Arch. Pharm. Res. Vol.21, No.3, pp. 305-309, 1998

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

Young Jin Chun

College of Pharmacy, Chung Ang University, Seoul 135-756, Korea

(Received February 19, 1998)

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 MeIQ 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, MeIQ

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 promutagens were examined in the newly developed strain as well as the original tester strain.

Correspondence to: Young Jin Chun, College of Pharmacy, Chung Ang University, 221, Huksuk-dong, Dongjak-ku, Seoul 156-756, Korea

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 MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*] 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 Fe^{2+} vs. Fe^{2+}-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 MeIQ 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 P450 17A (Sandhu *et al.*, 1994) (Fig. 1). Jenkins and Waterman (1994) showed that two soluble flavoproteins flavodoxin and NADPH-flavodoxin reductase can support the 17α-hydroxylase activities of heterologously expressed bovine P450c17. These results suggested that P450s expressed in *E. coli* show their catalytic activities

(A)

```
          1        10        20        30        40
Native    MALSQSVPFSATELLLASAIFCLVFW VLKGLRPRVPKGLK....
#1024     MA            LLLAVFLFCLVFW VLKGLRPRVPKGLK....
```

(B)

```
          1           10          20          30          40          50          60
Native    ATG GCA TTG TCC CAG TCT GTT CCC TTC TCG GCC ACA GAG CTT CTC CTG GCC TCT GCC ATC
#1024     ATG GCA                                             CTG TTA TTA GCA GTT TTT CTG
```

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.

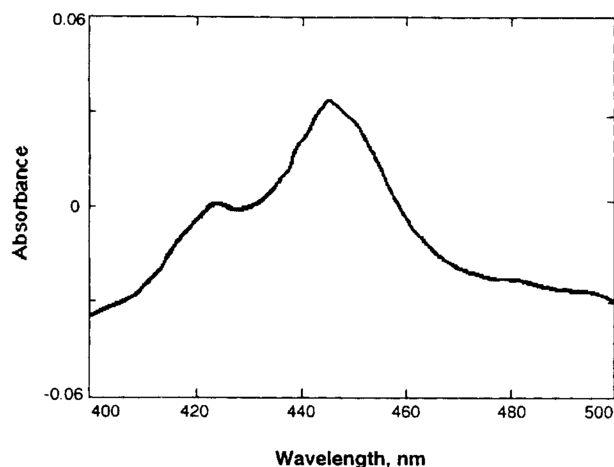

Fig. 2. Fe^{2+} vs. Fe^{2+}-CO difference spectra of *E. coli* WP2 uvrA membranes. Bacterial membranes were prepared and resuspended in 100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v) and 0.2% Emulgen 911 prior to recording the spectrum. The samples were saturated with bubbles of CO, and 1 mg of $Na_2S_2O_4$ crystal were added. Spectra were recorded between 400 nm and 500 nm until the 450 nm peak reached a maximum.

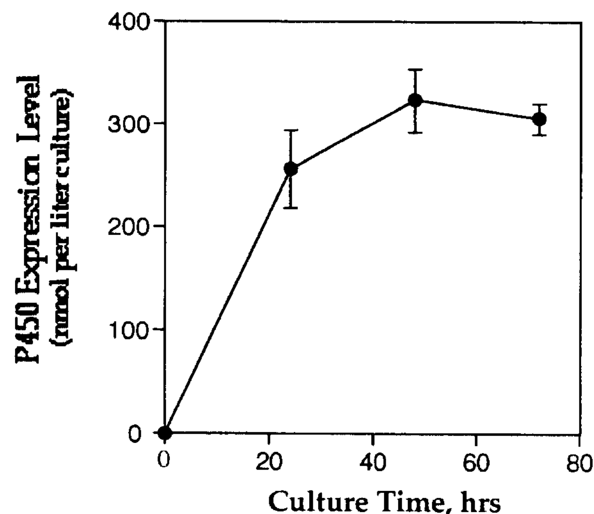

Fig. 3. Time course results of recombinant human P450 1A2 expression in *E. coli*. Bacterial cells were incubated for 0, 24, 48 and 72 hrs after seeding in terrific medium containing IPTG and other supplements for P450 expression. Bacterial membranes were prepared and CO-difference spectrum were recorded. Each data represents the mean$\pm$S. D. from four separate experiments.

without exogenous NADPH-P450 reductase, a common redox partner of P450 although the potency of electron transfer to P450 is approximately 10-fold less than NADPH-P450 reductase.

