BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 8(16)

Isoniazid and thioacetazone may exhibit anti-tubercular activity by binding directly with the active site of mycolic acid cyclopropane synthase: Hypothesis based on computational analysis

Dibyajyoti Banerjee* & Rajasri Bhattacharyya

Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, (PGIMER) Chandigarh-160012; Dibyajyoti Banerjee – Email: dibyajyoti5200@yahoo.co.in; Phone: 91-172-2755227; Fax: 91-172-2744401 *Corresponding author

Received August 06, 2012; Accepted August 20, 2012; Published August 24, 2012

Abstract:
Isoniazid and thioacetazone are the two important antitubercular drugs. In case of thioacetazone it is established that it inhibits mycolic acid cyclopropane synthase but the exact binding site accounting for such inhibition is presently unknown. In case of isoniazid its action on the said enzyme is unexplored. In this work we have analyzed the binding of isoniazid and thioacetazone with mycolic acid cyclopropane synthase (CmaA1 and CmaA2) using tools of computational biology. We have observed that thioacetazone fits well at the active site of CmaA1 and CmaA2 while isoniazid binds at the active site of CmaA1 only. We have recommended experimental validation of such results. If such results are proved to be fact it will explore the exact binding site of thioacetazone and discover a new mechanism of anti-tubercular action of isoniazid.

Keywords: Tuberculosis, Isoniazid, Thioacetazone, Mycolic acid, Pro-drug

Background:
Presently more than 1/3rd of world population is suffering from tuberculosis **[1, 2]**. Isoniazid (INH) is one of the popular first line anti-tubercular drugs available for management of tuberculosis. There is experimental evidence to believe that INH is a prodrug, either mammalian lactoperoxidase or Mycobacterial catalase peroxidase (KatG) is required for its activation **[3]**. In the presence of the nicotinamide coenzyme, the INH oxidation produces the formation of INH-NAD(H) adducts which are potential competitive inhibitors of the enoyl-acyl carrier protein reductase InhA, an INH target in the biosynthetic pathway for mycolic acids, an important cell wall biomolecule for the bacteria to survive **[4]**. There is experimental evidence to believe that INH-NAD adduct is a slow, tight-binding competitive inhibitor of InhA **[5]**. With this experimental evidence it appears that KatG activity is an absolute requirement for anti-tubercular activity of INH **[6]**. But at the present moment there is experimental evidence to believe that neither catalase nor peroxidase activities, the two inherent enzymatic functions of KatG are absolute determinants of isoniazid resistance at least in in-vitro condition **[7]**. There is one study to show that for the N138S trans-dominant mutant, the catalase-peroxidase activity is significantly decreased while the sensitivity to INH is retained **[8]**. Therefore, INH inhibits mycolic acid synthesis by some other way or not needs to be explored.

Mycolic acid cyclopropane synthase is an important enzyme for mycolic acid biosynthesis and the known drug target for thioacetazone, one of the second line antitubercular drugs **[9]**. It is presently known that thioacetazone directly binds with the mycolic acid cyclopropane synthases but its exact binding site is presently unknown. It is unknown whether INH inhibits this enzyme or not. If it is found that INH can bind with this enzyme that can account for an alternative anti-tuberculosis activity mechanism even in situations with diminished catalase-peroxidase activity of KatG.

In this paper we have analyzed binding of thioacetazone and INH with the active site of mycolic acid cyclopropane synthase and observed that analogous to thioacetazone, INH also binds to the active site of the enzyme which can account for its anti-tubercular activity even in case of KatG mutant cases with diminished catalase peroxidase activity.

