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CYP2A13 Metabolism of Coumarin and Carcinogens

Cytochrome P450 enzymes are commonly known for oxidative metabolism of both endogenous and exogenous organic compounds, such as drugs, carcinogens, and steroids. Fifty-seven kinds of P450 enzyme are found in humans alone, and most organisms also have P450s. Enzymes in the P450 2A subfamily are particularly interesting for their role in the activation of nitrosamines, which are found in cured tobacco. After activation, the nitrosamine metabolites can bind to DNA and are carcinogenic. Currently, we are investigating P450 2A13, found in the human lung, because it has been shown to efficiently catalyze the oxidation of nitrosamines. By studying its active site, and comparing its metabolic activity to a mutant form, we can better understand this carcinogenic pathway. We are using coumarin, a P450 2A substrate, to assess the enzymatic activity, and HPLC to measure the products. The mutant P450 2A13 has an asparagine to alanine change at amino acid 297 (Asn297Ala), which is predicted to affect substrate orientation.

The primary pathways of coumarin metabolism by P450 2A13 are hydroxylation at the 7' position, forming 7-hydroxycoumarin, and metabolism at the 3' position, leading to an unstable 3,4-epoxide, which breaks down to form O-HPA or it conjugates to other compounds. In order to measure this second pathway, we plan to trap the reactive epoxide using the reducing agent glutathione. When glutathione is present, it can form a glutathione-epoxide conjugate, which forms a single, quantifiable peak on HPLC. The HPLC of P450 2A13 metabolism of coumarin still shows unidentified peaks, and if these disappear in the presence of glutathione, forming the glutathione-epoxide conjugate, we may conclude that they were products of the epoxide pathway. The glutathione conjugate can be confirmed using mass spectrometry.

While the wild type P450 2A13 shows a circa 1:1 ratio of 7-hydroxylation to 3,4 epoxidation when metabolizing coumarin, the Asn297Ala mutant had remarkably less 7-hydroxylation. By trapping all of the 3,4 epoxide with glutathione, my goal is to quantify the 3,4 epoxidation pathway, for further comparison to the wild type. By measuring differences in hydroxylation and epoxidation of coumarin, comparing wild type to mutant, we can better understand the active site of P450 2A13 and how substrate orientation here affects product formation. This is valuable information as we continue to explore nitrosamine metabolism by P450 2A13, and more broadly, other carcinogenesis pathways by the P450 enzymes.

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