THYMIDYLATE SYNTHASE IN COMPLEX WITH N(4)-HYDROXY-2'-DEOXYCYTIDINE 5'-MONOPHOSPHATE: CRYSTAL STRUCTURE AND MOLECULAR MODELING

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Thymidylate synthase (TS) catalyzes the reductive methylation of dUMP, the reaction being the sole intracellular *de novo* source of thymidylate required for DNA synthesis. TS is an important target in chemotherapy, as its inhibition blocks DNA synthesis and prevents cellular proliferation. An analogue of dUMP, N(4)–hydroxy–2'–deoxycytidine 5'–monophosphate (N(4)–OH–dCMP), exhibited in the presence of the cofactor, N^5,N^{10}-methylenetetrahydrofolate (mTHF), a strong, time-dependent inhibition of TS [1]. In order to elucidate the inhibitory mechanism of N(4)–OH–dCMP on TS, X-ray crystallographic and molecular modeling studies were carried out.

The protein complex of mouse thymidylate synthase (mTS) with N(4)–OH–dCMP was crystallized by the vapor diffusion method. Diffraction data to 1.75 Å resolution were collected on the BESSY synchrotron in Berlin and processed with DENZO and SCALEPACK. The structure was refined with REFMAC5 of the CCP4 program suite using the 3IHI crystal structure of mTS as the search model. The quality of the final structure, with an *R*-factor of 21.5%, was analyzed with PROCHECK and SFCHECK.

The structure, consisting of one dimer per asymmetric part of the unit cell (Fig. 1), showed a strong similarity to the crystal structure of the complex mTS□dUMP, reflected by the backbone RMSD of 0.36 Å. The molecule of N(4) –OH–dCMP, which is defined by very good electron density, exhibits similar binding as the molecule of dUMP. Both molecules are anchored in the active site by several H-bonds to their phosphate moieties from four arginine and single serine residues. The orientation of N(4)–OH–dCMP, which is secured by H-bonds between the conserved Asn 220 of the enzyme and N(3)–H and N(4) moieties of the pyrimidine ring in N(4)–OH–dCMP, is the same as that of dUMP, with the positions of the pyrimidine rings in both molecules slightly shifted away from each other.

Molecular dynamics simulations were performed with SANDER module of AMBER8 for the complexes between TS from different sources, including human, rat, *E.coli* and *L.casei*, and (i) N(4)–OH–dCMP (binary systems), and (ii) N(4)–OH–dCMP and tetrahydrofolate (THF; analogue of mTHF) (ternary systems). The simulations were started from the respective crystal structures after replacing the molecule of dUMP with the one of N(4)–OH–dCMP. The binding mode of N(4)–OH–dCMP in each simulated complex was analyzed and compared with all other ones in the search for conformational and/or structural differences. The analysis revealed some differences in (i) the binding positions of N(4)–OH–dCMP among the binary systems and (ii) the binding alignments between N(4)–OH–dCMP and THF among the ternary systems. Those observations will help to construct a comprehensive model of the interaction of N(4)–OH–dCMP with TS and, in turn, to suggest a possible mechanism of the inhibitory action of this analogue of dUMP.

References

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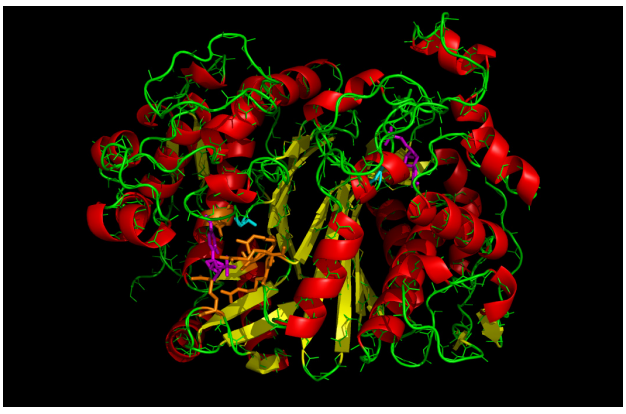

Figure 1. Crystal structure of mTS□N(4)–OH–dCMP (chains A and B). The ligand molecule depicted in magenta; Cys 189 in cyan; Arg 44, Arg 209, Ser 210, Arg 169', Arg 170' and Asn 220, the amino acids crucial in proper ligand binding and orientation, in orange.