

Crystal Structure of Yeast Cytosine Deaminase

Cytosine deaminase (CD) catalyzes the deamination of cytosine to uracil and that of 5-methylcytosine to thymine (Fig. 1). The antimetabolite 5-fluorouracil (5-FU) is one of the most active chemotherapeutic agents for cancer treatment, but it has limited efficacy due to gastrointestinal and hematological toxicities. Due to its ability to convert nontoxic 5-fluorocytosine (5-FC) into 5-FU and its absence in mammalian cells, the combination of 5-FC with CD in enzyme-prodrug gene therapy has been shown to effectively control tumor growth and is currently being evaluated in clinical trials. Here we have determined the yeast CD structure at 1.6 Å resolution at beamlines **BL12B2** and **BL41XU** [1,2].

The protein structure is composed of a central five-stranded β-sheet (β1-β5) sandwiched by six α-helices (αA-αF) (Fig. 2(a)). Surprisingly, even though yeast CD shares a higher sequence identity to cytidine deaminases (CDAs), its closest structural match is the subdomain 2 of the AICAR transformylase domain, due to the common αD helix and the same direction of the β5 strand (Fig. 2(b)). The strong conservation of tertiary structures suggests that these enzymes are descendants of a single ancestral gene, and thereby define a new superfamily.

Interestingly, the 426-residue hexameric *E. coli* CD belongs to

superfamily, whereas the 158-residue dimeric yeast counterpart is grouped into the CDA superfamily [3]. The active site of yeast CD contains one tightly bound zinc ion, which is tetrahedrally coordinated by His⁶², Cys⁹¹, Cys⁹⁴, and a bound inhibitor (Fig. 3). The complex structure reveals that yeast CD converts the inhibitor 2-hydroxypyrimidine into 4-(R)hydroxyl-3,4-dihydropyrimidine, which is enantiomeric to the configuration observed in *E. coli* CD. Therefore, the crystal structures of bacterial and fungal CDAs provide an excellent example of convergent evolution, in that they have evolved from unrelated ancestral proteins but have achieved the same deamination reaction.

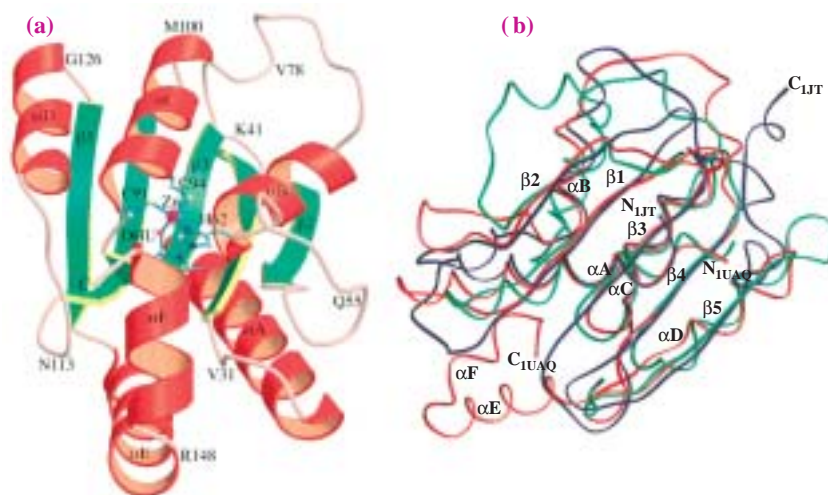


Fig. 2. (a) The monomeric structure of yeast CD with the zinc ion shown as a magenta sphere with its ligands and the inhibitor (DHU) as ball-and-stick representations. (b) Structural superposition of yeast CD (red), *B. subtilis* CDA (blue), and the subdomain 2 of AICAR transformylase (green) [4].

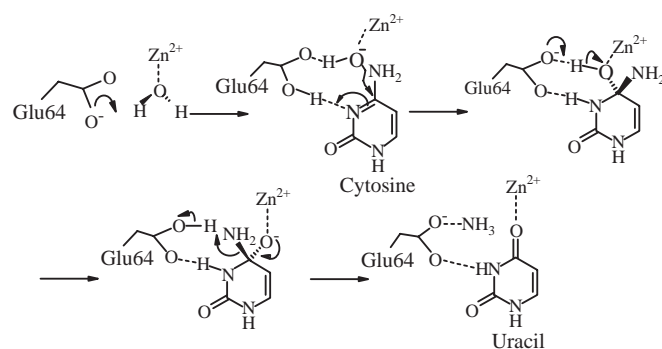


Fig. 1. Proposed catalytic mechanism for yeast CD.

Based on structural studies, we propose a deamination mechanism for yeast CD (Fig. 1). The substrate binds to an active site and induces a closed conformation to sequester the reaction from the solvent. The water molecule activated by the zinc ion attacks the C⁴ atom with the assistance of Glu⁶⁴ serving as a proton shuttle. The product uracil moves toward the zinc ion for ligation and this would weaken its interaction with the C-terminal tail, allowing its release from the active site. The active site architectures of yeast CD and CDAs are strikingly similar. However, they contain unique substrate-recognition residues, in particular, C-terminal tails.

