CYTOCHROME P450, PSEUDOVIRAL NANOPARTICLES, AND CHEMOTHERAPY

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Introduction. Recently, the encapsulation of enzymes inside virus-like particles (VLPs) or other protein cages has been a fast growing topic because of its implications in biocatalysis as well as their potential as enzymatic delivery systems. We report for the first time the encapsulation of a CYP450, which belong to a family of enzymes medically and industrially important.

Results. Combined quantum mechanical and molecular mechanical (QM/MM) calculations were used to explore the electron pathway involved in the suicide inactivation of cytochrome CYPBM3 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. 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 CYPBM3 21B3.

The CYPBM3 21B3 from *Bacillus megaterium*, mutant "21B3", which has improved peroxygenase activity, as a model of this family of enzymes since it is stable and soluble in aqueous media, and it can be produced in large quantities, as opposed to human CYP. CYPBM3 21B3 has been encapsulated inside two different VLPs through a charge complementarity strategy. The VLPs used were CCMV and VP1 from murine poliomavirus, these capsids differ in size, porosity and charge of the inside surface. The encapsulation of CYP inside CCMV was favored by the fact that the enzyme (-) and the interior of the capsid (+) have opposite charge at pH 7.2. A range of molar ratio of viral protein to CYP were assayed (1.3-60:1) finding encapsulation at the 1.3:1 ratio. This particular stoichiometry has the characteristic of having a cero net charge. The VLPs formed have an average diameter of 19.9 nm, which is smaller than the reported for the empty capsid (28 nm).

For the VP1-CYP encapsulation three different molar ratio of viral protein to enzyme were assayed (3:1, 5:1, 10:1), only finding nanostructures with CYP activity in the 10:1 stoichiometry. In this case to promote the interaction of the CYP with the negatively charged interior of the VP1 capsid we chemically modified the surface of the enzyme with ethylenediamine to increase the number of positively charged groups at pH 8. The VLPs formed have an average diameter of 23.6 nm. It is still to be determined the exact number of CYPs encapsulated in each VLP, as well as complete enzymatic characterization of the biocatalytic VLPs.

Conclusions. We have demonstrated that it is possible to encapsulate the CYPBM3 inside CCMV and VP1 VLPs using a charge complementarity approach. The encapsulation of this CYP450 in viral structures will provide a model for the design of biocatalytical nanoparticles with potential medical applications.