Hydrogen Peroxide Supports Human and Rat Cytochrome P450 1A2-Catalyzed 2-Amino-3-methylimidazo[4,5-*f*]quinoline Bioactivation to Mutagenic Metabolites: Significance of Cytochrome P450 Peroxygenase

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We show that the naturally occurring hydroperoxide hydrogen peroxide is highly effective in supporting the cytochrome P450 1A2 peroxygenase-catalyzed metabolic activation of the heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) to genotoxic metabolites. Mutagenicity was assessed by the Ames assay with *Salmonella typhimurium* strain YG1012 and an activation system consisting of hydroperoxides plus either 3-methylcholanthrene-induced rat liver microsomes (rP4501A) or human P450 1A2-containing microsomes (hP4501A2). The mutagenic response was dependent on the concentration of microsomal protein, IQ, and hydroperoxides. The addition of hydrogen peroxide or *tert*-butyl hydroperoxide to rP4501A greatly enhanced the yield of histidine prototrophic (His^+) revertants. This increase was inhibited, in a concentration-dependent manner, by α-naphthoflavone, a P450 1A inhibitor. Hydrogen peroxide was the most effective peroxygenase cofactor, particularly with hP4501A2 ($K_m = 0.1$ mM). The hydroperoxide-supported activation of IQ produced reactive intermediates which bound to 2′-deoxyguanosine; LC/MS analysis of the adducts revealed the same major (protonated) adduct at $m/z = 464.4$ as previously reported for the DNA adduct formed (*in vivo* or *in vitro*) by the mixed function-catalyzed bioactivation system. None of the peroxidase-catalyzed IQ metabolites (nitro-, azo-, or azoxy-IQ) were detected. In conclusion, hydrogen peroxide in the physiological/pathological concentration range may be able to support the metabolic activation of arylamines to genotoxic products through the cytochrome P450 peroxygenase pathway.

Introduction

Heterocyclic aromatic amines (HAAs[1]) are formed in proteinaceous foods as a result of pyrolysis during cooking (*1*). Seventeen HAAs have been identified so far in a variety of foods at parts per billion levels (*1*, *2*). IQ and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) are abundant HAAs, potent bacterial mutagens, and rodent carcinogens (*2*, *3*); IQ is also a liver carcinogen in monkeys (*4*). HAAs, like many carcinogens, must be metabolically activated in order to exert their genotoxic effects. Metabolic activation occurs through N-oxidation of the exocyclic amine group catalyzed primarily by cytochrome P450 1A2 (*5*−*8*).

Cytochrome P450 (P450) enzymes are extraordinarily versatile hemoproteins that catalyze the oxidation of physiological substrates and foreign chemicals, such as drugs, pesticides, polycyclic aromatic hydrocarbons, and HAAs (*9*, *10*). The versatility also extends to the oxygen donor. Molecular oxygen serves as the natural donor when electrons are supplied to cytochrome P450 by NADPH via NADPH-cytochrome P450 reductase in the P450 monooxygenase pathway (eqs 1 and 2). In the overall reaction, equimolar amounts of substrate, O_2, and NADPH are consumed and equimolar amounts of oxidized substrate, H_2O, and $NADP^+$ are formed (*11*).

$$P450{-}Fe^{III} + O_2 + 2NADPH + 2e^- \rightarrow P450^{\bullet+}(Fe^{IV}{=}O) + H_2O + 2NADP^+ \quad (1)$$

$$P450^{\bullet+}(Fe^{IV}{=}O) + X{-}H \rightarrow P450{-}Fe^{III} + X{-}OH \quad (2)$$

Cytochrome P450 can also utilize reduced oxygen equivalents, e.g., hydroperoxides, to support the oxidation of various substrates, in a reaction that is independent of molecular oxygen, NADPH, and NADPH-cytochrome P450 reductase (*12*−*18*). Cytochrome P450 functions as a "peroxygenase" in this reaction and forms iron−oxygen species identical to the iron−oxene complex in the P450 monooxygenase pathway and analogous to peroxidase compound I, as shown in eqs 3 and 4 (*12*, *19*, *20*).

$$P450{-}Fe^{III} + ROOH \rightarrow P450^{\bullet+}(Fe^{IV}{=}O) + ROH \quad (3)$$

$$P450^{\bullet+}(Fe^{IV}{=}O) + X{-}H \rightarrow P450{-}Fe^{III} + X{-}OH \quad (4)$$

Analysis of the microsomal hydroperoxide-supported oxidation reaction has provided valuable insights into the

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[1] Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; P450, cytochrome P450; 3-MC, 3-methylcholanthrene; HAAs, heterocyclic aromatic amines; His^+, histidine-prototrophic; MROD, methoxyresorufin O-demethylation; hP4501A2, human P450 1A2-containing microsomes; rP4501A, 3-MC-induced rat liver microsomes; tBHP, *tert*-butyl hydroperoxide.

