



A RATIONAL DESIGN OF AN IMPROVED CYTOCHROME P450 FOR PESTICIDE TRANSFORMATION

Rafael Vazquez-Duhalt
Instituto de Biotecnología UNAM
Av. Universidad 2001, Col. Chamilpa, Cuernavaca. Mor. Mexico

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Introduction. Cytochromes P450 (CYP) are heme-containing enzymes able to introduce an oxygen atom to a variety of substrates. All CYP enzymes exhibit similarity in their structure and general mechanism of action. However, there are significant differences in the detailed function of each individual enzyme as well as in the structure and properties of their active sites. Special attention has been given to investigate the role of CYPs in the transformation of drugs and pollutant compounds, such as PAHs, PCBs and pesticides. The diversity of substrates for CYP enzymes offers the opportunity to develop enzymatic systems for detoxification and biotransformations of toxic and pollutant compounds. The aim of this work was to improve by a rational design the capacity of mutant CYPBM3 “21B3” to transform organochlorine and organophosphorus pesticides, which are major environmental pollutants and represent a public health risk.

Methods. Combined quantum mechanical and molecular mechanical (QM/MM) calculations were used to explore the electron pathway involved in the suicide inactivation of cytochrome P450BM3 from *Bacillus megaterium*. An extensive mapping of residues involved in electron transfer routes was obtained from density functional calculations on activated heme (i.e. Compound I) and selected amino acid residues. Identification of oxidizable residues (electron donors) was performed by selectively activating/deactivating different quantum regions. This method allowed a rational identification of key oxidizable targets in order to replace them for less oxidizable residues by site-directed mutagenesis.

Results. This evolved CYP from *B. megaterium* is able to transform a variety of structurally different pesticides. The catalytic parameters for two organochlorine; dichlorophen ($k_{cat} = 9.2 \text{ min}^{-1}$, $K_M = 64.1 \text{ }\mu\text{M}$) and linuron ($k_{cat} = 226.5 \text{ min}^{-1}$, $K_M = 468.2 \text{ }\mu\text{M}$), and two organophosphorus compounds; parathion ($k_{cat} = 10.9 \text{ min}^{-1}$, $K_M = 59.3 \text{ }\mu\text{M}$) and chlorpyrifos ($k_{cat} = 9.2 \text{ min}^{-1}$, $K_M = 226.5$

μM) were determined giving catalytic efficiencies between 0.143 and $1.107 \text{ min}^{-1} \mu\text{M}^{-1}$. CYPBM3 “21B3” has the ability to both activate and detoxify organophosphorus pesticides, as demonstrated by the chemical nature of the reaction products.

On the other hand, the QM/MM calculations were used to explore the electron pathway involved in the suicide inactivation of cytochrome P450BM3 from *B. megaterium* allowed a rational identification of key oxidizable targets in order to replace them for less oxidizable residues by site-directed mutagenesis. The residues W96 and F405 were consistently predicted by the QM/MM electron pathway to hold high spin density; single and double mutants of P450BM3 on these positions (W96A, F405L, W96A/F405L) resulted in a more stable variants in the presence of hydrogen peroxide, displaying a similar reaction rate than P450BM3 21B3. Furthermore, mass spectrometry confirmed these oxidation sites and corroborated the possible routes described by QM/MM electron transfer (ET) pathways.

In conclusion, the algorithm “QM/MM ET pathway” was successfully used to map the intramolecular electron transfer involved in the radical migration that induces the suicide inactivation of cytochrome P450BM3 21B3. Rational enzyme stabilization was performed by site directed mutation of selected amino acid residues based on their redox properties. A double variant, showing to be 260 times more stable to hydrogen peroxide inactivation than the parental enzyme, was produced. The rational approach with QM/MM tools could be extended to the study of other peroxidases.

References.

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