Crystal structures of mouse thymidylate synthase in complexes with inhibitor

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Thymidylate synthase (TS; EC 2.1.1.45) catalyzes the conversion of deoxyuridine monophosphate (dUMP) and N(5,10)-methylenetetrahydrofolate (mTHF) to deoxythymidine monophosphate (dTMP) and dihydrofolate (DHF) via reductive methylation, in which mTHF serves as both methyl donor and reducing agent. The reaction is a terminal step in the *de novo* pathway leading to dTMP (one of the four building blocks of DNA). Inhibition of TS blocks DNA synthesis and prevents cellular proliferation. Therefore, targeting of TS for inactivation in TS-expressing cells, such as tumor cells, has become a reasonable strategy in the development of drugs for chemotherapy. Many compounds modeled after the substrate (dUMP) or cofactor (mTHF) have been tested as TS inhibitors and some have advanced to clinical trial or become licensed drugs (5-fluorouracil, Raltitrexed, Pemetrexed).

Crystallographic experiments have been shown to be a useful tools for analyzing the mechanism of inhibition, and relationship between inhibitor structure and specificity.

N(4)-OH-dCMP is a substrate analogue, being a potent mTHF-dependent, thus mechanism-based, slow-binding inhibitor of TS (K_i ~ 50nM). Similar to FdUMP, incubated with the cofactor and the enzyme it was shown to form a ternary complex. However, when N(4)-OH-[5 $^{-3}$H]dCMP replaced dUMP in the reaction mixture, ^{3}H abstraction from the uracyl ring C(5) was not apparent, suggesting, the reaction to be inhibited at an earlier stage that with FdUMP. In solution the equilibrium between rotamers around the C(4)-N^4 bond is significantly shifted towards *syn* rotamer (relative to pyrimidine N(3), but surprisingly only the *anti* isomer appeared to be the active inhibitor form.

In order to learn the inhibition mechanism, structural studies of TS complexes with N(4)-OH-dCMP were undertaken. Structures of two mouse TS (mTS) complexes with the inhibitor were solved, based on crystals formed by the enzyme protein in the presence of either only N(4)-OH-dCMP or both N(4)-OH-dCMP and mTHF. The former structure (1,75Å resolution) revealed the mTS-N(4)-OH-dCMP binary complex, as expected, but the latter (1,35Å resolution) showed the enzyme to be involved in a ternary complex with N(4)-OH-dCMP and DHF instead of expected mTHF, suggesting the inhibition to result from an abortive enzyme-catalyzed reaction. In accord with the previous results, in both complexes the *anti* inhibitor isomer was found covalently bound in the active center, and in the ternary complex no indication of proton release from C(5) was apparent.

Acknowledgements: The work was supported by the Ministry of Science and Higher Education, grant number N N401 0240 36.