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mechanism of oxygen activation by P450 (*12*, *19*, *21*), but the significance of this pathway in intact cells remains unresolved. Recently, we reported that cytochrome P450-catalyzed xenobiotic metabolism in, and acute cytotoxicity to, isolated rat hepatocytes could be enhanced markedly by the addition of a nontoxic concentration of the alkyl hydroperoxide *tert*-butyl hydroperoxide (tBHP) (*22*). The peroxidase or peroxygenase nature of xenobiotic oxidation was not studied in this preliminary report, although further studies with intact hepatocytes showed that the P450-catalyzed homolytic cleavage of hydroperoxides initiates the formation of cytotoxic radical species (*23*). The objective of the present study was to determine whether hydrogen peroxide or fatty acid hydroperoxides, which are being produced *in vivo* particularly during the course of oxidative cell stress (*24*), can support the bioactivation of promutagens/procarcinogens to genotoxic metabolites through the cytochrome P450 peroxygenase pathway. Previously it was shown that hydrogen peroxide was much less effective that alkyl hydroperoxides in supporting cytochrome P450 peroxygenase activities (*25*−*27*). However in the following,[2] low concentrations of hydrogen peroxide were shown to effectively support the cytochrome P450 1A2-catalyzed metabolic bioactivation of a heterocyclic arylamine to DNA-reactive mutagenic metabolites.

Experimental Procedures

Chemicals. IQ (67730-10-3) and nitro-IQ were purchased from Toronto Research Chemicals (Downsview, ON). *tert*-Butyl hydroperoxide and 3-methylcholanthrene were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Hydrogen peroxide, linolenic acid, linoleic acid, resorufin, methoxyresorufin, sodium azide, and soybean lipoxygenase were obtained from Sigma Chemical Co. (St. Louis, MO). HPLC grade solvents were purchased from Calden (Georgetown, Ontario). **Caution:** *IQ, nitro-IQ, and their metabolites are carcinogenic to rodents and should be handled with the appropriate safety precautions.*

Microsomal Preparations. Adult male Sprague−Dawley rats, 250−300 g, were obtained from Charles River Canada Laboratories (Montreal, Quebec), fed *ad libitum*, and allowed to acclimatize for 1 week on clay chip bedding. Animals were pretreated by daily injection of 3-methylcholanthrene (3-MC) (25 mg/kg of body wt in corn oil, ip) or vehicle (0.5 mL of corn oil, ip) for 3 days. The animals were anesthetized by sodium pentobarbital (60 mg/kg of body wt) on day 4. Livers were removed under sterile condition and perfused with KCl solution (1.18%, w/v, 4 °C). Hepatic microsomes were prepared as described (*28*). The microsomal pellet was suspended and homogenized in sterile potassium phosphate buffer:KCl solution (50 mM KH_2PO_4 and 0.23% (w/v) KCl, pH 7.4) and stored at −80 °C. Microsomal preparations containing human P450 1A2 (hP4501A2) (from AHH-1 TK +/− cell line expressing human P450 1A2 cDNA) and control vector microsomes (from AHH-1 TK +/− cell line transfected with l-histidinol-resistance vector without human P450 1A2 cDNA) were obtained from Gentest Corp. (Woburn, MA). hP4501A2 and its control vector microsomes were sterilized by γ radiation (680 Gy) which had no effect on P450 1A2 enzymatic activity. The total cytochrome P450 content was 880 and 41 pmol of P450/mg of protein for the 3-MC-induced rat liver microsomes (rP4501A) and hP4501A2, respectively. The ethoxyresorufin O-deethylation activities of rP4501A, control rat liver microsomes, hP4501A2, and control vector microsomes were 12 500, 253, 0.6, and 155 pmol of resorufin min^{-1} (mg of microsomal protein)$^{-1}$, respectively.

Mutagenicity Assays. *Salmonella typhimurium* strain YG1012 (TA1538 1,8-DNP pYG213) was graciously provided by Dr. T. Nohmi, National Institute of Hygienic Sciences, Tokyo, Japan. This strain expresses elevated levels of acetyl-CoA: arylamine *N*-acetyltransferase and has high sensitivity for detection of mutagenic nitroarenes and aromatic amines (*29*, *30*). The mutagenicity assay was performed as described previously (*31*−*33*) except that arachidonic acid and ram seminal vesicle microsomes were replaced by hydroperoxides and rP4501A or hP4501A2, respectively. In brief, IQ, microsomes, and bacteria were preincubated in 0.5 mL of potassium phosphate buffer (100 mM, pH 7.4) at 37 °C for 2 min and then incubated for an additional 30 min following hydroperoxide addition. The mixture was plated on minimal glucose medium and incubated at 37 °C for 72 h, prior to scoring of His^+ revertant colonies with the Optimas (Microsoft Windows-based) videoimage analysis system.

