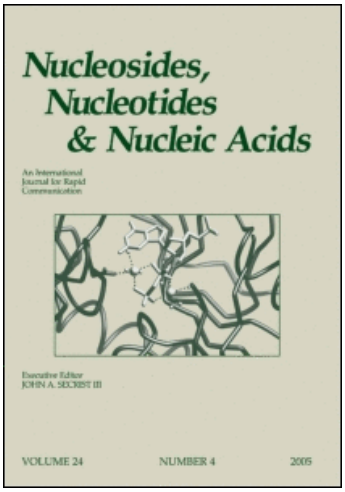

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Towards New Thymidine Phosphorylase/PD-ECGF Inhibitors Based on the Transition State of the Enzyme Reaction

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ABSTRACT

Computational studies have been conducted to built a closed form of TPase and to characterize the transition state of the phosphorylisis reaction catalyzed by TPase. The results obtained point to a crucial role of His-85 and the O2 of thymine in the catalysis. This modelled transition state forms the basis for the design of new TPase inhibitors.

Key Words: Thymidine phosphorylase; Transition state; Thymidine; 2-Thiothymidine.

Platelet-derived endothelial cell growth factor (PD-ECGF)/thymidine phosphorylase (TPase) is a nucleoside-processing enzyme that stimulates endothelial cell migration in vitro and angiogenesis in vivo. TPase is overexpressed in several human tumors, and in many cases these levels have been correlated with an aggressive pro-

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gression of the tumor (metastasis). TPase is involved in the catabolic/salvage pyrimidine nucleoside pathway, and catalyses the reversible phosphorolysis of thymidine to thymine and 2-deoxyribose-1-phosphate. To date, few TPase inhibitors have been described, and most of them are uracil-based substrate analogues. We have recently reported on the first multisubstrate inhibitors that are able to interact simultaneously at the phosphate and the pyrimidine binding sites.[1] We have now started a programme to characterize the transition state of the enzyme reaction in order to prepare new and potent TPase inhibitors.

In the X-ray crystal structure of TPase from *E. coli*, the phosphate and the pyrimidine binding sites, which are located in different domains, are separated by more than 8 Å. During catalysis, TPase must undergo a significant conformational change from an ''open'' to a ''closed'' form. In the absence of an experimentally determined structure for the latter form, we have conducted a number of computational studies that are briefly summarized as follows. First, a model of the ''closed'' form of TPase was built by means of targeted molecular dynamics (tMD) based on the structural homology between this enzyme and pyrimidine nucleoside phosphorylase, whose crystal structure has been reported for the ''closed'' form.[2] Second, the transition state (TS) of the phosphorolysis reaction has been characterised by ab initio calculations. The optimized TS was then built into the enzyme active site and the complex was subjected to molecular dynamics simulations. Then, the enzymatic reaction was studied in the protein environment to determine which aminoacid residues are mostly involved in the catalysis. Finally, the relative stabilities of the closed conformations of TPase complexed with either the substrates, the TS or the products were measured. The results obtained point to a SN_1 mechanism in the TS of the phosphorolysis reaction with notable changes between Lys-190 and the thymine O2. It seems that in the TS complex it is the protonated His-85 that binds to the O2, playing an important role in the catalysis. In the mechanism proposed, His-85 could transfer a proton from the phosphate to the O2 of thymine so that the first product of the reaction should be the C2-enol form of thymine. To test this hypothesis, both thymidine (Thd) and 2-thiothymidine (2S-Thd) were evaluated as substrates of *E. coli* TPase. It was found that 2S-Thd is degraded faster than thymidine. Moreover, when competition experiments were performed with 2S-Thd and Thd, 2S-Thd prevented the degradation of Thd in a concentration-dependent manner. These experimental data could be explained by the higher polarisation of thioamides and thioureas as compared to amides and ureas, so that the interaction with the protonated His-85 is favoured with 2S-Thd as compared to Thd, and this could facilitate breaking of the glycosidic bond. The modelled TS is currently forming the basis for new inhibitor design.

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