

Glucosamine-6-phosphate synthase, a novel target for antifungal agents. Molecular modelling studies in drug design

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Fungal infections are the growing problem in the contemporary medicine, yet there are still only a few antifungal agents used in the clinical practice. Thus searching not only for improved versions of existing drugs but also for completely new targets has become an urgent need. In our laboratory we proposed as such new and promising target for antifungals the enzyme L-Glutamine:D-fructose-6-phosphate amidotransferase, known under the trivial name of glucosamine-6-phosphate synthase (EC 2.6.1.16). This protein is a key enzyme in a pathway leading to the formation of UDP-GlcNAc, an important participant in the process of assembly of the microbial cell wall, so its selective and irreversible inhibitors can become the valuable antifungal drugs. The structure of this enzyme consists of two domains N-terminal and C-terminal catalysing glutamine hydrolysis and sugar-phosphate isomerisation respectively.

In our laboratory a series of potent selective inhibitors of GlcN-6-P synthase have been designed and synthesised. One group of these compounds including N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) behaves like glutamine analogs acting as active site directed inactivators, blocking the N-terminal, glutamine binding domain of the enzyme. The second group of GlcN-6-P synthase inhibitors mimics the transition state of the reaction taking place in the C-terminal - sugar isomerising domain. Surprisingly, in spite of the fact that glutamine is the source of nitrogen for a number of enzymes it turned out that glutamine analogue FMDP and its derivatives are selective against GlcN-6-P synthase and they do not block other enzymes, even belonging to the same family of glutamine amidotransferases. Since high specificity is the critical factor in rational drug design, elucidation of the mechanism of the selective action of these compounds became very important for the design of novel antifungal agents.

Because the crystal structure of the enzyme was not known yet, we applied the active analogue approach to solve the problem of this selectivity and searched for the common conformational space of the set of GlcN-6-P synthase inhibitors. Our calculations revealed that even within the family of related enzymes there may exist the substantial difference in the geometry of the active site. In the case of glutamine amidotransferases family it looks that glutamine binding site of GlcN-6-P synthase fits to the different region of the glutamine conformational space than other amidotransferases.

Only recently the structure of the enzyme of bacterial origin has been solved so the more thorough analysis is now possible. For a set of known inhibitors we carried out a series of docking experiments followed by the molecular dynamics calculations of the resulting complexes. Detailed analysis of the interaction pattern for the best known, so far, inhibitor of the sugar isomerising domain, namely 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP), allowed us to suggest the changes in the structure of the inhibitor that should improve the interaction pattern. The novel ligand has been designed and synthesised. Biological experiments confirmed our predictions. The new compound named ADMP is much better inhibitor of the glucosamine-6-phosphate synthase than ADGP.