The presence of an α D helix in yeast CD leads strands β 4 and β 5 to be parallel and this results in the C-terminal tail moving back sharply towards the active site to accommodate only the cytosine base within the same molecule (Fig. 4(a)). On the other hand, the lack of an α D helix in *B. subtilis* CDA leads strands β 4 and β 5 to be antiparallel and this results in an opposite direction for the C-terminal tail, which enlarges the active site of the adjacent molecule to accommodate a larger cytidine substrate (Fig. 4(b)).

Previous studies have demonstrated that yeast CD has a greater therapeutic potential than the bacterial enzymes in the enzyme-prodrug strategy. The C⁵ atom of the pyrimidine ring is adjacent to a hydrophobic cluster and faces toward Phe¹¹⁴, with a distance of 4.1 Å. When a fluorine atom is attached to an aromatic ring, it will make it very hydrophobic. Therefore, the hydrophobic cluster in yeast CD should enhance the binding of 5-FC. On the other hand, the C⁵ atom faces toward Asp³¹⁴ in *E. coli* CD, with a distance of 3.6 Å between C⁵ and Asp314 O^{δ1}. Close contact with such a polar residue would be unfavorable to 5-FC binding. These structural observations are consistent with enzyme kinetic measurements.

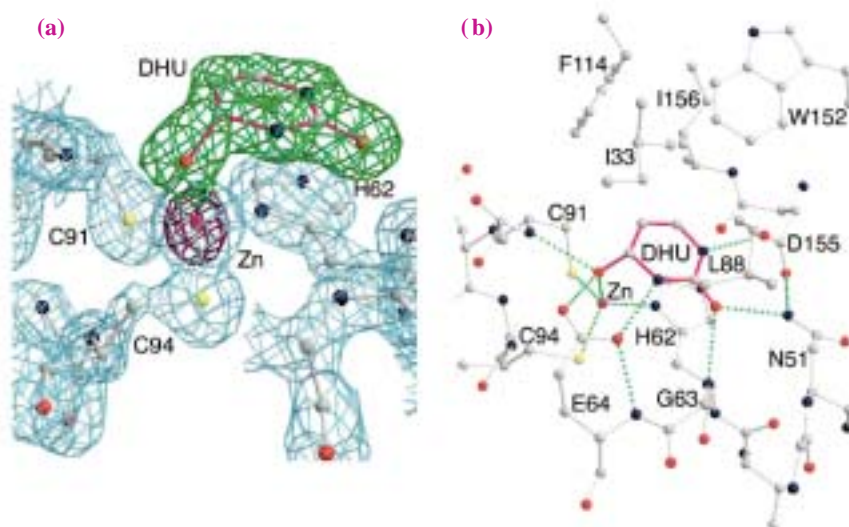


Fig. 3. Active site. (a) The $2F_o - F_c$ map at 1.5σ level in cyan, the difference anomalous map for the zinc ion at 30σ level in purple, and the densities for the inhibitor (DHU) in green. (b) The interaction networks in the active site. There are six direct hydrogen bonds between the protein and the inhibitor [4].

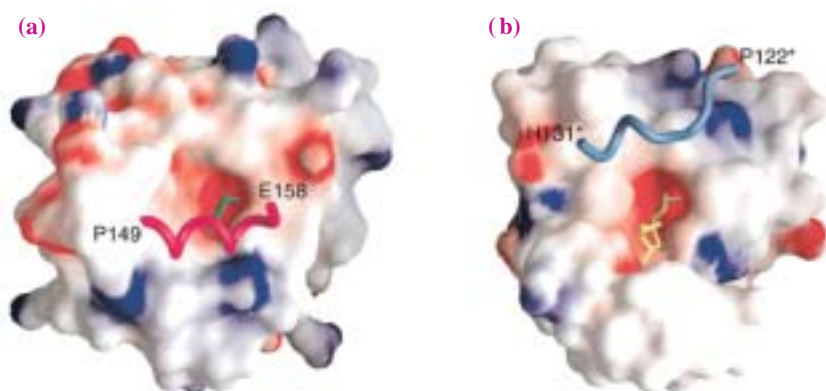


Fig. 4. Molecular surfaces of yeast CD (A) and *B. subtilis* CDA (B) colored for electrostatic potentials from $-10 k_B T$ (red) to $10 k_B T$ (blue). The C-terminal tail (residues 149-158) of yeast CD is shown explicitly as worms as for the C-terminal residues (residues 122*-131*) from one adjacent subunit in *B. subtilis* CDA. The analogues for cytosine and cytidine are colored green and yellow, respectively. The CDAs have space to accommodate the ribosyl sugar of cytidine, whereas yeast CD does not have space due to the blocking by the C-terminal tail [4].

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References

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