BIOACTIVATION OF TOXICANTS BY CYTOCHROME P450-MEDIATED DEHYDROGENATION MECHANISMS

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INTRODUCTION

Normal cytochrome P450-mediated metabolism of toxicants proceeds through an electron or hydrogen atom abstraction mechanism that generally produces hydroxyl radical equivalents bound to prosthetic heme iron and eventually leads to oxygenated products through a hydroxyl rebound mechanism (Ortiz de Montellano, 1995). In a growing number of examples, certain P450 enzymes initiate oxidation of toxicants through the first step of one-electron abstraction (or hydrogen atom abstraction), but subsequently catalyze a second-electron oxidation that leads to dehydrogenated (desaturated) products. Many of these products are highly reactive electrophiles that initiate toxicities through binding to proteins and/or DNA (Yost, 1997; Guengerich and Kim, 1991; Lewis et al., 1996; Han et al., 1990). The precise chemical environments of the active sites of the enzymes that direct selective dehydrogenation, rather than hydroxylation, are not known. Several of the enzymes that catalyze dehydrogenation of toxicants are selectively expressed in respiratory tissues (Pelkonen and Raunio, 1997; Mace et al., 1998), and much of our work (Thornton-Manning et al., 1996; Lanza et al., 1999) has addressed the mechanisms of dehydrogenation by several human lung-expressed enzymes such as CYP2F1 (Nhamburo et al., 1990) and CYP4B1 (Nhamburo et al., 1989).

The precise mechanisms that control the production of dehydrogenated intermediates by selective P450 enzymes, and the mechanisms that are responsible for cell death after alkylation of critical protein and/or DNA targets have not been adequately elucidated. A working hypothesis of our studies is that the spatial and electronic parameters of these enzymes must direct two-electron oxidation rather than oxygenation through facilitated electron transport from ferryl heme and/or proton relay mechanisms. Presented below are several examples of dehydrogenation reactions that are catalyzed by P450 enzymes, including recent work in our laboratory that has evaluated the formation of a dehydrogenated
product of 3-methylindole (Skiles and Yost, 1996; Thornton-Manning et al., 1996; Lanza et al., 1999), and the formation of adducts of this product with thiols (Skordos et al., 1998a) and with deoxynucleosides (Regal et al., 1999).

**DEHYDROGENATION OF ALKANES**

Oxidation of substrates that are simple hydrocarbons would appear to be the most difficult type of dehydrogenation reactions because of the relatively strong "unactivated" C-H bond that must be broken, and because intermediate radicals or radical cations are not resonance stabilized. Resonance stabilization of intermediates in the dehydrogenation of typical aromatic substrates such as acetaminophen, butylated hydroxytoluene, and 3-methylindole would be extensive. Thus, it is surprising that hydrocarbons are substrates for this process.

However, the formation of alkenes from alkyl groups of substrates such as lauric acid can be catalyzed by P450 enzymes. The CYP4B1 enzyme from rabbits possess a unique propensity to dehydrogenate lauric acid (Guan et al, 1998) and valproic acid (Rettie et al., 1995) with only a modest preference for formation of the hydroxylated metabolites (Figure 1). Dehydrogenation of valproic acid occurs through selective hydrogen abstraction at the omega-1 methylene position, as demonstrated by elegant intramolecular deuterium isotope effects (Rettie et al., 1995), which is presumably followed by a second hydrogen abstraction at the methyl carbon.

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**Figure 1.** Dehydrogenation of a fatty acid and valproic acid that illustrate dehydrogenation of hydrocarbons by cytochrome P450 enzymes. Dehydrogenation of valproic acid leads to production of the putative toxic alkene intermediate. (Pr = propyl)