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PROSTAGLANDIN H SYNTHASE AND XENOBIOTIC OXIDATION*

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HISTORICAL PERSPECTIVES

Exposure to environmental chemicals and other toxicants is generally recognized as an important cause of human cancer and disease. For carcinogenesis and other toxic effects, numerous investigations indicate that chemicals must be metabolized to exert their effects. For carcinogens, the accepted hypothesis is that chemical carcinogens are converted to electrophilic metabolites that react covalently with nucleophilic DNA. These DNA adducts are the ultimate molecular lesion that leads to the activation of oncogenes and, eventually, neoplasia.

The oxidation of a chemical, however, can not only enhance its toxicological activity, but also lead to a loss of activity, or result in its detoxification. The introduction of an oxygen can serve as a means of attaching polar moieties such as glutathione and glucuronic acid, which increase water solubility and hence its excretability. The metabolism of a xenobiotic is an important determinant in chemical toxicity.

Extensive literature testifies to the importance of the cytochrome P-450 (P-450)[1] monooxygenases in the metabolic activation of chemicals to reactive electrophiles. However, a 1975 report by Marnett, Wlodawer & Samuelsson (1) showed that during the oxidation of arachidonic acid (AA) to prostaglandins by prostaglandin H synthase (PHS) chemicals were oxidized. These reactions were termed "cooxidation" and the oxidized chemicals "cosubstrates." The dissimilarity between P-450 and PHS with respect to the physical properties, tissue distribution, and response to inhibitors implied that PHS could serve as an alternate enzyme for chemical metabolism, particularly in many extrahepatic tissues that are low in monooxygenase activity. In the years since this initial report, the cooxidation of chemicals during AA metabolism has been investigated in detail. These investigations indicate that multiple mechanisms exist for the metabolism of chemicals during AA oxidation, and these events can play an important role in determining the eventual toxicity of a chemical.

BIOCHEMISTRY OF PHS

AA is converted into a number of biologically active metabolites, including prostaglandins, prostacyclin, thromboxane, and leukotrienes, via either the cyclooxygenase or lipoxygenase pathways (2). PHS is the initial enzyme in the cyclooxygenase pathway that commits AA to the formation of prostaglandins (Figure 1). As such, this enzyme's activity is of primary importance in controlling the formation of prostaglandins and accordingly has received vigorous attention from researchers. Two catalytic activities copurify with PHS—cyclooxygenase and peroxidase (3). The cyclooxygenase catalyzes the addition of two moles of oxygen to one mole of AA and forms a cyclic endoperoxide hydroperoxide, prostaglandin (PG) G_2. The peroxidase sub-

[1]Abbreviations used in order of appearance: PHS, prostaglandin H synthase; AA, arachidonic acid; P-450, cytochrome P-450; PG, prostaglandin; HRP, horseradish peroxidase; PAH, polycyclic aromatic hydrocarbons; BP, benzo(a)pyrene; BP-7,8-diol, 7,8-dihydroxy-7,8-dihydroBP; anti-BPDE, anti-9,10-epoxy-7,8-dihydroxy-7,8,9,10-tetrahydroBP; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CCP, cyclopenteno[c,d]pyrene; AFB, aflatoxin B_1; AFB-2,3-epoxide, 2,3-dihydro-2,3-epoxy-AFB; FANFT, N-[4-(5-nitro-2-furyl)-2-thiazole]formamide; ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; DES, diethylstilbestrol; Z,Z-DIES, β-dienestrol; SHE, Syrian hamster embryo; SCE, sister chromatid exchange; GS·, glutathionyl free radical; GSH, glutathione; GST, glutathione S-transferase; MFO, monooxygenase; 2-NA, 2-naphthylamine; RSV, ram seminal vesicle; ESR, electron spin resonance; Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole; IQ, 2-amino-3-methylimidazo[4,5-f]-quinoline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline; Trp-P-1, (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole); Trp-P-2, (3-amino-1-methyl-5H-pyrido[4,3-b]indole); BA, benzo(a)anthracene; BA-3,4-diol, 3,4-dihydroxy-3,4-dihydrobenzo(a)-anthracene; 15-HPETE, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid.

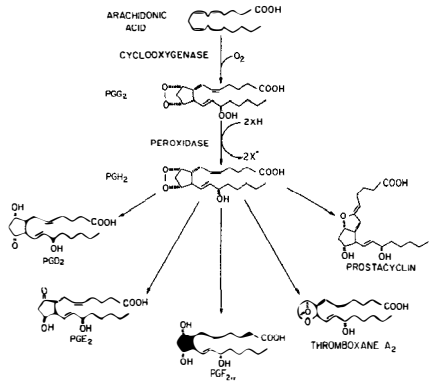

Figure 1 The metabolism of arachidonic acid to prostaglandins

sequently reduces the hydroperoxide to the corresponding alcohol, PGH_2, which is then converted into various other prostanoid metabolites, including thromboxanes, prostacyclin, and prostaglandins E_2 and $F_{2\alpha}$.

