

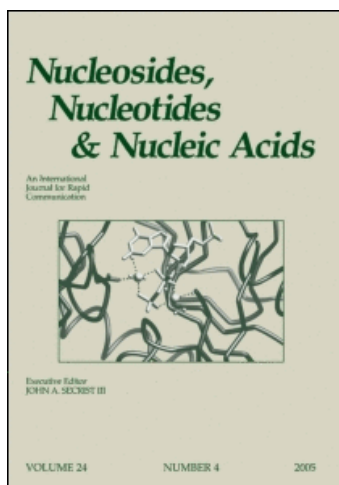
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A New Look at the Molecular Mechanism of Thymidylate Synthase and Its Interaction with Nucleotide Analogues

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A NEW LOOK AT THE MOLECULAR MECHANISM OF THYMIDYLATE SYNTHASE AND ITS INTERACTION WITH NUCLEOTIDE ANALOGUES

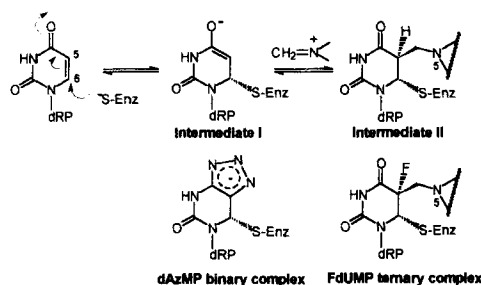
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The increasing availability of the crystal structures of a variety of complexes of thymidylate synthase¹ (TS) and its mutants with nucleotide and folate analogues, has greatly advanced our understanding of the mechanism of catalysis, and the detailed interaction of the enzyme with inhibitors.

TS catalyzes the formation of thymidylate (dTMP) from deoxyuridylylate (dUMP) in a complex process involving two distinct reactions: 1. one carbon transfer from 5,10-CH₂H₄folate to dUMP, and 2. reduction of the one carbon unit to CH₃ by H₄folate produced in the first reaction. At the onset, the enzyme activates both the substrate and the cofactor to form the respective nucleophilic and electrophilic reactants. Activation of the substrate involves nucleophilic addition of the ionized SH-group² of **Cys146** across the 5,6-double bond of dUMP, to form an enolate of the pyrimidine ring covalently linked to the enzyme *via* a thioether bond (intermediate **I**). Ionization of the SH-group is facilitated by electron donation by N¹ of the imidazole ring of **His207**, *via* a charge relay, using the OH-group of **Ser167** as a proton shuttle. Activation of the cofactor involves general acid catalyzed opening of the CH₂-bridge of 5,10-CH₂H₄folate by **Glu58**, with the possible assistance of a water molecule, to form a quaternary aldimine, with the reactive CH₂-iminium ion at N-5. The reaction of the activated cofactor with the enzyme-linked substrate yields a covalent ternary complex (intermediate **II**).

The transition state analogue 1-(β -D-2'-deoxyribofuranosyl)8-azapurin-2-one 5'-monophosphate³ (dAzUMP) inactivates TS by covalent interaction with Cys146, forming an analogue of intermediate **I**. The 5'-monophosphate of the anticancer agent 5-fluoro-2'-deoxyuridine (FdUMP), inactivates TS by a suicide substrate mechanism, forming an analogue of the second covalent intermediate (**II**). A comparison of the crystal structures of the ternary complex of TS, FdUMP and 5,10-CH₂H₄folate, with that of the complex of the dAzUMP-inactivated *E. coli* TS,⁴ revealed information about the possible roles of the active site residues in the catalytic mechanism.



Keto-enol tautomerization of intermediate **II** sets the stage for β -elimination of H₄folate. The tautomerization is likely catalyzed by **Tyr94**, as a general acid assisted by a water molecule. The acidity of **Tyr94** is modulated by the adjacent **His147**, possibly by cation- π bond formation. The β -elimination of H₄folate is initiated by abstraction of the proton from the enol intermediate (**III**) by Glu58, assisted by a water molecule, serving as a proton shuttle. The leaving group ability of H₄folate is enhanced by general acid **Asp169**, which may promote enolization of NH-3/CO-4 of the cofactor and intramolecular proton transfer from OH-4 to N-5. The exocyclic CH₂-group of the resulting quinoid intermediate (**IV**) is reduced by hydride transfer from CH-6 of H₄folate, facilitated by the negative charge developed by **Asp169** in the previous step. Elimination of Cys146 by reversal of the first step concludes the turnover of the enzyme.

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