Enzyme Kinetic Parameters for Hydrogen Peroxide Generation (Auto-Oxidation) in P450-Related Microsomal Electron Transport Chains

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It is well recognized that a microsomal electron transport chain with terminal oxidase cytochrome P450 enzymes (CYP's) generates hydrogen peroxide. This reaction requires NADPH and oxygen and proceeds via autooxidation without metabolizing substrates or by uncoupling with metabolizing substrates. Rates of hydrogen peroxide generated by this system in rat liver microsomes depend on multiple factors including the specific set of expressed CYP's. Earlier studies by our laboratory showed that the CYP3A family is one of the most active CYP's producing hydrogen peroxide. Of the CYP3A family, rat liver microsomes from male Sprague Dawley (SD) rats contain only CYP3A2; using the Amplex Red/HRP assay, we found that the Vmax for hydrogen peroxide generation by these microsomes in the absence of substrates was 4.0 nmol/min/mg protein with NADPH as the electron donor. Significantly increased hydrogen peroxide generating activity was found in liver microsomes from dexamethasone treated male rats where CYP3A1 and CYP3A2 are highly inducible (Vmax = 14-16 nmol/min/mg protein). Clotrimazole, a form-selective inhibitor of rat CYP3A1 and CYP3A2, suppressed 60% of hydrogen peroxide generation in liver microsomes from dexamethasone treated male rats. The Km for NADPH for hydrogen peroxide generation was similar (≈ 2.0 μM) in microsomes from control and dexamethasone treated male rats. These data indicate that increases in content of CYP 3A family enzyme in rat liver microsomes are largely responsible for the higher rates of hydrogen peroxide formation. Moreover, similarities in the Km for NADPH may represent similar affinities of CYP3A subfamily enzymes for the NADPH-cytochrome P450-reductase during the formation of hydrogen peroxide. *Supported by NIH grants U54AR00573 and R25ES020721.*