Tissue and Subcellular Localization of PHS

PHS activity is found in almost every mammalian tissue investigated, as well as in certain vertebrate and invertebrate species. The highest concentrations of PHS, and therefore the primary source for investigative studies, are found in ram or bull seminal vesicles. Other cells and tissues containing relatively high levels of PHS include kidney medulla, platelets, vascular endothelial cells, the alimentary tract, brain, lung, and bladder (4–8). PHS is an integral membrane protein and is present in the microsomal fraction of tissue homogenates. Immunocytochemical studies on the subcellular localization of PHS show it to be present in both the endoplasmic reticulum and nuclear membrane (9–11). The PHS active site is oriented on the cytoplasmic side of these membranes (12). In smooth muscle cells PHS is associated with plasma membrane (13), while in renal medulla some activity is also associated with the mitochondrial fraction (14).

Physical/Chemical Characteristics of PHS

PHS has been isolated and purified from bull and ram seminal vesicles (3, 15–17). The subunit molecular weight of the purified enzyme is about 70,000 daltons. Ram-derived PHS exists as a homodimer in detergent solutions and is a glycoprotein containing 3.5% carbohydrate with mannose and N-acetylglucosamine as the only sugar constituents (16). The carbohydrate chains are of the oligomannoside type containing six to nine mannose residues (18). The enzyme requires heme to exhibit maximal activity. Full catalytic activity is exhibited with as little as 0.53 mol heme/subunit, but greater

amounts of heme will bind to the enzyme (19–22). PHS appears to have two high-affinity binding sites for metalloporphyrins, only one of which is necessary for catalytic activity in the presence of heme (19). Other hemoproteins in addition to hematin (iron protoporphyrin IX) also support PHS activity; these include oxyhemoglobin, methemoglobin, and metmyoglobin (23). Interestingly, manganese protoporphyrin IX will support cyclooxygenase activity but not peroxidase activity.

PHS exhibits a K_m of 5 μM for both AA and oxygen (24, 25). The K_m for hydroperoxide ranges from about 2 μM for 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE) and PGG_2 (26) to almost 20 μM for 15-hydro-peroxy-8,11,13-eicosatraenoic acid (127). These differences may reflect the use of different cosubstrates in the assay, or even different concentrations of the same cosubstrate. Kulmacz recently demonstrated that K_m values for H_2O_2 and 15-HPETE varied with the concentration of the cosubstrate tetra-methylphenylenediamine present in the assay (28). Electron-donating cosubstrates significantly increase the rate of reaction by increasing the rate of reduction of higher peroxidase oxidation states and/or by protecting the enzyme from self-inactivation (29, 30). For example, phenol enhances the specific activity of purified PHS by approximately threefold (1542 mol AA/mol PHS/min with phenol-stimulated enzyme compared to 578 with native enzyme).

Before 1988 little was known about the primary structure of PHS. Roth et al (31, 32) demonstrated that aspirin acetylates a specific site on PHS (a serine residue) and sequenced a 22-amino acid segment surrounding this site, presumed to be near the active site since aspirin inhibits cyclooxygenase activity. In 1986, Hla et al (33) isolated a partial cDNA (2.2 kb pairs) coding for human PHS. In 1988, three separate groups used radiolabeled oligonucleotide probes to isolate full-length cDNAs (2.8 kb pairs) coding for PHS from ram seminal vesicles (34–36). These cDNAs were sequenced and the nucleotide and amino acid sequence of PHS are now known. The precursor peptide consists of 600 amino acids, including a 24-residue signal peptide. The serine residue susceptible to acetylation by aspirin is located near the enzyme's C-terminus. There are four potential asparagine-linked glycosylation sites as well as several hydrophobic sites that may represent points of interaction with the membrane. Using controlled tryptic digestion, PHS has been separated by HPLC into two separate fragments (33 and 38 kDa) (37). The 38-kDa fragment retains the aspirin-labeling site and the structural features responsible for the cyclooxygenase activity. The heme prosthetic group that controls PHS peroxidase activity may bind to a histidine residue located immediately adjacent to the trypsin cleavage site (38).

Neither the nature of the active site of PHS nor the nature and number of binding sites for peroxides and cosubstrates have been elucidated. Evidence

suggests that multiple sites may exist for the binding of cyclooxygenase and peroxidase substrates (24) and for the action of nonsteroidal antiinflammatory agents (39). In addition, PHS isozymes may exist in certain tissues with different catalytic properties and metabolite profiles (40). Future studies with the PHS gene, such as using site-directed mutagenesis, should help resolve fundamental questions regarding the active site of PHS.

