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CHARACTERIZATION OF A HUMAN FUSION PROTEIN CONTAINING CYTOCHROME P450 1A1 AND NADPH-P450 REDUCTASE EXPRESSED IN *ESCHERICHIA COLI*

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Cytochrome P450 enzymes are integral membrane proteins responsible for the metabolism of numerous endogenous and exogenous chemicals. Among various P450s, P450 1A1 has been of considerable interest because of its inducibility by several environmental toxicants and a possible relationship to human lung cancer. To characterize the enzymatic properties of human P450 1A1, a fusion protein containing human P450 1A1 and rat NADPH-P450 reductase were high-level expressed in E. coli DH5*α*. *E. coli* membranes of transformed cells showed strong P450 1A1-dependent monooxygenase and NADPH-P450 reductase activities. The fusion protein was purified from detergent-solubilized bacterial membranes using DEAE and 2',5'-ADP agarose chromatography. The purified fusion protein catalyzed benzo[*a*]pyrene 3-hydroxylation, 7-ethoxyresorufin O-deethylation, and zoxazolamine 6-hydroxylation. The respective V_{max} and K_m values measured for the oxidations of 7-ethoxyresorufin, benzo[*a*]pyrene, and zoxazolamine were 0.65, 1.04 and 0.91 nmol product formed min^{-1} (nmol enzyme)$^{-1}$ and 0.34, 19, and 7 mM. Catalytic activity was not increased in the presence of added NADPH-P450 reductase, cytochrome b_5, or phospholipid. The fusion protein could also transfer electrons to cytochrome c and b_5 but not P450 1A2. The same oxidation products of benzo[*a*]pyrene were formed with the purified fusion protein and the fusion protein functioning in bacterial cells. The purified fusion protein also oxidized (+)- and (-)-benzo[*a*]pyrene 7,8-dihydrodiols and eight aryl and heterocyclic amines to genotoxic products, in the absence of added NADPH-P450 reductase. The demonstration of catalytic activities of the human fusion proteins within bacterial cells suggests the prospect of utilizing such cellular systems for human drug metabolism studies.