SITE-SPECIFIC CHARACTERIZATION OF P450CAM SUBSTRATE RECOGNITION *VIA* 2D IR SPECTROSCOPY

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Determining the mechanism by which Cytochrome P450s (P450s) can catalyze oxidation reactions of substrates with differing specificity and regioselectivity is crucial for aiding drug development and understanding drug metabolism. Cytochrome P450cam (P450cam), a model P450, catalyzes the hydroxylation of *d*-camphor to 5-exo-hydroxycamphor with high specificity and regioselectivity. P450cam can also act upon camphor-like analogs at the expense of regioselectivity. To investigate the contribution of conformational dynamics to varying regioselectivity of hydroxylation, we characterized specific locations throughout P450cam when the enzyme was in complex with camphor or norcamphor, substrates acted upon with 100% and 45% regioselectivity respectively. Linear and two-dimensional IR spectroscopy were applied to measure P450cam side-chain dynamics with high spatial and temporal resolution. Cyanophenylalanine was used as a vibrational probe and incorporated in five distinct locations of P450cam, three sites in the active site and two progressively distal from the active site. The results suggest norcamphor binding does not induce the same large-scale conformational change associated with the closed enzyme state found for the camphor-bound complex. Additionally, probes located in the active site of the enzyme report distinct, localized changes that, in some cases, can be directly correlated to hydroxylation product distribution. Overall, this study illustrates the utility of site-selective infrared spectroscopy to address questions of functional protein dynamics.