Catalytic Properties

The cyclooxygenase and peroxidase activities of PHS are intimately related. Early studies with soybean lipoxygenase indicated that lipid hydroperoxides, the end product of lipoxygenase reactions, initiate the catalytic activity of this enzyme (41). This is apparent by the presence of a short lag period after the addition of substrate before full catalytic activity is seen. Prostaglandin biosynthesis is similarly triggered by a variety of lipid hydroperoxides, particularly those formed by lipoxygenase as well as PGG_2 (42, 43). The concentration of lipid hydroperoxides (10^{-8} M) required to initiate cyclooxygenase activity (44) is almost 100-fold lower than that needed to sustain peroxidase activity (10^{-6} M). Thus, at low concentrations of lipid hydroperoxide, cyclooxygenase activity is stimulated and increases the concentration of PGG_2, while at higher concentrations, PHS peroxidase activity is initiated and limits the concentration of lipid hydroperoxides attained in the cell.

Self-inactivation is another unusual kinetic feature of PHS, in addition to the lag period before prostaglandin synthesis begins. Under standard incubation conditions, PHS is irreversibly inactivated within 1 to 2 min after AA is added. Both the cyclooxygenase and the peroxidase are sensitive to inactivation (30). Preincubation of PHS with micromolar amounts of AA or lipid hydroperoxides inactivates PHS. Inactivation of PHS requires peroxidase turnover and can be prevented by the presence of a reducing cosubstrate, e.g. 15-HPETE inactivates PHS with an I_{50} of 4 μM in the absence of phenol compared with an I_{50} of 92 μM in the presence of phenol (30). Both cyclooxygenase and peroxidase are inactivated by 15-HPETE at similar concentrations. PHS apoenzyme is not sensitive to inactivation, indicating the importance of the heme prosthetic group in the inactivation process (30). Also, the number of hydroperoxide molecules turned over by the enzyme appears to be more important for inactivation than the absolute concentration of hydroperoxide, because the same result is obtained whether the hydroperoxide is added in small aliquots or all at once. Marshall et al (45) suggested that there is a finite limit of turnovers (1300 mol AA/mol enzyme) before inactivation occurs. The cause of this inactivation is not known, although hydroperoxide-derived enzyme-bound free radicals have been detected by electron spin resonance (ESR) (46) and may be responsible.

The cyclooxygenase activity of PHS catalyzes the insertion of 2 mol of

oxygen into 1 mol of AA. Cyclooxygenase activity starts with hydrogen abstraction at the allylic C-13 of AA. The resulting carbon-centered radical rearranges to C-11, detected with ESR using spin traps (47, 48). The C-11 radical is attacked by molecular oxygen and forms a cyclic 9,11-endoperoxide and a C-15 radical, which is attacked by a second mol of oxygen forming a peroxyl radical that then abstracts hydrogen to form PGG_2. (For detailed descriptions of this mechanism see 49, 50). Karthein et al (51) recently provided evidence that the initial step, the hydrogen abstraction at C-13, is catalyzed by a tyrosyl radical. The mechanism of PHS peroxidase is discussed below.

PHS cyclooxygenase is the site of action of the nonsteroidal anti-inflammatory drugs (52). Several different classes of chemicals inhibit the cyclooxygenase portion of PHS activity (53), some of which are substrate analogs, while others have little apparent structural relationship to AA. Markey et al (30) demonstrated that there was no relationship between the ability of a given compound to serve as a reducing cosubstrate and its ability to inhibit PHS. Enantiomers with identical ability as reducing cosubstrates had widely differing inhibitory potential, suggesting significant differences in the orientation of compounds at cyclooxygenase inhibitory sites and the peroxidase oxidation site(s). Phenolic compounds stimulate PHS cyclooxygenase activity at low concentrations but inhibit at higher concentrations. In contrast to PHS cyclooxygenase, there are no known inhibitors specific for PHS peroxidase. Compounds that inhibit PHS peroxidase-dependent cooxidation reactions, such as methimazole, probably do so by directly reducing cosubstrate free radicals rather than by inhibiting the enzyme (54).

Regulation

The regulation of PHS expression and catalytic activity is a complex, multifaceted process, which may occur at multiple levels, e.g. through the availability of substrate. AA, the physiological substrate for PHS, occurs at low levels in cells in the unesterified form (55). When radiolabeled AA is introduced into cells, less than 2% remains as free fatty acid (56). The bulk of the AA is esterified into glycerophospholipids by an acyltransferase. In addition, cytosolic proteins bind free AA and make it unavailable to PHS (45, 57). Thus, in order to initiate PHS activity, AA must be released from membrane stores by a phospholipase. In platelets this occurs via the action of two enzymes, phospholipase C and diglyceride lipase (58). Agents that stimulate the release of AA from membranes, either directly or indirectly through the phospholipase, stimulates PHS activity; these include calcium ionophores, bradykinin, cyclic AMP, and hormones (59). Conversely, agents that inhibit phospholipase activity block PHS activity. Corticosteroids inhibit AA metabolism, presumably by stimulating the synthesis of lipocortin that

directly inhibits the phospholipase (60). Recent studies, however, have questioned these conclusions (61, 62).

