Folate Binding Site of Flavin-dependant Thymidylate Synthase and the Mechanistic Implications

Flavin-dependant thymidylate synthases (FDTSs) are a class of recently identified family of thymidylate synthases that employ novel mechanism for the thymidylate synthase reaction (1.2). Thymidylate synthases use N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate (CH_2H_4folate) to reductively methylate 2'-deoxyuridine-5'-monophosphate (dUMP) producing 2'-deoxythymine-5'-monophosphate (dTMP). dTMP is one of the four DNA building blocks and is crucial for survival of all organisms. Unlike other deoxynucleotides, dTMP cannot be directly synthesized by a ribonucleotide reductase, and its *de novo* biosynthesis requires the enzyme thymidylate synthase (3). Therefore, inhibition of thymidylate synthesis stops DNA production, arresting cell cycle and eventually leading to "thymineless" cell death. The human enzyme has long been recognized as a target for anticancer drugs (3).

Since FDTS enzymes are mainly found in very pathogenic microbes including the pathogens causing leprosy, botulism, diarrhea, anthrax, pneumonia, syphilis, etc., the FDTS enzyme is an attractive target for antibiotic drugs (1,2). The FDTS catalysis involving the binding and release of three different molecules and the participation of another molecule deeply buried in the enzyme is challenging and several mechanisms have been proposed for the FDTS reaction (2). The large conformational changes occurring during catalysis adds complexity and underscores the importance of structural information for various intermediate states.

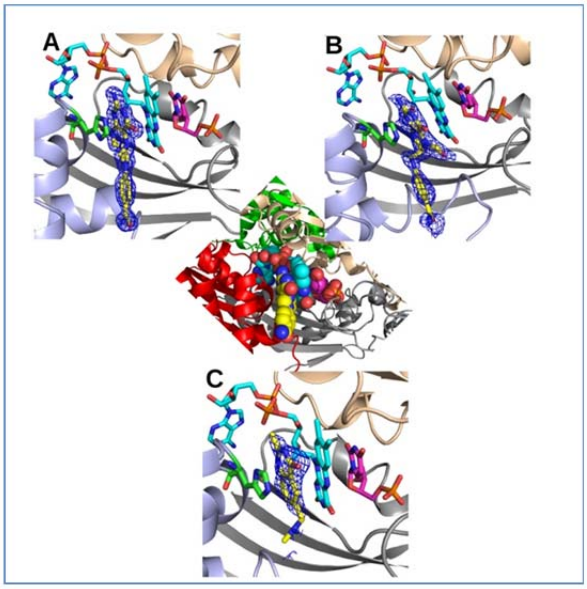

Figure 1. The Active site view of the FDTS enzyme in complex with FAD, dUMP and folate derivatives. A view of the omit map contoured at 3 sigma for CH_2H_4folate (A), Leucovorin (Folinic Acid) (B), and Tomudex (Raltitrexed) (C). Ribbon drawings for the protein chains and stick representation for FAD (cyan), dUMP (magenta), Folate (yellow), and His53 (green).

Even though the first structure of the flavin-dependent thymidyalte synthases was reported a decade ago (4), researchers were unable to isolate and crystallize a complex of the enzyme with the important methyl donor, CH_2H_4folate, and this limits understanding of the molecular mechanism and the design of inhibitors. The studies of the human enzyme utilized the CH_2H_4folate binding site for inhibitor design and several of the cancer drugs are targeted against the CH_2H_4folate binding site (5).

Using SSRL Beam Lines 9-2 and 12-2, we determined the structures of FDTS with CH_2H_4folate and the cancer drugs tomudex and leucovorin (Figure 1). These structures, highest resolution reported for any FDTS enzyme structures, show molecular details of the folate interactions with the enzyme. The folate is bound between the flavin ring of the FAD and a well conserved histidine side chain (Figure 1). The presence of conserved residues near the CH_2H_4folate binding site and their interactions reveal the importance of the CH_2H_4folate binding site and the potential for using this site for the inhibitor design.

The detailed structural and biochemical studies with several mutants explain how the currently determined binding site could also act as a binding site for nicotinamide adeninine nucleotide during the early stages of the FDTS catalysis. Using the structural and biochemical information, we are able to propose a computational model that shows how CH_2H_4folate binds in the active site to perform the intricate FDTS catalysis. The present study sheds light on the cofactor binding and function. The new structural data will likely facilitate further elucidation of FDTS's mechanism and the design of structure-based inhibitors as potential leads to new antimicrobial drugs.

Primary Citation

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