Allostery and Heteroinhibition of Human Thymidylate Synthase

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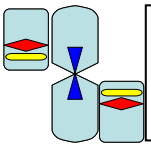
Thymidylate synthase (TS) is a homodimer which shows strong negative cooperativity between subunits. Unique property of human TS (hTS) among TS enzymes it that its active site loop (residues 181-197) can flip 180 degrees producing an inactive conformation [1]. Solution fluorescence studies have shown equilibrium between the active and inactive conformers [2]. We have developed bisphosphonate inhibitors that stabilize the inactive conformation and bind between dimers leading to the formation of hTS tetramers (but not higher oligomers) in solution. These inhibitors show positive cooperativity with antifolate inhibitors used in chemotherapy, which bind only to the active conformer. These data are consistent with a model in which hTS exists preferably as an asymmetric dimer with one subunit in the active conformation of loop 181-197 and the other in the inactive conformation.



Model of hTS homotetramer in which two subunits are connected by bisphosphonate inhibitor stabilizing the inactive conformation and two are inhibited by an antifolate with dUMP.

[1] Schiffer C. A., Clifton I. J., Davisson V. J., Santi D. V., Stroud, R. M., *Biochemistry,* 1995, **34**, 16279. [2] Phan J., Steadman D. J., Koli S., Ding W. C., Minor W., Dunlap R. B., Berger S. H., Lebioda L., *J. Biol. Chem*., 2001, **276**, 14170.

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