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Detection of ligand binding to cytochrome P450 BMP labeled with a fluorophore

Cytochromes P450 are a vast class of heme-thiolate enzymes highly relevant to pharmaceutical, environmental and biocatalytical applications. The availability of a fast method able to measure substrate binding to these enzymes is an important target for rapid screening of various classes of compounds. Here the heme domain of cytochrome P450 BM3 (BMP) and two site directed mutants (C62S and C156S) are used as a model system for site-specific labeling with the fluorescent probe N,N'-dimethyl-N-(iodoacetyl)-N'-(7-nitrobenz-2-oxa-1,3-diazol-4-Yl)-ethylenediamine (IANBD amide). Ligand binding studies were carried out using known substrates such as arachidonic acid (AA) and propranolol (PRP). AA is one of the physiological substrates of P450 BM3 and binding can be optically detected by the low-to-high spin shift from 419 to 397 nm of the Soret peak. On the other hand PRP is a non-physiological substrate of P450 BM3 and its binding does not give rise to the spin shift. Here we investigate as to whether in the absence of an absorbance shift due a spin change the IANBD probe can reveal the binding of a substrate by fluorescence emission. Titrations with AA and PRP of IANBD-labeled wt and mutants show significant changes in the fluorescence emission of the probe. The fluorescence emission increased by 55-140% upon titration with AA and decreased by 21-38% (all values corrected for the background buffer) with PRP. Titrations with lauric acid and chlorzoxazone, two substrates known to behave like AA and PRP respectively, confirmed the same pattern in fluorescence changes. In all cases the dissociation constants (K_D) were calculated and the results, spanning from the μM to the mM range, were found to be in good agreement with the literature. Control experiments carried out with with diclofenac and ibuprofen, known not to be ligands of the enzyme, and imidazole, known to be an inhibitor, gave negligible variations of fluorescence in the same range of the control titrations with the buffer (<20%).