The irreversible self-inactivation of PHS during catalysis also regulates the quantity of prostaglandins formed. Once inactivation occurs, new protein synthesis is required before PHS activity resumes. Recent studies suggest that several agents, including platelet-derived growth factor, epidermal growth factor, interleukin-1, interleukin-2, and phorbol esters (63–67), can stimulate the de novo synthesis of new PHS. The factors involved in controlling the expression of the PHS gene are unknown.

As mentioned earlier, low concentrations of hydroperoxides are necessary to stimulate PHS cyclooxygenase activity. Agents that increase the cellular concentration of lipid hydroperoxides likewise stimulate cyclooxygenase activity. Lands (68) has estimated that intracellular concentrations of lipid hydroperoxides may be as low as 10^{-10} M. When this concentration rises to above 10^{-8} M, cyclooxygenase activity is turned on. Relatively mild vitamin E deficiency, which would raise lipid hydroperoxide levels, increases prostacyclin formation in vascular endothelial cells (69). A major cellular enzyme for lowering lipid peroxide levels is glutathione peroxidase. Unlike PHS peroxidase, which is a low capacity, high K_m enzyme, glutathione peroxidase has a lower apparent K_m and is thus more effective in controlling intracellular peroxide levels. In vitro incubations with PHS show that glutathione peroxidase is an effective inhibitor of PHS (70). Glutathione peroxidase also increases the inhibitory potency of phenolic compounds (71). The ratio of glutathione peroxidase /PHS in a given tissue may therefore be important in estimating that tissue's capacity to synthesize prostaglandins (45).

Similarly, the availability and concentration of cosubstrates can influence PHS activity. For example, acetaminophen, which is used clinically because it can inhibit cyclooxygenase activity, can also stimulate PHS activity. In vitro, acetaminophen stimulates cyclooxygenase activity at low concentrations and inhibits only at high concentrations. Likewise, depending on the dose, acetaminophen given in vivo can either stimulate or inhibit cyclooxygenase activity in a particular tissue (72). As mentioned above, this stimulation/inhibition phenomenon is typical of most phenolic compounds.

MECHANISM OF COOXIDATION

Peroxidase-Derived

Chemicals cooxidized during AA metabolism by PHS can be separated into classes depending upon their ability to donate electrons to the peroxidase. Many aromatic amines and phenols are excellent electron donors to the peroxidase, serving as reducing cosubstrates during the reduction of PGG_2 to PGH_2. During the donation of electrons, these cosubstrates are oxidized to

free radical metabolites. The classical reaction catalyzed by peroxidases is shown as:

$$\mathrm{ROOH + 2DH \xrightarrow{Peroxidase} ROH + 2D\cdot + H_2O.} \quad 1.$$

However, under some conditions, peroxidases can also catalyze peroxide reduction by direct transfer of the peroxide oxygen to an acceptor molecule as shown (73, 74):

$$\mathrm{RO^{18}O^{18}H + A \xrightarrow{Peroxidase} RO^{18}H + AO^{18}.} \quad 2.$$

The purification of PHS (3, 22) in sufficient quantities has made possible investigations into the mechanisms responsible for peroxide reduction at the expense of a cosubstrate. Early studies indicate that PHS peroxidase is very similar to horseradish peroxidase (HRP). Studies on chemical oxidations catalyzed by HRP serve as a mechanistic framework in understanding chemical oxidations catalyzed by PHS peroxidase (75, 76). As seen in Equation 1, stoichiometry between the peroxide and the reducing cosubstrate is predicted to be 1:2.

Upon reaction with peroxide, HRP and other peroxidases with ferric protoporphyrin IX, which normally exist in the Fe(III) state, undergo a two-electron oxidation. With HRP, this intermediate (compound I) is in the Fe(V) state and contains a porphyrin (π) cation radical with the structure [(protoporphyrin IX)$\cdot^{+}Fe^{IV}O$]. Compound I is reduced to compound II at the expense of an electron donor (cosubstrate) that undergoes a one-electron oxidation. Compound II has the structure [(protoporphyrin IX) $Fe^{IV}OH^{-}$] and is reduced to the resting enzyme (Fe(III)) at the expense of a second electron donor molecule. In some cases, compound I can directly transfer the oxygen atom to an acceptor molecule and is reduced to the resting enzyme. Thus, electron or oxygen transfer can occur. Because the electron donor transfers a single electron to the peroxidase, a free radical metabolite of the electron donor is generated.

