infectious diseases.

Keywords: ATPase, cancer drug design, membrane protein crystallization

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Human thymidylate synthase: Conformational stabilization and dimer asymmetry

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Loop 181-197 of human thymidylate synthase (hTS) populates two conformational states. In the first state, Cys195, a residue crucial for catalytic activity, is in the active site (active conformer); in the other conformation, it is about 10 Å away, outside the active site (inactive conformer). We have designed and expressed an hTS variant, R163K, in which the inactive conformation is destabilized. The activity of this mutant is 33% higher than that of wt hTS suggesting that at least 1/3 of hTS populates the inactive conformer. Crystal structures of R163K in three different crystal forms, with 6, 5 and 2 subunits per asymmetric part of the unit cells, have been determined. All subunits of this mutant are in the active conformation while wt hTS crystallizes as the inactive conformer in similar mother liquors. The structures show differences in the environment of catalytic Cys195, which correlate with Cys195 thiol reactivity, as judged by its oxidation state. One of the dimers is asymmetric with a phosphate ion bound in only one of the subunits. In the absence of the phosphate ion, that is in the inhibitor-free enzyme, the tip of loop 47-53 is about 11 Å away from the active site. The structures of crystals soaked in solutions with dUMP and FdUMP show variable occupancy of the active sites.

Keywords: thymidylate synthase, cancer drug design, inhibitor interactions

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Structure for an HIV-1 reverse transcriptase RNase H inhibitor bound at the active site

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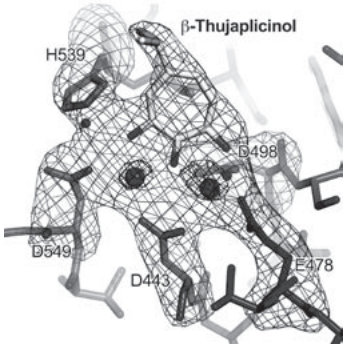
HIV-1 reverse transcriptase (RT) has been a key target of anti-AIDS drugs, because this enzyme is essential to the life cycle of HIV, the causative agent of AIDS. RT converts single-stranded viral RNA into double-stranded DNA suitable for integration into the host cell's genome. To do this, RT uses two enzymatic activities: (1) a DNA polymerase which can use either RNA or DNA as a template, and (2) an RNase H (RNH) that degrades the viral RNA after it is no longer needed as either a template for the first DNA strand or a primer for the second strand. RT inhibitors are typically included as part of a cocktail of therapeutic agents. The efficacy of these therapies has been reduced by the rapid emergence of drug-resistant viral strains. Antiretroviral inhibitors are therefore needed that target novel functions not affected by existing drugs. All clinically used RT inhibitors target the enzyme's polymerase activity, not its RNH activity. We have previously reported a structure for an RNH inhibitor (RNHI) that binds near the polymerase active site of RT. Here, we present a 2.8 Å resolution crystal structure for an RNHI, beta-thujaplicinol, that binds at the RNH active site.



Keywords: HIV-1 reverse transcriptase, inhibitor binding, X-ray crystal structure analysis

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Alanine racemase as a template for drug design against tuberculosis

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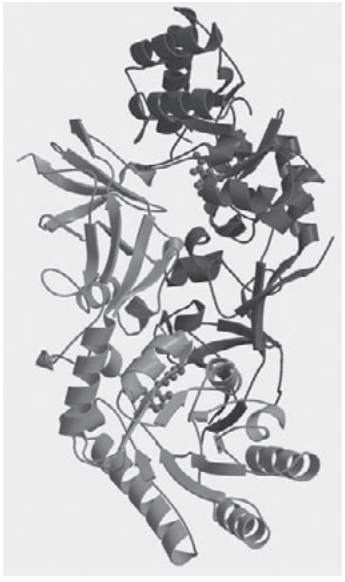
We present progress from an academic structure-aided drug design program aimed at developing new agents for the treatment of tuberculosis. We will present crystallographic data from five alanine racemase structure determinations, including M. tuberculosis and P. aeruginosa. We will analyze this data in terms of pharmacophore development, interface structure, and water conservation. We will review the creation of pharmacophore models that incorporate the results of molecular dynamics simulations. These pharmacophore models have been used to survey, in silico, chemical databases for racemase inhibitors. We will also present new results on inhibitor soaking experiments. From our most recent structures, including the alanine racemase from M. tuberculosis, we present evidence for a conserved substrate entryway that may result in improved pharmacophore development.



Keywords: alanine racemase, structure aided drug design, tuberculosis