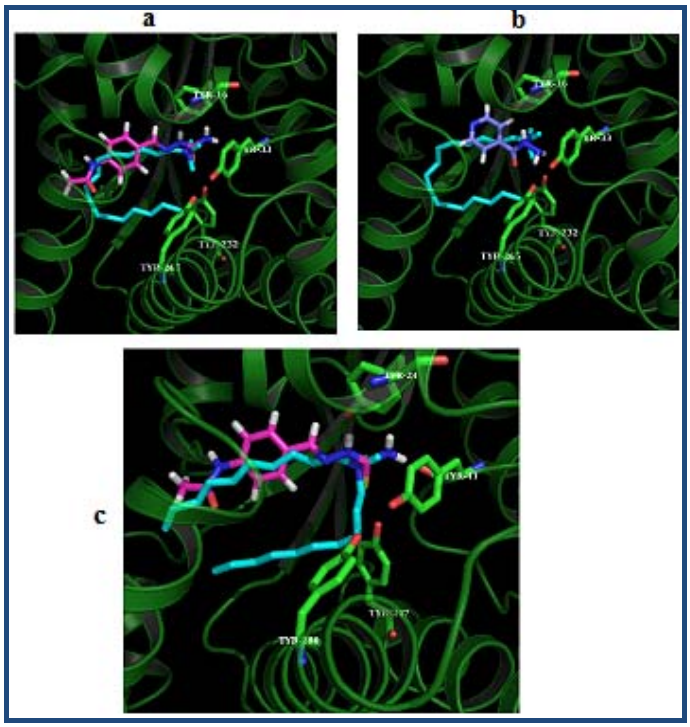

Figure 1: Interaction of **a)** Thioacetazone and **b)** Isoniazid drugs at the active site of CmaA1 and **c)** thioacetazone at the active site of CmaA2 are represented. Three tyrosine residues (in green colored and labeled) at the active sites, reference ligands CTAB and DDDMAB (in cyan) and the two drugs are represented in stick mode while the nitrogen and oxygen atoms are shown in blue and red color. The receptors are represented in green cartoon.

Methodology:

The structures of the receptor, mycolic acid cyclopropane synthases - CmaA1 and CmaA2 are taken from Protein Data Bank (PDB) **[10]** having PDB code 1KPG and 1KPI **[11]**. The structures of the drugs – thioacetazone, ethambutol, isoniazid, amoxycilin are taken from PubChem database **[12]**. The receptor-drug complexes are built by using docking software GOLD **[13]**. The cetyltrimethylammonium bromide (CTAB) and didecyldimethylammonium bromide (DDDMAB) located at the active site of the crystal structure of CmaA1 and CmaA2, respectively, are considered as reference ligands while Tyr265 and Tyr280 are chosen as one of the active site residues of the receptor for the docking study. Pictorial representations of the receptor-drug complexes are done by pymol **[14]**.

Discussion:

The active site of cyclopropane synthase is constituted by cofactor and substrate binding sites **[11]**. Further it is known that quaternary ammonium ion of substrate is stabilized by cation-π interaction with receptor molecule **[11]**. Thioacetazone and isoniazid drugs have amino group. We have observed that amino group of these drugs may interact with Tyr33 of CmaA1 through N-H-π interaction **(Figure 1a, b)**. N-H-π interaction plays a significant role in protein-protein and protein-ligand interactions **[15, 16]**. The quaternary ammonium group of CTAB and primary amino group of thioacetazone and isoniazid are geometrically oriented in same way at the quaternary ammonium ion binding region facilitating cation-π and N-H-π interactions. The long alkyl chain of CTAB makes hydrophobic interactions with cyclopropane synthase **[11]**. The methyl-phenyl acetamide and pyridine moieties of thioacetazone and isoniazid drugs are oriented almost in the same fashion like the alkyl chain of CTAB at the active site. In case of CmaA2, we have observed that thioacetazone is interacting through N-H-π interaction **(Figure 1c)** like DDDMAB but isoniazid has failed to fit at the active site.

Melamine, which is structurally similar to INH and diethylamine, a primary aliphatic amine are also considered. But these ligands are not fitted at the active sites of both CmaA1 and CmaA2. Structural homologues, thioacetazone-sulfinic acid and thioacetazone-carbodimide are the two metabolites of thioacetazone. In case of thioacetazone-sulfinic acid the amino group can also participate in N-H-π interaction with cyclopropane synthase. However in thioacetazone-carbidimide the nitrogen in amide group is sp2 hybidised. As a result the strength of N-H-π interaction is much stronger. So thioacetazone-carbidimide is much more active than thioacetazone-sulfinic acid **[17]**. Rifampicin, another anti-tubercular drug has complex structure in compare to thioacetazone and isoniazid and polar groups are distributed. We have also docked rifampicin at this active sites but it is not fitted there.