Fatty Acid Hydroperoxides Preparation. Linoleic and linolenic acid hydroperoxides were prepared by the enzymatic peroxygenation of the corresponding fatty acid by soybean lipoxidase and purified as described (*34*). The P450 peroxidase activity for each fatty acid hydroperoxide was determined by measuring the rate of oxidation of tetramethylphenylenediamine as described previously (*14*). A rate of 8.30 and 2.54 μmol of oxidized tetramethylphenylenediamine min^{-1} (mg of uninduced rat liver microsomal protein)$^{-1}$ was detected for linoleic and linolenic acid hydroperoxides, respectively.

Enzymatic Assays. Microsomal protein was determined by the method of Lowry et al. (*35*), and the total cytochrome P450 content was assayed as described by Omura and Sato (*36*). Methoxyresorufin O-demethylation (MROD), an enzymatic probe for the P450 1A2 activity (*37*), was determined as follows. Briefly, microsomes were diluted with 0.1 M potassium phosphate buffer (pH 7.4) to a concentration of 50 μg/mL for rP4501A and 0.1 mg/mL for hP4501A2, which provided a linear rate of reaction for at least 10 min. Methoxyresorufin (in DMSO) was then added (0.5 μM final concentration), and the mixture was incubated at 37 °C for 3 min. A stable baseline was established, and the reaction was initiated by the addition of various concentrations of hydroperoxides. Due to the high microsomal-associated catalase activity, the catalase inhibitor sodium azide (1 mM) was added to the incubation mixture just before hydrogen peroxide addition. The increase of resorufin fluorescence was recorded using a Shimadzu RF5000U spectrofluorophotometer, with excitation and emission wavelengths of 530 and 586 nm, respectively. The fluorometer was calibrated with 100 pmol of resorufin in each experiment, and the K_m and V_{max} for each hydroperoxide were obtained using a Lineweaver−Burk plot of the reciprocal rate of reaction ($1/V$) versus the reciprocal hydroperoxide concentration (*38*).

LC/MS Analysis of IQ Metabolite:2′-Deoxyguanosine Conjugates. For identification of the deoxyguanosine:IQ metabolite adducts, a reaction mixture containing 3-MC-induced microsomes (0.2 mg/mL), 2′-deoxyguanosine (5 mM), and IQ (1 mM) in potassium phosphate buffer (pH 7.4) was preincubated for 2 min at 37 °C. Hydroperoxides (5 mM) were added in four equal portions during the 30 min incubation, and the reaction was stopped by the addition of equal volumes of acetonitrile. In the case of hydrogen peroxide, 1 mM sodium azide was added to inhibit microsomal catalase activity. Samples were centrifuged at 15000*g* for 10 min to pellet the proteins. The supernatants were concentrated under a stream of nitrogen at room temperature. The residues were dissolved in 100 μL of water:methanol (50%, v/v) and analyzed by LC/MS and selective ion monitoring (SIM), as previously described (*22*). Briefly, narrow-bore C18 Phenomenex columns (Torrance, CA) packed with 5 μm i.d. Ultracarb ODS 30 were used. This packing does not require use of triethylamine, which prevents the "tailing" of amines but severely inhibits ionization in mass spectral analysis. The dimensions of the columns used were 2 × 100 mm with a 2 × 30 mm guard column. A mobile phase of acetonitrile:water:acetic acid (30:70:1, v/v) including 1 mM ammonium acetate was used.

Statistical Analysis. One- and two-way ANOVA followed by the Scheffe's test was used for comparison among the

[2] Part of this work was presented at the 87th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 1996.

