6A, 5A, NW12/2004G342

Crystal Structure of A Bifunctional Deaminase and Reductase from *Bacillus subtilis* Involved in Riboflavin Biosynthesis

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Introduction

Flavin coenzymes are ubiquitous in all organisms because of their involvements in central metabolic pathways. 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). The D domain of bacterial RibG was expected to belong 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. These deaminases catalyze the hydrolytic deamination of cytosine, guanine, adenine moieties and several of their therapeutically useful analogues. Furthermore, the structural fold of the R domain was predicted to be similar to dihydrofolate reductase (DHFR). DHFR catalyzes the NADPH-utilizing reduction of DHF to tetrahydrofolate. 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. To gain structural insights into the inhibitor design, substrate specificity and evolution, we have solved the BsRibG structure at 2.41-Å resolution.

Results and Discussion

Analytical ultracentrifugation experiments clearly demonstrated that BsRibG exists as a tetramer in solution as well as in crystal form, where the enzyme forms a tetrameric ring-like structure. Molecules A and B interact with each other through their D domains, while molecule A makes extensive contacts with molecule C through their R domains. The active site of the D domain contains one tightly bound endogenous zinc ion, of which the anomalous data provided sufficient phase information for structure determination. The active-site architecture resembles those of the CDA members. The C-terminal segment beyond the β4 strand, is quite diverse and may make a major contribution to the structural plasticity and functional diversity among the CDA members. A structure-based sequence alignment of the CDA members was constructed, revealing not only the unique signatures in each family member for gene annotation, but also putative substrate-interacting residues for RNA-editing deaminases such as the loss-of-function point mutants of acid-induced deaminase in patients with hyper-IgM syndrome type 2.

The strong conservation of the tertiary structures of DHFRs and BsRibG suggests that the two reductases involved in the riboflavin and folate biosyntheses are descended from a single ancestral gene and thereby define a new superfamily. They share four conserved regions for NADPH binding, and the binding architecture of the nicotinamide ring is virtually identical, and hence with a similar enzymatic mechanism. There is no evidence for any dependence of the active sites within the RibG tetramer. Previous deletion mutants have demonstrated that the N-terminal 147 residues and the C-terminal 248 residues of BsRibG were sufficient for their respective enzyme activities. However, these truncated proteins could not be isolated due to poor stability. Therefore, even though the deaminase and the reductase are separate functional domains, the domain fusion is crucial for the enzyme activities through formation of a stable tetrameric structure.

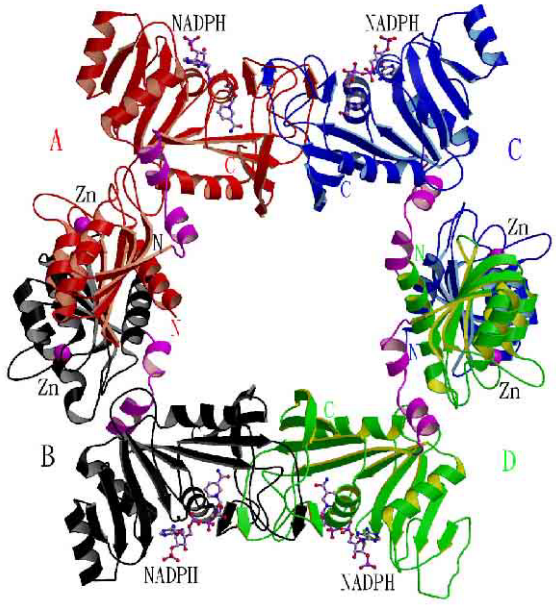

Fig. 1 The tetrameric BsRibG structure with the zinc ion in the D domain, the cofactor NADPH in the R domain, and the inter-domain linker highlighted in *magenta*.

References

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