Lambeir et al (77) investigated the reaction of PHS with 5-phenyl-4-pentenylhydroperoxide and observed two spectral intermediates resembling HRP compounds I and II. Ruf and his colleagues (51, 78) have extensively investigated the higher oxidation states of PHS using rapid spectroscopic methods and low temperature ESR. Two intermediates were detected and characterized (Figure 2). Intermediate I was proposed as [(protoporphyrin IX)$\cdot^{+}Fe^{IV}O$]) analogous to HRP compound I. Intermediate II resembles compound II of HRP but contains a tyrosyl radical with the proposed structure

[(protoporphyrin IX)($Fe^{IV}O$)tyr·], which is formed by an electron transfer from a tyrosine residue to the porphyrin cation radical. Intermediate II resembles the ES complex of cytochrome *c* peroxidase. Thus, intermediate II of PHS is different from compound II of HRP. A unique feature of PHS is that two enzymatic activities, the cyclooxygenase and the peroxidase, reside within a single protein. Ruf and coworkers (51, 78) have also proposed that the tyrosyl radical of intermediate II participates in the cyclooxygenase reaction as shown in the Figure 2. By this proposed mechanism, the tyrosyl radical initiates the cyclooxygenase by abstracting a hydrogen at C-13 of AA to form a carbon-centered radical. This radical rearranges and reacts with molecular oxygen to form a cyclic endoperoxide peroxyl radical that reoxidizes the tyrosine and yields the tyrosyl radical and PGG_2. Thus, the heme Fe maintains the same oxidation state during the cyclooxygenase reaction. This interaction between the peroxidase activity and cyclooxygenase activity may assist in understanding the self-inactivation of PHS that occurs during AA oxidation and may explain the protection that a reducing cosubstrate affords against this self-inactivation.

PHS peroxidase and HRP are mechanistically similar yet differences do exist. Studies on the oxidation of aminopyrine (79) by PHS peroxidase clearly indicate a stoichiometric ratio between peroxide reduction and cosubstrate oxidation of 1:2 (Equation 1). Other studies with sulindac sulfide, which is not an HRP cosubstrate, indicate a stoichiometry between peroxide reduction and sulfide oxygenation of 1:1 with the sulfoxide oxygen derived directly from the peroxide (Equation 2) (73, 80). PHS peroxidase can catalyze several different kinds of peroxide-dependent oxidations of cosubstrates and reduce a large variety of peroxides, including H_2O_2, alkylperoxides, and lipid peroxides (27). Noted exceptions are tertiary-peroxides such as cumene peroxide and phenylbutazone peroxide (81), which are not reduced by PHS. In the case

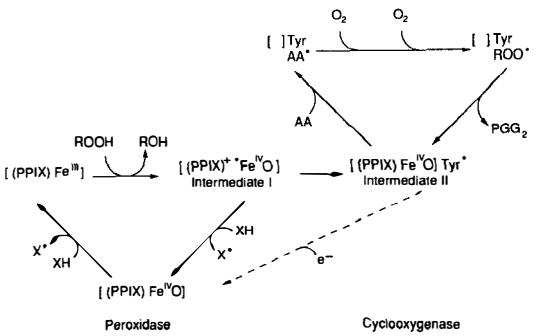

Figure 2 A possible reaction mechanism for prostaglandin H synthase

of phenylbutazone peroxide, the corresponding alcohol apparently forms by a Russell reaction rather than by direct peroxide reduction.

Markey et al (30) compared the peroxide reduction by HRP and PHS in the presence of various reducing cosubstrates and found their cosubstrate specificities very different. A plethora of reducing agents can function as cosubstrates for PHS peroxidase (27, 30, 49, 50). The ability of a given compound to serve as a cosubstrate depends on its lipid solubility (partition coefficient) as well as its redox potential. In general, any lipophilic compound with a relatively low oxidation potential (i.e. easily oxidized) will function as a cosubstrate in this reaction. This includes most phenolic compounds as well as many aromatic amines. In serving as reducing cosubstrates, these compounds donate one electron to form free radical metabolites.

Hydroperoxide-Derived Oxidation

Several xenobiotics are metabolized during oxidation of AA catalyzed by PHS, yet are not reducing cosubstrates as described above. These chemicals are oxidized by a hydroperoxide-dependent mechanism unrelated to PHS turnover. Examples include polycyclic aromatic hydrocarbons (PAH), aflatoxin, and diphenylisobenzofuran. Investigations into this mechanism of oxidation have primarily centered on the metabolism of the PAH and their metabolites (Figure 3).

During oxidation of AA by PHS, benzo(a)pyrene (BP), a very weak

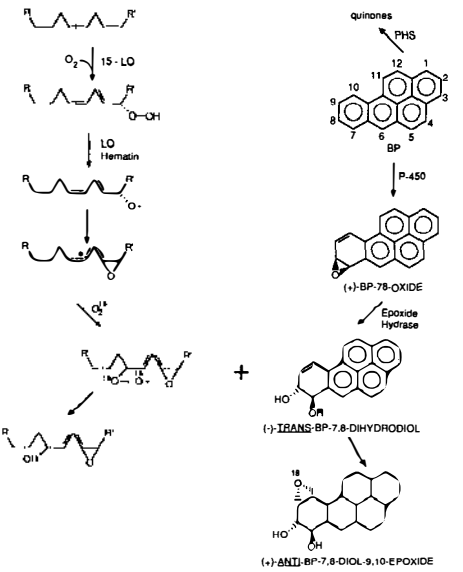

Figure 3 The metabolism of benzo(a)pyrene and its metabolites by peroxyl radicals