Modified human P450 1A2 cDNA (construct #1024) was transformed into *E. coli* WP2 uvrA, which is well known strain for bacterial mutagenesis assay. Human P450 1A2 construct containing native N-terminal sequence did not use for expression because of its low expression yield (less than 2 nmol P450 per liter culture). Because modified human P450 1A2 showed phenacetin O-deethylation activity similar to that of human liver microsomes, there is no large differences on P450 activity between native and modified P450 1A2 (Sandhu *et al.*, 1994). P450 expression in *E. coli* was determined by Fe^{2+} vs. Fe^{2+}-CO difference spectra of *E. coli* membrane fractions (Fig. 2). The expression levels in *E. coli* WP2 uvrA were ~300 nmol per liter culture. The published expression level of heterologously expressed human P450 1A2 in *E. coli* DH5α ranged from 245 nmol per liter culture to 950 nmol per liter culture (Sandhu *et al.*, 1994; Dong *et al.*, 1996). Maximal expression was seen at 48 hrs at 30 °C. After 48 hrs, P450 expression was slightly decreased (Fig. 3). In order to confirm high level expression of P450 1A2, bacterial membranes were analyzed by SDS-PAGE. Band at approximately 56 kDa (P450) is present in membrane derived from cultures transformed by P450 1A2 expression vector but not in membrane from cells transformed by pCW control vector (Fig. 4).

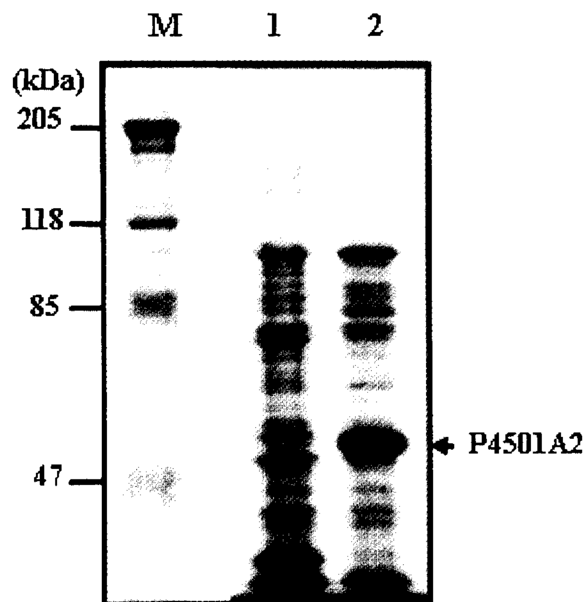

Fig. 4. SDS-PAGE analysis of *E. coli* WP2 uvrA membranes. M, protein size marker; 1, *E. coli* membrane transformed by pCW control plasmid; 2, *E. coli* membrane expressing recombinant human P450 1A2. *Arrowhead* indicates P450 1A2.

Bacterial mutagenesis

2-AA and MeIQ increased the revertant colonies of *E. coli* WP2 uvrA expressing P450 1A2 in a dose-dependent manner without rat liver S9 fraction (Fig. 5A and B). However, no change of numbers of revertants was shown in pCW-transformed cells by mutagens.