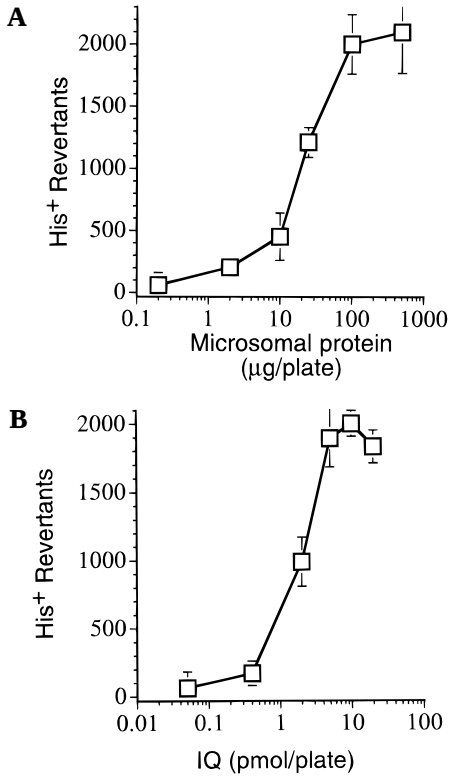


Figure 1. Hydrogen peroxide-supported P450-catalyzed mutagenic activation of IQ. (A) Enzyme concentration−response curve: The frequency of His^+ revertants was determined using various concentrations of rP4501A in the presence of hydrogen peroxide (50 nmol/plate) and IQ (10 pmol/plate) as described in Experimental Procedures. (B) Mutagen concentration−response curve: The frequency of His^+ revertants was determined using rP4501A (25 μg/plate) in the presence of hydrogen peroxide (50 nmol/plate) and various concentrations of IQ as described in Experimental Procedures. Values represent means ± SE of three separate experiments.

multiple-treated groups and the relevant controls. Results represent the mean ± standard error of the mean of triplicate samples.

Results

The bioactivation system used in the Ames assay usually comprises rat liver S9 fraction and an NADPH-generating system (*39*). We used 3-MC-induced rat liver microsomes or human P450 1A2-containing microsomes (*40*) but replaced the NADPH-generating system with hydrogen peroxide or an organic hydroperoxide. The complete system of microsomes, IQ, and hydroperoxide gave a strong mutagenic response, which was dependent on the concentrations of microsomal protein (Figure 1A), IQ (Figure 1B), and hydroperoxide (Figure 2). Using a concentration of microsomal protein within a linear range (25 μg/plate), the effect of various concentrations of naturally occurring hydroperoxides (fatty acid hydroperoxides and H_2O_2) as well as *tert*-butyl hydroperoxide on the mutagenicity of IQ (10 pmol/plate, 5 nM) was investigated (Figure 2). Hydrogen peroxide showed the highest efficacy in this system with a maximum mutagenic response of 1650 His^+ revertants/plate, whereas *tert*-butyl hydroperoxide showed a maximum mutagenic response of 500 His^+ revertants/plate. Linoleic and linolenic acid hydroperoxides were not effective in supporting the P450 1A2-catalyzed mutagenic bioactivation of IQ ($P > 0.05$, compare to control group without hydroperoxide). α-NF, a competitive inhibitor of P450

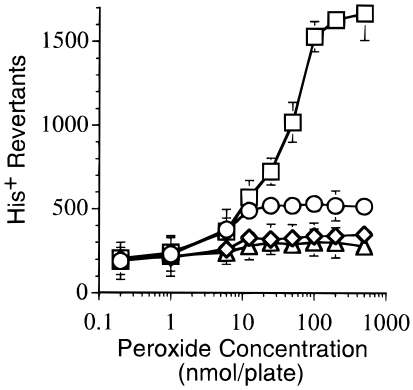


Figure 2. Effectiveness of various hydroperoxides at supporting the mutagenic activation of IQ. The frequency of His^+ revertants was determined using rP4501A (25 μg/plate) in the presence of IQ (10 pmol/plate) and various concentrations of hydrogen peroxide (□), tBHP (○), linoleic acid hydroperoxide (◇), or linolenic acid hydroperoxide (△) as described in Experimental Procedures. Values represent means ± SE of three separate experiments.

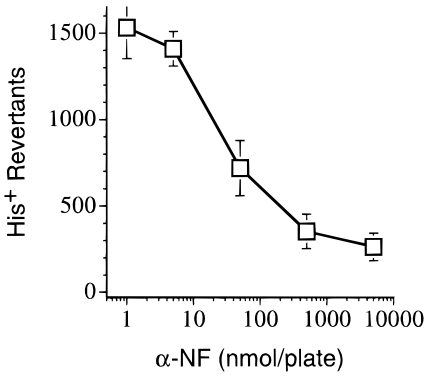


Figure 3. Hydrogen peroxide-supported P450-catalyzed mutagenic activation of IQ: effect of α-NF. The frequency of His^+ revertants was determined using rP4501A (25 μg/plate) in the presence of IQ (10 pmol/plate), hydrogen peroxide (50 nmol/plate), and various concentrations of α-NF as described in Experimental Procedures. Values represent means ± SE of three separate experiments.

