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Crystal Structure of Chorismate Synthase: A Novel FMN-Binding Protein Fold and Functional Insights

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Introduction

Chorismate synthase catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate to chorismate in the shikimate pathway [1], which represents an attractive target for discovering antimicrobial agents and herbicides [2]. Chorismate serves as a common precursor for the synthesis of aromatic amino acids and many aromatic compounds in microorganisms and plants. Chorismate synthase requires reduced FMN as a cofactor but the catalyzed reaction involves no net redox change. Here we have determined the crystal structure of chorismate synthase from *Helicobacter pylori* in both FMN-bound and FMN-free forms. It is a tetrameric enzyme, with each monomer possessing a novel "β–α–β sandwich fold." Highly conserved regions, including several flexible loops, cluster together around the bound FMN to form the active site. The unique FMN-binding site is formed largely by a single subunit, with a small contribution from a neighboring subunit. The isoalloxazine ring of the bound FMN is significantly non-planar. Our structure illuminates the essential functional roles played by the cofactor.

Results and Discussion

Chorismate synthase monomer has a novel fold

Each monomer of chorismate synthase consists of nine α-helices and 18 β–strands, which fold into a large single domain with several protruding parts (Figure 1). The core of each monomer may be termed a three-layered, "β–α–β sandwich fold". This novel fold is composed of two antiparallel five-stranded β–sheets and four α-helices that are sandwiched between these two β–sheets. The unusual "β–α–β sandwich fold" provides a framework for building the unique cofactor binding site, which also requires dimerization. Four independent monomers in the crystallographic asymmetric unit forms a tight tetramer of 222 point group symmetry.

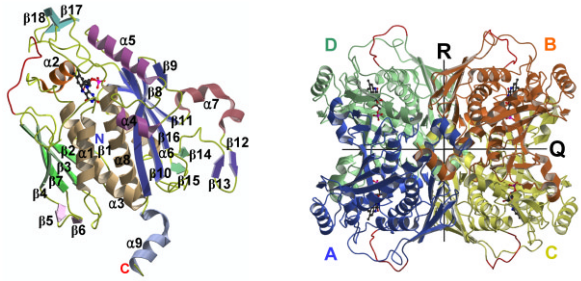

Figure 1: Ribbon diagram of chorismate synthase monomer and tetramer.

Flexibility of active site

The six flexible loop regions F1-F6 are clustered around or near the bound cofactor (Figure 4(B) and (C)). These flexible regions are rich in strictly or highly conserved residues (Figure 4(B) and surround the FMN-binding sites.

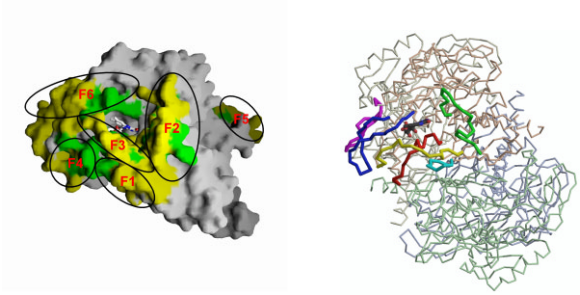

Figure 2: The active site at both monomer and tetramer surrounded by six flexible regions.

Conclusion

We have determined the first crystal structure of chorismate synthase. It reveals several interesting features. First, each monomer is folded into a novel three-layered, "β–α–β sandwich fold" in its core. Second, highly conserved regions that include several flexible loops cluster together to form the active site with a unique FMN binding pocket. Binding of FMN causes little overall structural changes except in three flexible loop regions (F2–F4). Third, most part of the cofactor is bound deeply within the protein, and conserved residues interact with the cofactor.

References

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[2] Kishore, G. M. & Shah, D. M. Annu. Rev. Biochem. 57, 627-663 (1988).

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