., Cohen L., Löwik C., Papapoulos S., *Biochem. Biophys. Res. Commun.* 1999, 264, 108.
[2] Keller R.K., Fliesler S.J., *Biochem. Biophys. Res. Commun.* 1999, 266, 560.
[3] Bergstrom J.D., Bostedor R.G., Masarachia P.J., Reszka A.A., Rodan G., *Arch. Biochem. Biophys.,* 2000, 373, 231.

MS03 P02

Crystal Structure of α-Carbonic Anhydrase from *Chlamydomonas reinhardtii*

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Keywords: carbonic anhydrase, crystal structure, *Chlamydomonas*

Carbonic anhydrase (CA) plays important roles in biological processes such as photosynthesis, respiration, secretion of HCO_3^-, pH homeostasis and ion exchange. The proteins commonly contain a zinc ion in the active site for catalyzing the hydration of CO_2 and *vice versa*. It is known that there are three classes of CA, designated α-, β- and γ–CAs, depending on the amino acid sequence similarities. The α-class is different from others in the structural architecture. Furthermore, even in the α-class, the enzyme from unicellular green alga, *Chlamydomonas reinhardtii* (*ch*CA) is unique in post-translational modifications that it is glycosylated and spliced into two peptides. Such glycosylations are found in only mammalian CAs but they are not spliced. To reveal the structural details and the role of N-glycosylation, an X-ray analysis of *ch*CA has been performed.

The wild type *ch*CA was isolated and purified, and then crystallized by the hanging drop vapor diffusion method. X-Ray diffraction data were collected at 100K with synchrotron radiation. The initial structure was determined by the MAD method of zinc atoms, and the atomic parameters including water molecules were refined to R=18.3 (R_{free}=21.9) % at 1.88 Å resolution.

*ch*CA is a homodimeric protein, the two subunits being crystallographically independent. In each subunit, residues from Ser298 to Asn345 are spliced to separate into long and short peptides. The two subunits are, however, linked together by a disulfide bond. In the catalytic site, a zinc ion is bound to the three conserved His163, His165 and His182 in a tetrahedral configuration. A water molecule is trapped at the fourth position of the Zn atom.

As seen in the figures, the electron density maps indicate that N-glycosylations occur at the three sites, Asn101, Asn135 and Asn297. A sequence comparison with other organisms shows that the first and the third N-glycosylations are conserved in mammalian CAs. The present structure is the first example of CA attached to N-glycosides. The additional N-glycosylation at the second site of Asn135 is also unique in chCA.

In the crystal, *ch*CA molecules are interacted to each other with a six-fold screw symmetry to form a long column. Furthermore, these columns are fused through the lateral interactions like a beehive. Each catalytic site is exposed to the central tunnel. It suggests that *ch*CA in the crystalline state also catalyze the reaction.

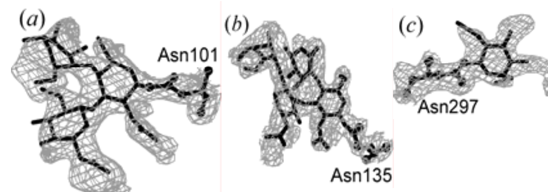

Figure. $2|F_o|-|F_c|$ maps contoured at 1 level for N-glycosylation sites at Asn101(*a*), at Asn135(*b*) and at Asn297(*c*).

MS03 P03

Structural studies of Holliday junction resolvases from yeast and archaea

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Holliday junctions (four-way junctions) are universal intermediates in repair and reorganization of DNA by homologous recombination. They are mobile links between two homologous DNA duplexes and generate new segments of heteroduplex DNA by branch-migration. A crucial step is the final resolution of the junction without loss of nucleotides. Holliday junction-resolving enzymes mediate the termination process by recognizing DNA four-way junctions and introducing symmetrical