Table 1. Hydrogen Peroxide- and NADPH-Supported Human P450 1A2-Catalyzed Metabolic Activation of IQ in *S. typhimurium* Strain YG1012[a]

treatment			
hP4501A2	IQ	cofactor	His^+ revertants
+	+	H_2O_2	2200 ± 83[b]
−[c]	+	H_2O_2	44 ± 5
+	−	H_2O_2	23 ± 4
+	+	−	225 ± 37
+	−	H_2O_2	33 ± 3
+	+	NADPH	1186 ± 75
−[c]	+	NADPH	25 ± 3

[a] The Ames assay was performed with IQ (10 pmol/plate, 5 nM), human P450 1A2-containing microsomes (2.05 pmol of P450/plate, 1.025 nM), and hydrogen peroxide (50 nmol/plate, 25 μM) or NADPH (1 mM) as described in Experimental Procedures. In the absence of an activation system, nitro-IQ (0.2 pmol/plate) gave 1570 ± 22 revertants/plate. [b] Data represent the means of three separate experiments ± SE. [c] Microsomes from the AHH-1 TK +/− cell line transfected with vector without human P450 1A2 cDNA were used as a negative control.

1A isoforms (*40*), inhibited the H_2O_2-dependent mutagenicity of IQ, in a concentration-dependent manner (Figure 3). Human P450 1A2-containing microsomes were also effective in the H_2O_2-dependent system (Table 1). Again, all three components of the bioactivation mixture (P450 1A2, IQ, and hydrogen peroxide) were required for

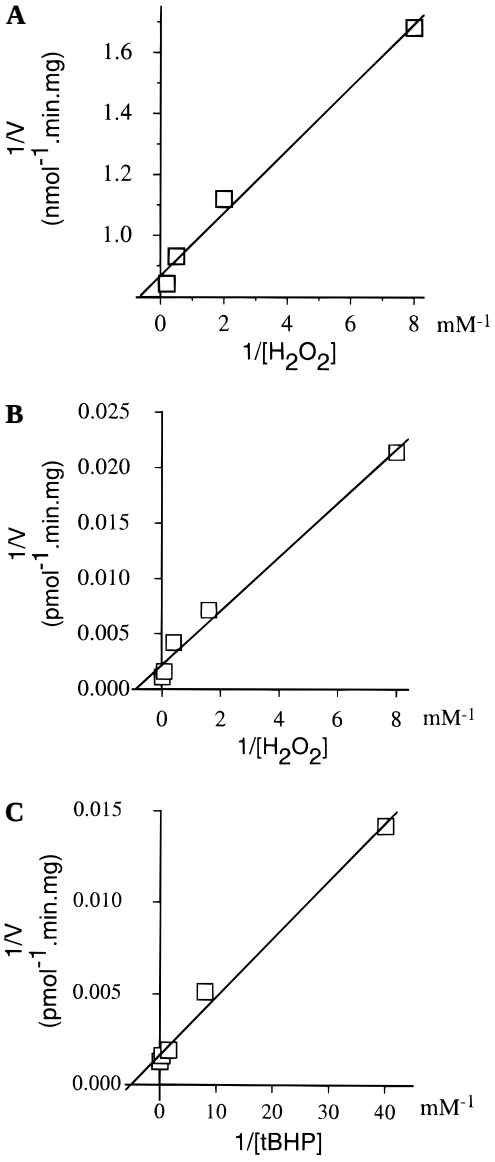


Figure 4. Kinetic analysis of hydroperoxide-supported methoxyresorufin O-demethylation: (A) hydrogen peroxide and human P450 1A2, (B) hydrogen peroxide and rat liver microsomal P450 1A2, (C) tBHP and rat liver microsomal P450 1A2. Incubation mixtures contained methoxyresorufin (0.5 mM), rP4501A (50 mg/mL) or hP4501A2 (0.1 mg/mL), and hydroperoxides. Reciprocal rate of MROD is plotted against reciprocal hydroperoxide concentration. The linear regression equations were (A) $y = 0.103x + 0.001$, $r^2 = 0.9821$, $V_{max} = 1000$ pmol min^{-1} mg^{-1}, $K_m = 103$ μM; (B) $y = 2.416x + 0.002$, $r^2 = 0.9868$, $V_{max} = 500$ pmol min^{-1} mg^{-1}, $K_m = 1208$ μM; (C) $y = 0.316x + 0.002$, $r^2 = 0.9895$, $V_{max} = 500$ pmol min^{-1} mg^{-1}, $K_m = 158$ μM. Values represent the average of two separate experiments.

the induction of the mutagenic response, and the mutagenic response was almost double that obtained by the NADPH-supported monooxygenase system under similar conditions.