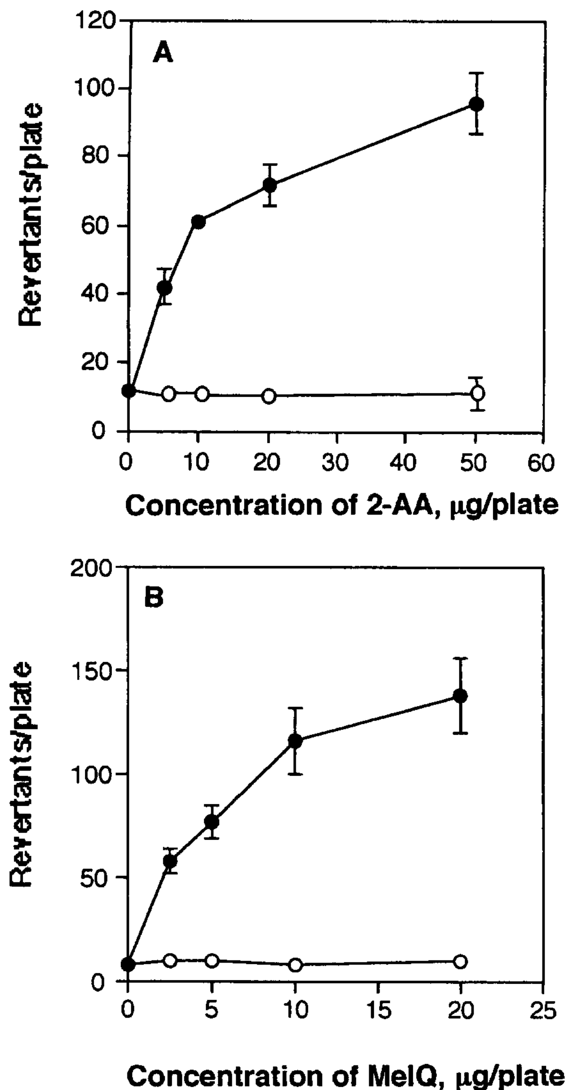

Fig. 5. Mutagenicity of heterocyclic amines in an *E. coli* WP 2 uvrA strain. Solid circles, *E. coli* WP2 uvrA expressing human P450 1A2.; open circles, control strain. (A) 2-AA (B) MeIQ. Each data represents the mean±S.D. from three separate experiments.

When 10 μg of 2-AA or MeIQ were incubated with *E. coli* WP2 uvrA in the presence of rat liver S9 fractions, 380±24 or 512±86 revertant colonies were shown.

To study the mechanism of the mutagenesis and carcinogenesis of new developing drugs, it is necessary to develop the bacterial test strain expressing human drug metabolizing enzymes. Human P450 1A2 is known to involve the bioactivation of many arylamine procarcinogens and heterocyclic amines. Thus, it might be a good candidate to express in mutagenicity testing bacterial cells. Although 2-AA and MeIQ increased the revertant colonies in 1A2-expressed cells, the response was weaker than for the cells with S9 fraction. Commercially available S9 fraction is prepared from male rat liver administered with Aroclor 1254. It contains a lot of P450 1A1 and 1A2, and electron transfer to P450 by NADPH-P450 reductase is efficient. Lower response of mutagens in expressed cell may be caused by the low electron transport from NADPH to P450 by bacterial flavoproteins. Therefore, expression of mammalian NADPH-P450 reductase with P450s in testing strains might increase the sensitivity of mutagenicity by promutagens and procarcinogens. Recently, several approaches to express both P450 and NADPH-P450 reductase in intact cells were successful. Chun and his associates (1996) have expressed an active fusion protein of human P450 1A 1 with rat NADPH-P450 reductase in *E. coli.* Human P450 3A4 with rat NADPH-P450 reductase was also expressed in *E. coli* (Fisher *et al.*, 1992; Guengerich *et al.*, 1996). Recently, an active human P450 1A2: rat NADPH-P450 reductase fusion protein was purified and characterized following heterologous expression in *E. coli* (Parikh and Guengerich, 1997). Moreover, bicistronic coexpression of P450 and NADPH-P 450 reductase was reported (Parikh *et al.*, 1997). If these coexpression plasmids are introduced into *E. coli* WP2 uvrA strain, the response will be much greater.

Josephy *et al.* (1995) reported that *S. typhimurium* strains (originated from TA1538) carrying the genes for aromatic amine N-acetyltransferase and human P 450 1A2 are quite sensitive to aromatic amines. In *S. typhimurium* TA1535 expressing rat GST 5-5 or human GSTT1-1, mutagenicity of several dihaloalkanes was enhanced (Thier *et al.*, 1993, 1996). While other systems that express human drug-metabolizing enzymes in bacterial mutagenicity test strains have been known, the new strain of *E. coli* WP2 uvrA expressing a human P450 1A2 may be a useful to consider the mutagenic potencies of new drugs and environmental chemicals although improvement of systems is required further.

Taken together, recombinant human P450 1A2 was successfully expressed in *E. coli* bacterial mutagenicity tester strain. This new developed strain will provide valuable information to elucidate the details of the mechanisms of several mutagens and carcinogens.

REFERENCES CITED

Barnes, H. J., Arlotto, M. P. and Waterman, M. R., Expression and enzymatic activity of recombinant cytochrome P450 17α-hydroxylase in *Escherichia coli. Proc. Natl. Acad. Sci. USA*, 88, 5597-5601 (1991).

Butler, M. A., Iwasaki, M., Guengerich, F. P. and Kadlubar, F. F., Human cytochrome P-450PA (P-450IA 2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci. USA*, 86, 7696-7700 (1989).

Butler, M. A., Lang, N. P., Young, J. F., Caporaso, N.

E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F. and Kadlubar, F. F., Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics,* 2, 116-127 (1992).

Chun, Y. J., Shimada, T. and Guengerich, F. P., Construction of a human cytochrome P450 1A1: rat NADPH-cytochrome P450 reductase fusion protein cDNA and expression in *Escherichia coli*, purification, and catalytic properties of the enzyme in bacterial cells and after purification. *Arch. Biochem. Biophys.*, 330, 48-58 (1996).