reducing cosubstrate, is oxidized to a mixture of quinones (82–84) by a one-electron oxidation mechanism. The quinones are formed via the intermediacy of a BP cation radical (84) and 6-hydroxyBP (85, 86). This oxidation is also catalyzed by heme or heme-containing proteins and lipid hydroperoxides. A P-450-derived metabolite of BP, 7,8-dihydroxy-7,8-dihydroBP (BP-7,8-diol), which is not a reducing cosubstrate for PHS, is epoxidized to the proposed ultimate carcinogen, anti-9,10-epoxy-7,8-dihydroxy-7,8,9,10-tetrahydroBP (anti-BPDE) with PHS, in the presence of either AA or 15-HPETE (87–89). Dix et al (90, 91) used hematin, the prosthetic group of PHS, to investigate this mechanism of BP-7,8-diol epoxidation. Hematin in the presence of lipid hydroperoxides catalyzed epoxidation of BP-7,8-diol predominantly to the anti-BPDE. For epoxidation to occur, this model system requires atmospheric oxygen, a fatty acid hydroperoxide with at least one double bond α to the hydroperoxide carbon, and detergents that solubilize the hydrophobic PAH and stimulate the catalytic activity of hematin. Atmospheric oxygen is required because the epoxide oxygen is derived from the atmosphere and not from the hydroperoxide oxygen. The epoxidation is inhibited by BHA, which suggests the mechanism involves peroxyl radicals. From these observations, Dix et al (90, 91) proposed that this mechanism of epoxidation, as catalyzed by hematin, occurs by the donation of one electron from hematin to the hydroperoxide, forming an alkoxyl radical with loss of a hydroxyl anion (Figure 3). The alkoxyl radical cyclizes to an epoxy allylic radical, which traps oxygen forming a peroxyl radical, a known epoxidizing agent. Alkoxyl radicals are generally not epoxidizing agents, as shown by the absence of epoxidation in the presence of alkoxyl radicals generated by the homolytic cleavage of cumene hydroperoxide, hydrogen peroxide, or saturated fatty acid hydroperoxides (92). Thus, formation of a peroxyl radical is a requirement for epoxidation of BP-7,8-diol to occur.

Epoxidation of BP-7,8-diol by peroxyl radicals to the anti-BPDE occurs by a stereoselective mechanism, apparently determined by the orientation of the 8-hydroxyl group. The importance of the 8-hydroxyl group on the stereoselectivity of the peroxyl radical-mediated epoxidation is observed in the epoxidation of 7,8-dihydroBP (93). Nearly identical quantities of anti- and syn-9,10-epoxy-7,8,9,10-tetrahydroBP are formed in incubations of this analog of BP-7,8-diol, PHS and AA. This mechanism is distinct from the epoxidation catalyzed by P-450, which depends more on the orientation of the PAH within the active site of the enzyme (94). The stereoselectivity of the epoxidation mediated by peroxyl radicals and P-450 can be differentiated using the (+) and (−) enantiomers of BP-7,8-diol (93, 95–99). Peroxyl radicals and P-450 epoxidize (−)-BP-7,8-diol to the (+)-anti BPDE while (+)-BP-7,8-diol is epoxidized to (+)-syn-BPDE by P-450 and (−)-anti-

BPDE by peroxyl radicals. Thus, (+)-BP-7,8-diol can be used as a probe to determine the pathway of epoxidation in different systems. This approach was used to examine the oxidation of (+)-BP-7,8-diol in mouse epidermal homogenates, keratinocytes, and intact skin in vivo (98, 99).

Other sources generate peroxyl radicals that epoxidize BP-7,8-diol. These include microsomal lipid peroxidation in the presence of either ascorbic acid or NADPH (100) and cumene hydroperoxide with microsomes (101). Studies in our laboratory and others (102, 103) indicate that 15-lipoxygenase catalyzes the epoxidation of BP-7,8-diol. The mechanism of this epoxidation appears to be analagous to the hematin/hydroperoxide system. Lipoxygenases are found in human lung epithelial cells (104) and thus may be involved in PAH-induced carcinogenesis.

CHEMICAL REACTIONS INITIATED BY PHS

Since PHS cooxidation reactions were first described in the early to mid-1970s, a large body of literature has characterized known cooxidation reactions. Although the list of reactions is extensive, diverse, and ever expanding, one common trait is shared: PHS peroxidase cooxidations are initiated as one-electron oxidations of the reducing cosubstrate to free radical metabolites. Any subsequent reactions of these free radicals will be determined by their physical and chemical properties.

Our emphasis here is toward analysis of the possible significance of selected cooxidation reactions in biological systems, rather than a detailed overview of known cooxidation reactions, which are given elsewhere (see 49, 50). A unifying theme of this article is that PHS-peroxidase catalyzed oxidation of any given reducing cosubstrate to a free radical metabolite should be viewed as the primary reaction. Cooxidation reactions have generated considerable interest because they result in the formation of reactive species, in particular, free radicals. The primary oxidation often is essentially not of great interest, but the subsequent reactions of the reactive species produced during cooxidation may often be of biological importance.