We also evaluated hydroperoxide-supported P450 activity by direct enzyme assay (MROD; Figure 4). In the human P450 1A2-enriched microsomal system, MROD activity was efficiently supported by hydrogen peroxide (V_{max} = 1000 pmol of resorufin min^{-1} (mg of protein)$^{-1}$, $K_m = 103$ μM H_2O_2). With a rat liver microsomal system, MROD was also supported by hydrogen peroxide (V_{max} = 500 pmol of resorufin min^{-1} (mg of protein)$^{-1}$, K_m = 1208 μM) or tBHP (V_{max} = 500 pmol of resorufin min^{-1} (mg of protein)$^{-1}$, $K_m = 158$ μM). MROD activity however was not supported by linoleic or linolenic acid hydroperoxide (MROD activity of less than 1 pmol of resorufin min^{-1} (mg of protein)$^{-1}$). The formation of reactive metabolites from IQ was examined by trapping with the deoxyribonucleoside 2′-deoxyguanosine; adducts were analyzed by LC/MS. The major deoxyguanosine adduct was characterized on the basis of its protonated molecular ions and is probably either *N*-(deoxyguanosin-8-yl)-2-amino-3-methylimidazo[4,5-*f*]quinoline or (deoxyguanosin-N^2-yl)-2-amino-3-methylimidazo[4,5-*f*]quinoline (Figure 5). Selective ion monitoring ($m/z = 464.4$) confirmed the formation of a single major adduct ($t_R = 2.49$ min; Figure 5). Distinguishing between the two possible isomeric forms of the adduct will require further investigation. Adduct yield was dependent on hydroperoxide (Table 2), in a manner which paralleled the results of the mutagenicity and enzyme assay experiments. Neither the NADPH-supported microsomal P450 1A2 monooxygenase system nor the hydrogen peroxide-supported horseradish peroxidase system produced deoxyguanosine adducts.

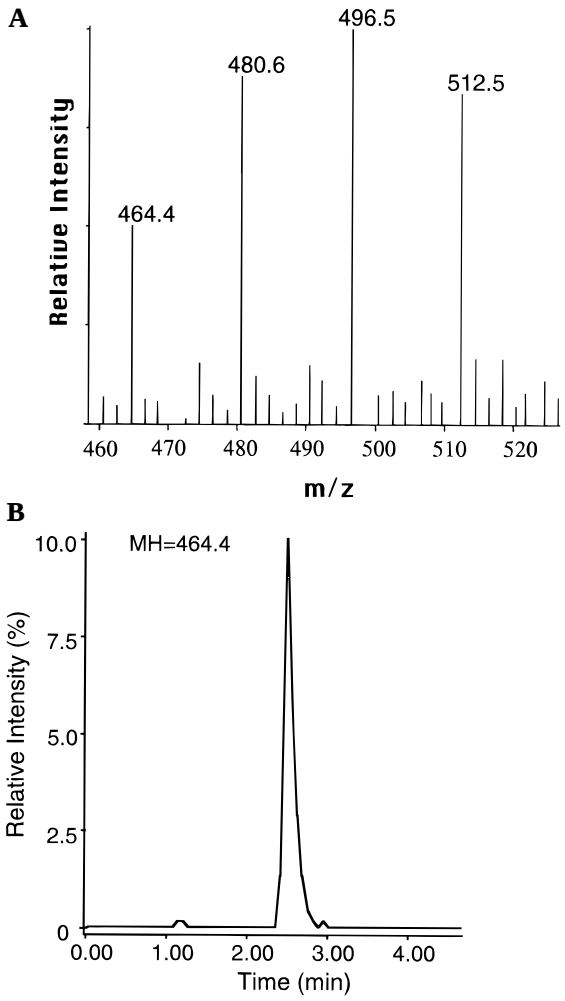


Figure 5. Analysis of IQ:deoxyguanosine adducts. (A) LC/MS analysis: Adducts were formed by the microsomal metabolism (rP4501A, 0.2 mg/mL) of IQ (1 mM) in the presence of deoxyguanosine (5 mM). Major protonated adduct peaks (MH^+) occurred at $m/z = 464.4$, 480.6, 496.5, and 512.5 and reached up to 14.8% of the total ion current. (B) Selective ion monitoring at $m/z = 464.4$: Experimental conditions were as in panel A; t_R of major peak = 2.49 min.

Discussion

Following our finding that P450 peroxidase/peroxygenase can bioactivate xenobiotics in isolated hepatocytes (*22*), we have investigated whether naturally occurring

Table 2. Hydroperoxide-Dependent Formation of IQ:Deoxyguanosine Adducts[a]

treatment			IQ:deoxyguanosine conjugates at *m/z*			
rP4501A	IQ	cofactor[b]	464.4	480.4	496.2	512.4
+	+	H_2O_2, 5 mM	1.0×10^5 [c]	1.9×10^5	2.2×10^5	1.7×10^5
+	+	−	ND[d]	ND	ND	ND
−[e]	+	H_2O_2, 5 mM	ND	ND	ND	ND
+	+	tBHP, 1 mM	2.4×10^4	4.4×10^4	8.3×10^4	7.5×10^4
+	+	linoleic HP, 0.5 mM	ND	ND	ND	ND
+	+	linolenic HP, 0.5 mM	ND	ND	ND	ND
+	+	NADPH, 1 mM	ND	ND	ND	ND

