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Complex Structures of *Bacillus subtilis* RibG with Substrates

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Introduction

Hundreds of flavoproteins have been reported to catalyze a variety of biological redox reactions, and hence their flavin coenzymes are ubiquitous in all organisms. Therefore, the enzymes involved in riboflavin biosynthesis have the potential to become attractive candidates for the design of new defenses against antibiotic-resistant pathogens. Most eubacteria contain a bifunctional protein involved in the second and third steps in the riboflavin biosynthesis; for instance the *Bacillus subtilis* RibG (BsRibG) is composed of an N-terminal deaminase domain (D domain) and a C-terminal reductase domain (R domain). Recently, we have solved a tetrameric ring-like structure of *Bacillus subtilis* RibG (BsRibG). The D domain belongs to the cytidine deaminase (CDA) superfamily. The CDA superfamily consists of the mononucleotide deaminases involved in nucleotide metabolism, and the RNA (DNA)-editing deaminases involved in gene diversity and in anti-virus defense. The R domain displays significant structural similarity to dihydrofolate reductase (DHFR). Many DHFR inhibitors such as methotrexate, pyrimethamine and trimethoprim, have long been used clinically in the treatment of cancer, rheumatoid arthritis, malaria, and bacterial and fungal infection. Therefore, the R domain may become an important target for new drug design. To gain structural insights into the inhibitor design, substrate specificity and catalytic mechanisms, we have solved the BsRibG structure in complex with its substrate (product) at 2.56-Å resolution.

Results and Discussion

The complex structures of *Bacillus subtilis* RibG at 2.56-Å resolution provide insights into the substrate specificity and catalytic mechanisms. Upon the product binding to the N-terminal deaminase domain, significant conformational changes were observed in two loops moving toward for interaction with the ribosyl and phosphate moieties, respectively. The phosphate forms hydrogen bonds with Asn^{23}, His^{49}, His^{76}, Lys^{79}, and Thr^{80}, while the ribosyl group contacts with Asp^{101} and Asn^{103}. These substrate-binding residues are all highly conserved in the eubacterial RibGs. However, the fungal Rib2 deaminases apparently contain different substrate-interacting residues because of their distinct substrate with a ribityl group instead of a ribose.

Unexpectedly, the electron density map demonstrates a ribitylimino intermediate bound at the C-terminal reductase domain. Both the pyrimidine ring and phosphate form extensive interactions with Lys^{151}, Ser^{167}, Ile^{170} and Thr^{171}, and Arg^{183}, Ser^{202}, Leu^{203}, and Arg^{206}, respectively, while the ribityl group with Asp^{199} and Glu^{290}. Glu^{290} but not previously predicted Asp^{199} assists in proton transfer during catalysis. The strong conservation of the active-site architectures of the reductase domain and the pharmaceutically important dihydrofolate reductase suggests that these two reductases involved in the riboflavin and folate biosyntheses share a similar catalytic mechanism and have a close evolutionary relationship.

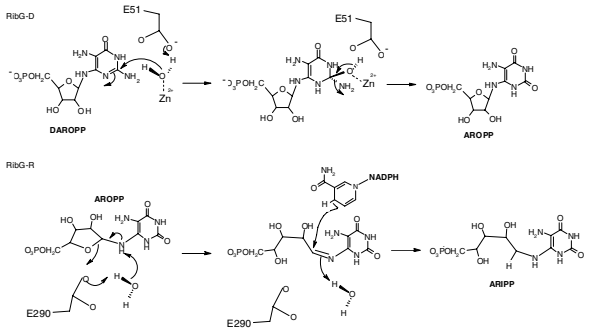

Figure 1. The proposed deamination and reduction mechanisms for RibG.

Figure 2. The monomeric BsRibG structure with the zinc ion shown as a sphere (*magenta*) and the substrates (*cyan* or *black*) as *ball-and-stick* representations.

References

[1] T. Ko et al., *J. Biol. Chem.* **278**, 19111 (2003).
[2] S. Liaw et al., *J. Biol. Chem.* **279,** 35479 (2004).
[3] S. Chen et al., *J. Biol. Chem.* **281**, 7605 (2006).
[4] S. Chen et al., manuscript in preparation.
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