Dong, M. S., Bell, L. C., Guo, Z., Phillips, D. R., Blair, I. A. and Guengerich, F. P., Identification of retained N-formylmethionine in bacterial recombinant mammalian cytochrome P450 proteins with the N-terminal sequence MALLLAVFL...: roles of residues 3-5 in retention and membrane topology. *Biochemistry*, 35, 10031-10040 (1996).

Gillam, E. M. J., Baba, T., Kim, B. R., Ohmori, S. and Guengerich, F. P., Expression of modified human cytochrome P450 3A4 in *Escherichia coli* and purification and reconstitution of the enzyme. *Arch. Biochem. Biophys.,* 305, 123-131(1993).

Green, M. H. and Muriel, W. J., Mutagen testing using trp$^+$ reversion in *Escherichia coli. Mutat. Res.,* 38, 3-32 (1976).

Guengerich, F. P., Analysis and characterization of enzymes, In Hayes, A. W. (Ed.). *Principles and Methods of Toxicology.* Raven Press Ltd., New York, pp. 1259-1313, 1994.

Guengerich, F. P., Martin, M. V., Guo, Z. and Chun, Y.-J., Purification of recombinant human cytochrome P450 enzymes expressed in bacteria. *Methods Enzymol.*, 272, 35-44 (1996).

Jenkins, C. M. and Waterman, M. R., Flavodoxin and NADPH-flavodoxin reductase from *Escherichia coli* support bovine cytochrome P450c17 hydroxylase activities. *J. Biol. Chem.*, 269, 27401-27408 (1994).

Josephy, P. D., DeBruin, L. S., Lord, H. L., Oak, J. N., Evans, D. H., Guo, Z., Dong, M. S. and Guengerich, F. P., Bioactivation of aromatic amines by recombinant human cytochrome P4501A2 expressed in Ames tester strain bacteria: a substitute for activation by mammalian tissue preparations. *Cancer Res.*, 55, 799-802 (1995).

Kudlubar, F. F., Butler, M. A., Kaderlik, K. R., Chou, H. C., and Lang, N. P., Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. *Environ. Health Perspect.*, 98, 69-74 (1992).

Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685 (1970).

Maron, D. M. and Ames, B. N., Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, 113, 173-215 (1983).

Omura, T. and Sato, R., The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.,* 239, 2370-2378 (1964).

Parikh, A. and Guengerich, F. P., Expression, purification, and characterization of a catalytically active human cytochrome P450 1A2: rat NADPH-cytochrome P450 reductase fusion protein. *Protein Expr. Purif.*, 9, 346-354 (1997).

Parikh, A., Gillam, E. M. J. and Guengerich, F. P., Drug metabolism by *Escherichia coli* expressing human cytochromes P450. *Nature Biotechnol.*, 15, 784-788 (1997).

Sandhu, P., Guo, Z., Baba, T., Martin, M. V., Tukey, R. H. and Guengerich, F. P., Expression of modified human cytochrome P450 1A2 in *Escherichia coli*: stabilization, purification, spectral characterization, and catalytic activities of the enzyme. *Arch. Biochem. Biophys.,* 309, 168-177 (1994).

Sasardic, D., Boodis, A. R., Edwards, R. J. and Davies, D. S., A form of cytochrome P450 in man, orthologous to form *d* in the rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br. J. Clin. Pharmacol.*, 26, 363-372 (1988).

Shet, M. S., Fisher, C. W., Holmans, P. L. and Estabrook, R. W., Human cytochrome P450 3A4: enzymatic properties of a purified recombinant fusion protein containing NADPH-P450 reductase. *Proc. Natl. Acad. Sci. USA*, 90, 11748-11752 (1993).

Thier, R., Pemble, S. E., Kramer, H., Taylor, J. B., Guengerich, F. P. and Ketterer, B., Human glutathione S-transferase T1-1 enhances mutagenicity of 1,2-dibromoethane, dibromomethane and 1,2,3,4-diepoxybutane in *Salmonella typhimurium. Carcinogenesis,* 17, 163-166 (1996).

Thier, R., Taylor, J. B., Pemble S. E., Humphreys W. G., Persmark M., Ketterer B. and Guengerich F. P., Expression of mammalian glutathione S-transferase 5-5 in *Salmonella typhimurium* TA1535 leads to base-pair mutations upon exposure to dihalomethanes. *Proc. Natl. Acad. Sci. USA*, 90, 8576-8580 (1993).