[a] Incubations contained rat liver microsomal preparation (0.2 mg/mL), IQ (1 mM), 2′-deoxyguanosine (5 mM), and hydroperoxides, as indicated. Adducts were measured by LC/MS analysis, as described in Experimental Procedures. [b] Fatty acid hydroperoxide cofactors: linoleic HP = linoleic acid hydroperoxide; linolenic HP = linolenic acid hydroperoxide. [c] Data represent the average of two measurements (arbitrary units) obtained from the area-under-curve of each adduct peak and should be used only for comparison of values for a given adduct. [d] Not detectable. [e] Microsomes from control rat livers (vehicle-treated) were used as a negative control.

hydroperoxides can support the P450-catalyzed metabolic activation of promutagens to DNA-reactive and genotoxic metabolites. Both human and rat P450 1A2 can utilize hydroperoxides and bioactivate IQ to mutagenic metabolites. The mutagenic response was dependent on each component of the bioactivation system (enzyme, peroxide, and promutagen). Hydrogen peroxide was much more efficient than *tert*-butyl hydroperoxide, though fatty acid hydroperoxides were ineffective.

Site-directed mutagenesis studies of rat P450 1A2 have demonstrated that the putative distal amino acid residues Glu 318 and Thr 319 are essential for high peroxygenase activity (*41*). Optical absorption spectroscopy studies indicated that the hydrogen peroxide O−O bond was cleaved heterolytically by the wild type rat P450 1A2, resulting in the formation of an oxo-P450−heme complex analogous to horseradish peroxidase compound I (*41*). The amino acid residues Glu 318 and Thr 319, in the oxygen-binding pocket, as well as neighboring residues are highly conserved between the rat and human 1A2 (*41*, *42*). Our results show for the first time that the human form of P450 1A2 can effectively catalyze the hydrogen peroxide-supported IQ bioactivation.

P450 isoforms which lack oxygen-binding pockets and utilize physiological lipid hydroperoxides as oxygen sources have recently been characterized in plants (*43*), platelets (*44*), and endothelial cells (*45*, *46*). The homolytic cleavage of the hydroperoxide O−O bond is involved in the catalytic cycle of these novel cytochrome P450 enzymes. Naturally occurring lipid hydroperoxides can also support the microsomal-catalyzed oxidation of retinoic acid (*47*) and diethylstilbestrol (*48*). However, linoleic and linolenic acid hydroperoxides did not support the mutagenic bioactivation of IQ by rat or human P450 1A2. It is known that the dioxygen bond in unsaturated fatty acid hydroperoxides is primarily metabolized homolytically by certain cytochrome P450 enzymes and P420 to free radicals which catalyze the above oxidations (*12*). Therefore, these hydroperoxides, although excellent peroxidase cofactors, cannot efficiently support the P450 peroxygenase-catalyzed metabolism of P450 substrates.

Hydrogen peroxide was also an efficient cofactor for another P450 1A2-catalyzed activity, i.e., methoxyresorufin O-demethylation (K_m = 0.103 mM for human P450 1A2 and 1.2 mM for 3-MC-induced rat liver microsomes). This was surprising as other investigators have reported that hydrogen peroxide was several orders of magnitude less effective that alkyl hydroperoxides in supporting microsomal P450-catalyzed benzphetamine N-demethylation (K_m = 250 mM) (*25*) or aniline hydroxylation (K_m = 18 mM) (*26*). The high concentrations of hydrogen peroxide required as a peroxygenase cofactor for P450 found by others may be attributed to catalase associated with the microsomal preparations and the possibility that it is the peroxide anion (HO_2^-) that is the reactive species interacting with other cytochrome P450 isoforms (*27*). The unusual high affinity of P450 1A2 for hydrogen peroxide warrants further investigation, particularly as such hydrogen peroxide concentrations may be formed *in vivo* by various oxidases and hydrogen peroxide formation could be higher during oxidative stress (*24*).

