ABSTRACT

Present doctoral thesis contributed to understanding of mechanistic principles of two enzymes participating in the process of carcinogenesis; DNA polymerase β (pol β) and cytochromes P450 (CYP). Pol β is part of the DNA base-excision repair mechanism (BER). The primary role of pol β in, the BER mechanism, is inserting a new nucleotide into a DNA strand according to Watson-Crick base pairing rules. Pol β plays an important role in the process of carcinogenesis, approximately 30 % of human tumors express pol β mutants. The ability of pol β to discriminate between "right" and "wrong" nucleotide during the insertion process is called fidelity. We employed computational methods to elucidate molecular basis of the fidelity of pol β. First, the relative free energy calculation method LRA was employed to compare differences in free energies between the "right" and "wrong" nucleotide during its insertion into DNA. The results indicated a better stabilization of transition-state of the nucleophilic substitution catalyzed by pol β in the case of the "right" versus "wrong" nucleotide. This difference resulted in an 80-fold contribution to its fidelity. Further, computational methods FEP and LIE were used to examine how mutations effect fidelity of pol β. Results were than correlated with experimental data obtained for six single-point mutants and wild-type form of pol β enzyme. These methods were found to be unable to predict subtle changes induced by distal single point mutations. Therefore, modifications were suggested to improve their performance leading to development of modified methods – FEP/LIE and "modified" FEP. These methods provided quantitative estimation of fidelity within acceptable margin of errors for most of the tested mutants. These new methods were further used for quantitative prediction of the effect of new distal mutations on fidelity of pol β.

Cytochromes P450 (CYP) represents a large group of enzymes metabolizing nonpolar xenobiotics, including carcinogenic compounds. The catalytic effect of CYP can be modified by cytochrome b_5 (cyt b_5). Primary role of this enzyme is a reduction of CYPs. Method of flexible protein-protein docking was used to examine the way cyt b_5 interact with CYP. Biological relevancy of the modeled complexes was further validated by stability evaluation performed using methods of classical molecular dynamics and steered molecular dynamics. CYPs also catalyze activation of certain xenobiotics that leads to metabolites with genotoxic properties. One of these compounds is Aristolochic Acid I (AAI). Method of flexible protein-ligand docking was used to explore possible activation mechanisms of CYP family 1 catalyzed nitroreduction of AAI. Experimental results showed much lower level of AAI activation by CYP1B1 compared to CYP1A1/2. Stepwise (two electrons and two protons) mechanism suggested for nitroreduction catalyzed by CYP requires a proton donor group in vicinity of nitrogroup. Potential source of these protons is a hydroxylic group of serine or threonine residue in the binary complex of AAI/CYP1A1 and CYP1A2, respectively. While CYP1B1 lacks a suitable proton donor group. This could explain substantial differences in AAI reduction activities between CYP1A1/1A2 and CYP1B1.