Once a reducing cosubstrate is oxidized, the resulting free radical can have several fates depending on the nature of the generated radical, i.e. its stability. This fate is further determined by whichever other chemicals are present for the free radical to react with in its environment. A few examples of general classes of cooxdination reactions catalyzed by PHS peroxidase illustrate this point.

A number of reducing cosubstrates are oxidized to carbon- or sulfur-centered free radicals that can trap molecular oxygen to form a peroxyl radical. The nonsteroidal anti-inflammatory agent phenylbutazone behaves

thus. As is illustrated in Figure 4, phenylbutazone (81) is oxidized to a carbon-centered free radical that then traps molecular oxygen to form a peroxyl radical. This peroxyl radical is a potent oxidizing agent as Reed et al demonstrated (105, 106) in studies showing that phenylbutazone oxidation by PHS peroxidase enhanced the formation of anti-BPDE in the presence of BP-7,8-diol. Peroxyl radicals can react with many different compounds; the exact fate of the phenylbutazone peroxyl radical depends upon the other compounds present in the incubation. Phenylbutazone is not the only compound whose peroxidase-catalyzed oxidation ultimately generates peroxyl radicals. (Bi)sulfite (107), retinoic acid (108), 3-methylindole (109), and indoleacetic acid (110) are other PHS peroxidase cosubstrates with this reaction mechanism.

Many phenolic compounds can serve as reducing cosubstrates for PHS peroxidase. These compounds are oxidized to phenoxyl radicals, which will undergo secondary reactions dependent upon their individual free radical chemistries. Large amounts of self-coupling products (i.e. polymeric material) are detected as major metabolites in many in vitro studies conducted with high concentrations of phenolic compounds under relatively simple incubation conditions. On the other hand, quite a different product profile is seen in an in vivo experiment where the concentration of the reducing cosubstrate is often much lower and there are more compounds present for the free radical to react with. Binding to macromolecules and reduction of the free radical by endogenous antioxidants such as glutathione commonly occur (see section VI). The same free radical is formed in vitro and in vivo, but if its environment is altered the secondary reactions it undergoes change dramatically.

Thompson et al's studies (111) with the phenolic antioxidants BHA and butylated hydroxytoluene (BHT) provide an interesting example of how the environment in which a free radical is generated affects its ultimate metabolic profile. BHA is extensively oxidized by PHS peroxidase while BHT is only marginally oxidized by the peroxidase (Figure 5). However, in incubations where both BHA and BHT are included, oxidation of BHT to the toxic BHT

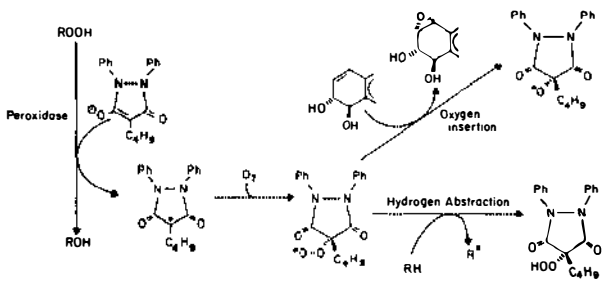

Figure 4 The oxidation of phenylbutazone by peroxidases

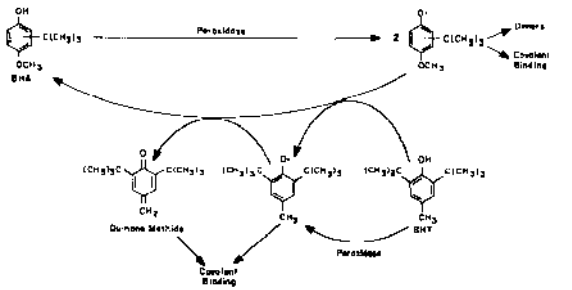

Figure 5 The metabolic interaction between BHA and BHT

quinone methide is significantly enhanced due to a direct interaction of BHT with the BHA-derived phenoxyl radical generated by the peroxidase.

Cooxidation of aromatic amines also illustrates the influences affecting the ultimate fate of the generated free radical. Aromatic amines that serve as reducing cosubstrates for PHS peroxidase are oxidized to nitrogen-centered free radicals (112–116). In N-alkyl-substituted aromatic amines, such as the anti-inflammatory agent aminopyrine (112) or the calcium indicator Quin-2 (115), dealkylation reactions are favored. In primary aromatic amines such as benzidine (116), the metabolic profile is often similar to that seen with phenolic compounds. When in vitro experiments use high concentrations of primary aromatic amines, the metabolic profile is dominated by polymeric products (117). Under in vivo conditions, the same free radical intermediates generated by cooxidation of primary aromatic amines will react with a wide variety of different compounds; high levels of binding to macromolecules are often seen in these systems.

