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Crystal structures of yeast cytosine deaminase and *Bacillus* guanine deaminase

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Introduction

One major challenge in the post-genomic era is to understand the strategies that nature uses to evolve new catalytic functions. Structure determination and comparison of superfamilies provides an efficient path to understand of the evolution history. Purine/pyrimidine bases and nucleotides serve as nitrogen and carbon sources and also take part in nucleotide synthesis. A deamination step is the first and the commitment step in the degradation and salvage pathways. Therefore, purine/pyrimidine deaminases play key roles in the nucleotide metabolism and have become important possibilities for anticancer and anti-microbial therapy. Interestingly, most of these deaminases belong to the cytidine deaminase (CDA) superfamily including fungal cytosine deaminase (CD), plant guanine deaminases (GD), CDAs, dCMP deaminases, RNA editing deaminases, and riboflavin biosynthesis proteins RibG. Here we have determined crystal structures of yeast CD (yCD) and *Bacillus subtilis* GD (bGD) by the Se-MAD methods.

Results and Discussion

Yeast Cytosine Deaminase

Yeast CD is an attractive candidate for anticancer gene therapy because of its catalysis of the deamination of the prodrug 5-fluorocytosine into 5-fluorouracil. The enzyme displays an unusually high structural homology to AICAR transformylase, and cytidine deaminases, and thereby may define a new superfamily. The unique C-terminal tail is involved in substrate specificity and controlling access to the active site. The complex structure reveals a closed conformation, suggesting that substrate binding seals the active site entrance so that the catalytic groups are sequestered from solvent. The bacterial and fungal cytosine deaminases provides an elegant example of convergent evolution, where starting from unrelated ancestral proteins, the same metal-assisted deamination is achieved through opposite chiral intermediates within distinctly different active sites.

B. subtilis Guanine Deaminase

GD, a key enzyme in the nucleotide metabolism, catalyzes the hydrolytic deamination of guanine into xanthine. The crystal structure of the 156-residue bGD has been solved at 1.17 Å resolution. Unexpectedly, the C-terminal segment is swapped to form an inter-subunit active site and an intertwined dimer with an extensive interface of 3900 $Å^2$ per monomer. The essential zinc ion is ligated by a water molecule together with His^{53}, Cys^{83} and Cys^{86}. A transition-state analog was modeled into the active-site cavity based on the tightly bound imidazole and water molecules, allowing identification of the conserved deamination mechanism and specific substrate recognition by Asp^{114} and $Tyr^{156'}$. The closed conformation also reveals that substrate binding seals the active-site entrance, which is controlled by the C-terminal tail. Therefore, the domain swapping has not only facilitated the dimerization, but has also ensured specific substrate recognition. Finally, a detailed structural comparison of the cytidine deaminase superfamily illustrates the functional versatility of the divergent active sites found in the guanine, cytosine and cytidine deaminases, and suggests putative specific substrate-interacting residues for other members.

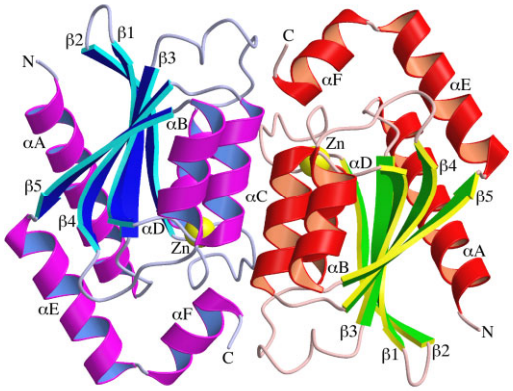

Fig. 1 The dimeric structure of yCD in complex with the inhibitor 2-hydroxypyrimidine at 1.6 Å resolution.

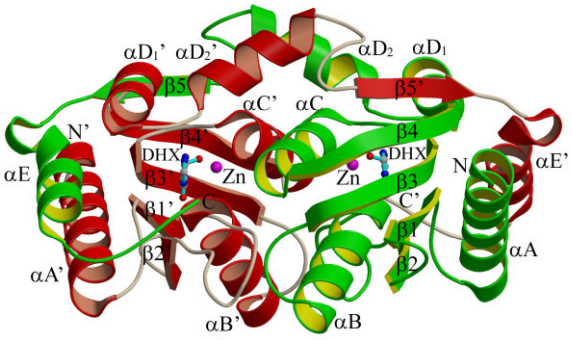

Fig. 2. The dimeric structure of bGD with a domain-swapped C-terminal segment.

References

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