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INVESTIGATION OF THE TERNARY COMPLEX FORMED BETWEEN RECOMBINANT RAT HEPATOMA THYMIDYLATE SYNTHASE, FdUMP OR S⁴FdUMP AND N⁵, N¹⁰-METHYLENETETRAHYDROFOLATE WITH THE USE OF ¹H AND ¹⁹F NMR

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ABSTRACT: Interactions of mammalian (rat hepatoma) thymidylate synthase with FdUMP and its 4-thio analogue were studied in solution with the use of ¹H and ¹⁹F NMR. The results pointed to formation of ternary covalent complexes of TS with either nucleotide in the presence of N⁵, N¹⁰-methylenetetrahydrofolate and only noncovalent binary complexes in the absence of the cofactor.

Thymidylate synthase (TS; EC 2.1.1.45), a target in chemotherapy, catalyzes the reductive methylation of dUMP at C(5), involving N⁵, N¹⁰-methylenetetrahydrofolate (CH₂H₄PteGlu) and resulting in dTMP synthesis. Recently rat hepatoma TS has been successfully expressed in *E. coli*, resulting in bacteria producing TS amounting to 10-20 % of total cellular protein¹ and enabling physicochemical studies on the enzyme of mammalian origin. Lately, in search of potent TS inhibitors, 4-thio analogue (S⁴FdUMP) of 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) was tested and showed the C(4)-substituent to influence enzyme inactivation specificity². In order to learn more about the mechanism of this influence, native binary complexes TS-FdUMP and TS-S⁴FdUMP, and ternary complexes TS-FdUMP- CH₂H₄PteGlu and TS-S⁴FdUMP- CH₂H₄PteGlu were studied with the use of ¹H and ¹⁹F NMR.

Recombinant TS was purified as previously described¹ and studied at concentration of 100 μ M in 50 mM borate buffer pH 7.4 (in 10 % D₂O), containing 2 mM dithiothreitol-d₁₀ and 1 mM EDTA. In ¹H and ¹⁹F NMR spectroscopy (Varian UNITYplus 500 MHz) experiments FdUMP or S⁴FdUMP were added to obtain different analogue to enzyme molar ratios, ranging from 1 to 10. Ternary complex formation was studied at 1 mM CH₂H₄PteGlu and analogue to enzyme molar ratio of 10. TS with either FdUMP or S⁴FdUMP showed in ¹⁹F NMR one signal of analogue's C(5)-fluorine in a dynamic equilibrium between the free and noncovalently bound states, indicating presence of only noncovalently bound states. Addition of CH₂H₄PteGlu resulted always in a new broad signal, ca. 14 ppm upfield, which could be ascribed to FdUMP or S⁴FdUMP covalently bound to TS. With FdUMP the half-width of the ¹H NMR proton signals increased ca. 2-fold upon adding of the enzyme, apparently due to binding of the nucleotide by the protein (not studied with S⁴FdUMP). Thus rat TS appears to form only noncovalent binary complex with either analogue studied. In contrast, ¹⁹F NMR studies of the enzyme from *Lactobacillus casei* showed also formation of a covalent binary complex with FdUMP^{3,4}.

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