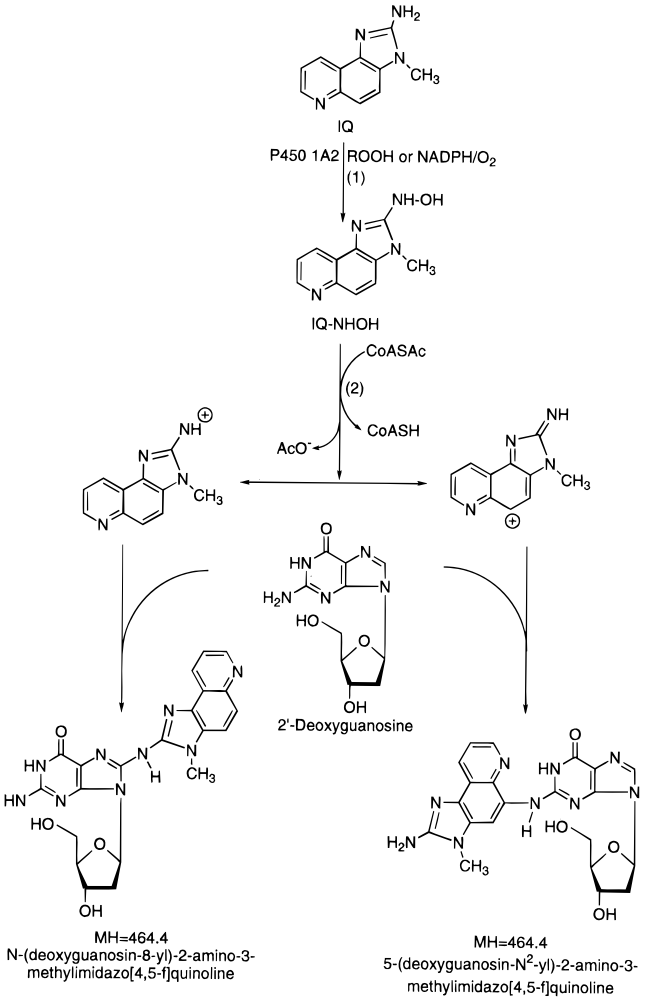
Covalent modification of DNA by genotoxins may be an initiating event in carcinogenesis (*49*). HAAs form DNA adducts primarily at the C-8 and N^2 atoms of guanine and to a lesser extent at the C-8 and N^6 positions of adenine (*50*−*52*). The activated metabolite (2-(hydroxyamino)-3-methylimidazo[4,5-*f*]quinoline, NHOH-IQ) formed by monooxygenase-dependent metabolism of IQ reacts with DNA primarily to form *N*-(deoxyguanosin-8-yl)-2-amino-3-methylimidazo[4,5-*f*]quinoline, with 5-(deoxyguanosin-N^2-yl)-IQ as a minor adduct (*53*, *54*) (Scheme 1). ^{32}P-Postlabeling analysis of DNA adducts of IQ formed *in vivo* (rodents, monkey) identified the same two 2′-deoxyguanosine adducts (*51*, *52*, *55*). We identified, by LC/MS, a major adduct which may be identical to these adducts. The formation of the deoxyguanosine adduct and its oxidized derivatives was dependent on the presence of all components of the bioactivation system. Again the order of potency for hydrogen peroxide and tBHP at supporting adduct formation was similar to that obtained in supporting the MROD reaction as well as enhancing IQ mutagenicity. Whether or not the deoxyguanosine adducts formed under these *in vitro* conditions are responsible for the mutagenic properties of IQ requires further investigation.

The *N*-hydroxy metabolites of IQ and related compounds require O-acetylation to generate a metabolite sufficiently reactive to form 2′-deoxyguanosine adducts (*56*). The bacterial strain used in this study expresses high levels of acetyl-CoA:arylamine *N*-acetyltransferase, which also catalyzes acetyl CoA-dependent O-acetylation. Prostaglandin H synthase metabolizes IQ to mutagenic products, via the peroxidase reaction; however, this metabolism proceeds by one-electron oxidation to form, ultimately, nitro metabolites (*30*, *57*, *58*). HPLC analysis of the hydrogen peroxide-supported P450 1A2-catalyzed IQ metabolites did not indicate the formation of nitro or nitroso metabolites.[3]

In conclusion, hydrogen peroxide and alkyl hydroperoxides can support the rat and human P450 1A2-catalyzed metabolic bioactivation of arylamines to reac-

[3] M. R. Anari and P. J. O'Brien, unpublished observations.

Scheme 1. Proposed Metabolic Pathway of IQ Bioactivation and Formation of Deoxyguanosine Adducts[a]



[a] (1) Cytochrome P450 1A2 monooxygenase/peroxygenase oxidation of IQ to hydroxylamine metabolite; (2) acetyl CoA:arylamine *N*-acetyltransferase-dependent formation of nitrenium- and carbon-centered cations.

tive genotoxic metabolites. P450 1A2 may be a particularly active peroxygenase with hydrogen peroxide, as a result of its distal active site structure, which favors the heterolytic cleavage of hydroperoxides. The activities of other human P450 enzymes toward hydroperoxide-supported oxidation reactions also require investigation.

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