In these days INH induced mycolic acid biosynthesis reduction is a proved fact in tuberculosis bacilli. But there are still possibilities of unidentified targets of INH other than its known target **[18]**. It is in this context our observed results are important. We have observed that INH can fit in the manner analogous to CTAB and thioacetazone at the active site of CmaA1 **(Figure 1).** CTAB is a known ligand for the active site of CmaA1, an important enzyme for mycolic acid biosynthesis in the tuberculosis bacteria **[11]**. It is possible that binding of INH if actually happening in reality at the active site of CmaA1, may inhibit the enzyme. At the present moment there is no experimental data to support the same but if it is proved to be true by experimental studies we will discover a yet not explored target for INH in the context of its anti-tubercular action which will explain its activity in KatG mutants with no significant catalase-peroxidase activity.

It is proved by experimental studies that thioacetazone inhibits mycolic acid biosynthesis by directly acting on the above

enzyme **[9]** although the exact binding site is not known. There is enough possibility that thioacetazone inhibits the enzyme by binding directly at the active site and that idea is strengthened by the docking results. In this context it may be noted that here thioacetazone is observed to be binding at the active site of both CmaA1 and CmaA2 but INH is fitting only at the active site of CmaA1. Moreover, the thioacetazone metabolites – thioacetazone sulfinic acid and thioacetazone carbodimide which have active anti-tubercular role may also bind at the active site by N-H-π interaction analogous to thioacetazone **[17]**. We also propose for experimental validation of our observed results with thioacetazone which will unravel the exact binding site of thioacetazone with CmaA1 and CmaA2. With increasing burden of tuberculosis throughout the globe experimental validation of such result is warranted.

Acknowledgement:
RB has done the computational analysis and DB developed the basic hypothesis. DB acknowledges DST, New Delhi for financial assistance.

References:

[1] http://www.who.int/mediacentre/factsheets/fs104/en/
[2] http://www.cdc.gov/tb/statistics/default.htm
[3] Singh AK *et al. J Biol Chem.* 2010 **285**: 1569 [PMID: 19907057]
[4] Bernadou J *et al. Ann Pharm Fr.* 2001 **59**: 331 [PMID: 11787427]
[5] Rawat R *et al. Proc Natl Acad Sci U S A.* 2003 **100**: 13881 [PMID: 14623976]
[6] Suarez J *et al. J Biol Chem.* 2009 **284**: 16146 [PMID: 19363028]
[7] Cade CE *et al. Protein Sci.* 2010 **19**: 458 [PMID: 20054829]
[8] Yu S *et al. J Biol Chem.* 2003 **278**: 14769 [PMID: 12586821]
[9] Alahari A *et al. PloS One.* 2007 **2**: e1343 [PMID: 18094751]
[10] Berman HM *et al. Nucleic Acids Res.* 2000 **28**: 235 [PMID: 10592235]
[11] Huang CC *et al. J Biol Chem.* 2002 **277**: 11559 [PMID: 11756461]
[12] http://pubchem.ncbi.nlm.nih.gov/
[13] www.ccdc.cam.ac.uk
[14] http://www.pymol.org
[15] Saha RP *et al. Proteins.* 2007 **67**: 84 [PMID: 17221868]
[16] Chakrabarti P *et al. Prog Biophys Mol Biol.* 2007 **95**: 83 [PMID: 17629549]
[17] Qian L *et al. Chem Res Toxicol.* 2006 **19**: 443 [PMID: 16544950]
[18] Vilchèze C *et al. Annu Rev Microbiol.* 2007 **61**: 35 [PMID: 18035606]

Edited by P Kangueane
Citation: Banerjee & Bhattacharyya, Bioinformation 8(16): 787-789 (2012)