Expression of Recombinant Human Cytochrome P450 1A2 in Escherichia coli Bacterial Mutagenicity Tester Strain

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Human cytochrome P450 1A2 is one of the major cytochrome P450s in human liver. It is known to be capable of activating a number of carcinogens such as arylamines and heterocyclic amines. In order to develop the new bacterial mutagenicity test system with human P450, a full length of human P450 1A2 cDNA inserted into pCW bacterial expression vector was introduced to Escherichia coli WP2 uvrA strain which is a well-known E. coli strain for bacterial reverse mutagenicity assay. Expressed human P450 1A2 showed typical P450 hemoprotein spectra. Maximum expression was achieved at 48 hrs after incubating at 30°C in terrific broth containing ampicillin, IPTG and other supplements. High level expression of P 450 1A2 in E. coli WP2 uvrA membranes was determined in SDS-PAGE. The well-known mutagens 2-aminoanthracene and MelQ increased the revertant colonies of E. coli WP2 uvrA expressing human P450 1A2 without an exogenous rat hepatic post-mitochondrial supernatant (S9 fraction) in a dose-dependent manner. The results show that the functional expression of human P450 in bacterial mutagenicity tester strain will provide a useful tool for studying the mechanism of the mutagenesis and carcinogenesis of new drugs and environmental chemicals.

Key words : Human cytochrome P450 1A2, Escherichia coli WP2 uvrA, High level expression, 2-Aminoanthracene, MelQ

INTRODUCTION

The cytochrome P450 (P450) enzymes are the major catalysts involved in the metabolism of most drugs. Cytochrome P450-mediated microsomal electron transport is responsible for oxidative metabolism of both endogenous compounds such as fatty acids, steroids, and eicosanoids, and exogenous compounds ranging from drugs and environmental chemicals to carcinogens. The P450 superfamily includes a group of at least 30 different enzymes expressed in each species, including humans, and these differ in their catalytic specificity towards individual substrates.

Among many human P450 enzymes, P450 1A2 is known to be capable of activating a number of known carcinogens, including arylamines and heterocyclic amines such as those found in broiled meat (Butler et al., 1989). This enzyme is inducible by compounds found in cigarette smoke, charbroiled meat, and cruciferous vegetables (Sesardic et al., 1988). Human P450 1A2 appears to be subject to genetic polymorphism (Butler et al., 1992). Its relevance to cancer risk has been the subject of much study (Kadlubar et al., 1992). Human P450 1A2 cDNA has been expressed in Escherichia coli (E. coli) DH5-α following introduction of modifications in the amino-terminal coding region (Sandhu et al., 1994).

The most widely used bioassay in genetic toxicology is the Ames test, which combines a bacterial mutagenicity assay with an exogenous bioactivation system such as rat hepatic S9 fractions. Recently, several approaches to replace mammalian tissue preparations by the engineered bacterial expression of recombinant drug metabolizing enzymes such as P450 and glutathione S-transferase (GST) have been successfully achieved. Expression of human theta class GSTT1-1 in Salmonella typhimurium (S. typhimurium) TA1535 revealed the contribution of this enzyme to the bioactivation of dihalomethanes, ethylene bromide, butadiene epoxides and other alkylating agents (Thier et al., 1996). Expression of human P450 1A2 was successful in S. typhimurium YG1019 (Josephy et al., 1995).

In this study, a new tester strain E. coli WP2 uvrA expressing human P450 1A2 was developed. Expression levels were determined by biochemical and spectrophotometric methods. The genotoxic activities of pro-mutagens were examined in the newly developed strain as well as the original tester strain.
MATERIALS AND METHODS

Chemicals

IPTG (isopropyl β-D-thiogalactoside), δ-aminolevulinic acid, thiamine, X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), and ampicillin were purchased from Sigma Chemicals (St. Louis, MO). 2-Aminoanthracene (2-AA) and MelQ (2-amino-3,4-dimethylimidazo[4,5-α]quinoline) were obtained from Wako Chemicals (Tokyo, Japan). All other chemicals used were of reagent grade and commercially available.

Expression of recombinant plasmid in E. coli

E. coli strain WP2 uvrA was provided by Dr. M.H.L. Green (University of Sussex, UK) (Green, and Muriel, 1976). A plasmid containing human cytochrome P450 1A2 cDNA was transformed into E. coli WP2 uvrA, and transformants were selected by growth on nutrient agar plates containing ampicillin (100 µg ml⁻¹) (Sandhu et al., 1994). A single ampicillin-resistant colony was grown overnight at 37°C in Luria-Bertani (LB) medium containing ampicillin (100 µg ml⁻¹). A 1-ml of aliquot was used to inoculate 100 ml of Terrific Broth (TB) containing 0.2% bactopeptone (w/v). The TB media was supplemented with ampicillin (100 µg ml⁻¹), 1 mM thiamine, 0.5 mM δ-aminolevulinic acid, and trace elements (Gillam et al., 1993). Induction of the tac promoters was done with 1.0 mM IPTG, and allowed to proceed for 48 hrs at 30°C with shaking at 250 rpm. Bacterial membrane fractions were prepared from the bacterial pellets by a series of fractionation and high-speed centrifugation steps (Guengerich et al., 1996).

Analysis of P450 expression

P450 expression in E. coli was studied by the spectrophotometric and biochemical analyses. P450 content in E. coli membrane was quantitated by Fe²⁺ vs. Fe³⁺-CO difference spectra according to the method of Omura and Sato (1964) using an extinction coefficient of 91 mM⁻¹ cm⁻¹ at ambient temperature. Proteins were separated by electrophoresis on 10% (w/v) SDS-PAGE according to modifications of Laemmli (1970) and Guengerich (1994). Proteins were visualized by staining with Coomassie Brilliant Blue.

Mutagenicity test

Bacterial reversion mutagenicity tests were performed as described previously (Green and Muriel, 1976; Maron and Ames, 1983). 2-AA and MelQ were dissolved in DMSO. Bacterial cells after culturing at 30°C for 24 hrs in complete TB medium were washed twice with fresh LB medium. For the assay, 2 ml of molten top agar containing 0.5% NaCl, 0.05 mM tryptophan, 0.1 ml of mutagen solution, and 0.1 ml of bacterial suspension were added to a 5-ml test tube. This mixture was stirred gently and then poured onto plates containing minimal glucose agar. After the top agar solidified, the plates were incubated at 37°C for about 48 hrs. The revertant colonies were counted after the incubation period. For the S9-dependent assays, S9 prepared from the livers of male Sprague-Dawley rats given Aroclor 1254 (500 mg/kg, i.p.) was purchased from Molecular Toxicology Inc. (Annapolis, MD), and stored at -70°C.

RESULTS AND DISCUSSION

Human P450 1A2 expression

Human cytochrome P450s have been expressed in heterologous systems such as E. coli. However, in general, modifications of the N-terminus coding region of cDNA sequence are necessary to achieve levels of expression in E. coli (Barnes et al., 1991). These approaches have now been applied to more than 30 P450s (Guengerich et al., 1996). High level expression of human P450 1A2 were achieved when the N-terminal sequence contained the MALLLAVFL sequence shown by Barnes et al. (1991) to yield high levels of expression of bovine P450c17. These results suggested that P450s expressed in E. coli show their catalytic activities

Fig. 1. N-Terminal amino acid sequences of human P450 1A2 expression constructs. (A) Amino acid sequences of native and modified P450 1A2 construct #1024. (B) Nucleotide sequences of native and modified P450 1A2 construct #1024.