Thymidylate synthase

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Introduction

Thymidylate synthase (TS) catalyzes the transfer of a methyl group from methylenetetrahydrofolate (CH2H4PteGlu) to dUMP forming TMP. Inhibition of TS results in apoptotic cell death due to intracellular thymidine depletion. Since cancer cells undergo rapid multiplication, they are much more sensitive to thymidine depletion and TS is the target of several anticancer agents used in colon, neck, and breast chemotherapy. Acquired resistance to TS inhibitors is a multifactorial process that can include amplification and mutation of the gene encoding thymidylate synthase. This project investigates variant TS proteins to study the mechanism of resistance and develop new inhibitors.

Methods and Materials

Typically, data were collected at the SBC-CAT beamline of the Advanced Photon Source at Argonne National Laboratory using x-rays of 0.9793 Å wavelength. Two sets of data were collected at high and low resolution (with a 3 x 3 CCD array detector) and indexed, integrated, and scaled with the HKL 2000 suit of programs. The Strategy option was utilized for collecting all possible independent reflections with minimum radiation damage to the crystal. In the first pass, oscillation frames were collected to record the high-resolution data. The second pass was used to remeasure reflections that suffered from intensity overflow during the first pass.

Results

Typically, high-resolution data were collected to 100% completion with a mosaic spread of 0.37º and an Rsymm of 12%. The completeness is also 100% in the highest resolution shell, 1.55–1.50, with 4008 reflections having $I > 2\sigma(I)$. The low-resolution set was 96.7% complete to 2.10 Å with an Rsymm of 10%. The data were merged after rejecting intensity-saturated and weak reflections to give a 99.9% complete set of data to 1.50 Å. Merging of the two data sets yielded an Rmerge of 6.6% and 91.1% of the reflections having $I/\sigma(I) > 2$. After several rounds of refinement and modeling, the 2Fo – Fc electron density map was superb; it revealed holes in aromatic and proline rings, and a bulge of density for every carbonyl group. However, electron density was missing for a few side chains, especially residues 19–22 at the β-bend located between helix A and strand I, which are partially disordered. The data were refined to a final R-factor of 22% and have been deposited to the Protein Data Bank as entry 1qqq.

Discussion

It can be speculated that mutations at residue 254 affect ligand binding and conformational isomerization by perturbing the movement of the C-terminal residues. However, the structure of P254S suggests a different mechanism. Our modeling indicates, that larger side chains at 254 must be accommodated by a movement of the side chain of Arg 48. This residue (although located on the surface and apparently not directly involved in catalysis, ligand binding, or dimer formation) is either conserved or replaced by lysine. This strongly suggests that its charge is essential for the proper functioning of TS. Larger side chains at 254 will push the guanidinium moiety of Arg 48 into the solvent region and the effect of its charge will be attenuated by the high dielectric constant of water [1].

The role of Ser 167 of *E. coli* TS in catalysis has been characterized by kinetic and crystallographic studies. Position 167 variants including S167A, S167N, S167D, S167C, S167G, S167L, S167T, and S167V were generated by site-directed mutagenesis. Only S167A, S167G, S167T, and S167C complemented the growth of thymidine auxotrophs of *E. coli* in medium lacking thymidine. Steady-state kinetic analysis revealed that mutant enzymes exhibited kcats that are 1.1–95-fold lower than that of the wild type enzyme. Relative to wild type TS, Kms of the mutant enzymes for 2'-deoxyuridylate (dUMP) were 5–90 times higher, while Kms for 5,10-methylenetetrahydrofolate (CH2H4folate) were 1.5–16-fold higher. The rate of dehalogenation of BrdUMP, a reaction catalyzed by TS that does not require CH2H4folate as cosubstrate, by mutant TSs was analyzed and showed that only S167A and S167G catalyzed the dehalogenation reaction and kcat/Kms for the mutant enzymes were decreased by 10- and 3000-fold, respectively. Analysis of pre-steady-state kinetics of ternary complex formation revealed that the productive binding of CH2H4folate is weaker to mutant TSs than to the wild type enzyme. Chemical transformation constants (kchems) for the mutant enzymes were lower by 1.1–6.0-fold, relative to the wild-type enzyme. S167A, S167T, and S167C crystallized in the I213 space group and scattered x-rays to either 1.7 Å (S167A and S167T) or 2.6 Å (S167C). The high-resolution data sets were refined to a Rcrys of 19.9%. In the crystals, some cysteine residues were derivatized with 2-mercaptoethanol to form S,S-(2-hydroxyethyl)thiocysteine. The pattern of derivatization indicates that in the absence of bound substrate the catalytic cysteine is not more reactive than other cysteines. It is proposed that the catalytic cysteine is activated by substrate binding by a proton transfer mechanism in which the phosphate group of the nucleotide neutralizes the charge of Arg 126', facilitating the transfer of a proton from the catalytic cysteine to a His 207-Asp 205 diad via a system of ordered water molecules.

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*****(crystallography)

References

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