Molecular Modelling and Protein Engineering of a C-terminally Truncated Human Flavin Containing Monooxygenase 3

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Human flavin-containing monooxygenase 3 (hFMO3) is a microsomal drug-metabolizing enzyme able to oxygenate drugs and xenobiotics containing a soft-nucleophile, usually sulphur or nitrogen. The structure of hFMO3 has not yet been solved and therefore molecular modelling was used in this work to assign a structural/functional role to the predicted secondary structure elements of the polypeptide sequence. A model of hFMO3 was built by combining *ab-initio* and homology modelling approaches using the structure of FMO from *Methylophaga sp*. as template (PDB ID: 2VQ7) sharing a 30% sequence identity with the human counterpart. The energy minimized and refined model was used for docking experiments to show how known substrates bind the catalytic site of the enzyme. Based on the hydrophobic nature of the carboxyl terminus, it was hypothesized that this region could function as a membrane anchor. Therefore, a C-terminal truncated form of hFMO3 (tr-hFMO3) was engineered at DNA level and subsequently cloned, expressed in *E. coli* and purified in order to compare its solubility and activity with that of the full-length wild type enzyme. The tr-hFMO3 was purified from the cytosolic fraction whereas the wild type protein was purified from the membrane fraction. Furthermore, catalysis experiments with the tr-hFMO3 showed that this enzyme is fully active and carries out the monooxygenation of substrates such as sulindac sulfide, benzydamine, tozasertib and danusertib. The results from docking experiments together with the success in rational design of the soluble and active tr-hFMO3 support the validity of the presented hFMO3 model.