Although peroxidases, in general, catalyze a one-electron oxidation of the reducing cosubstrates, a two-electron oxidation product is frequently observed in incubations with PHS. The chemistry of the initial free radical metabolite and the nature of the incubation mixture dictates or controls the formation of two-electron oxidation products. For some reducing cosubstrates, the free radical metabolite apparently undergoes a second one-electron oxidation. The aromatic amine tetramethylbenzidine is oxidized to a cation free radical that is subsequently oxidized to a diimine, a two-electron oxidation product (113, 114). As observed with aminopyrine, the initial free radical can also disproportionate to give a two-electron oxidation product, the iminium cation and the original substrate (112). The phenolic cosubstrate acetaminophen (118) is also oxidized by PHS peroxidase by a similar mechanism to a two-electron oxidation product, the quinone imine. Likewise, the suspected pulmonary toxicant eugenol is also oxidized by peroxidases to a quinone-methide, a two-electron oxidation product (119). Thus, for PHS

two-elcctron oxidation of cosubstrates occurs but is accomplished by consecutive reactions that occur by one-electron oxidation.

Chemical-chemical interactions are often seen with the free radical metabolites produced during cooxidation reactions and these secondary reactions may in fact be biologically significant. For example, although it may be interesting that the cooxidation of phenylbutazone results in the formation of a peroxyl radical, its subsequent reaction with other chemicals or biomolecules may be more consequential (120). Or, as with the BHA/BHT system, the biological importance may lie in the secondary reaction of the BHA-derived phenoxyl radical with BHT to form the very reactive BHT quinone methide. Oxidation of different compounds by PHS peroxidase almost invariably produces reactive intermediates. Whether or not these reactive intermediates ultimately undergo a chemical-chemical interaction will be a function of the chemistry of the free radical as well as the environment in which the reaction takes place.

Another interesting aspect of cooxidation reactions that is receiving some attention is the possible role of cooxidation in the detoxification of toxicants. Sulfur-centered free radicals, resulting from the peroxidase-catalyzed oxidation of compounds such as glutathione (121) and (bi)sulfite (107) or resulting from secondary reactions of thiols with free radicals generated during cooxidation reactions (122, 123), are capable of reacting with toxic compounds to form water-soluble conjugates. The secondary reactions of these sulfur-centered free radicals may be biologically beneficial. The mechanism and potential implications of these types of reactions are discussed below.

Thus, the fate of the primary free radical depends on the stability of the radical, the site of generation, and the environment in which it exists. The free radical can: (*a*) react with molecular oxygen as exemplified by carbon-centered free radicals, resulting in the formation of peroxyl radicals; (*b*) react with endogenous or exogenous thiols, reducing the free radical with the resultant formation of a thiyl free radical; (*c*) undergo coupling reactions resulting in the formation of oligomeric products; (*d*) undergo further oxidation to two-electron oxidation products, as shown for aminopyrine or benzidine; (*e*) react with a secondary compound to generate a secondary metabolite of potential toxicological interest, as illustrated by the interaction between BHA and BHT; and (*f*) react with macromolecules to form potentially toxic adducts, a subject discussed in detail in the following section.

BIOLOGICAL IMPLICATIONS

Although studies on the mechanisms by which chemicals are metabolized during AA metabolism are exciting and interesting, the ultimate goal is to establish the role and importance of these reactions in toxicity induced by

chemicals. The following is a review of the metabolism of selected chemicals by peroxidases and peroxyl radicals as related to the formation of toxic metabolites.

AROMATIC AMINES Aromatic amines are a class of chemicals present in the environment, the workplace, and in our food supply. Several aromatic amines are established as human carcinogens. Their importance as carcinogens is illustrated by the intense research effort devoted to understanding the mechanisms responsible for the induction of neoplasia by these agents. The central hypothesis underlying current thinking on the induction of cancer by aromatic amines originates in the work of the Millers in the 1960s (124, 125). They showed that aromatic amines are oxidized to electrophilic metabolites that covalently link to nucleophilic DNA. Since then, the characterization of the enzyme systems that activate aromatic amines and the chemical structures of the DNA adducts have been investigated. Many studies have focussed on the hepatic metabolism of aromatic amines and the induction of liver cancer by these compounds. Yet, many aromatic amines induce extrahepatic neoplasia, e.g. benzidine and its cogeners, which induce liver cancer in rats, also induce bladder cancer in humans and dogs (126–129).

Human and dog bladder epithelia have low P-450 activity but do contain appreciable PHS activity (130). Other regions of the urethral system can be rich in PHS activity. For rabbit kidney a clear segregation of PHS and P-450 activity occurs. The rabbit cortex has high P-450 and negligible PHS activity while the medulla possesses high PHS and low P-450 activity (131). These data coupled with the fact that aromatic amines can be excellent cosubstrates for peroxidases led to the hypothesis that PHS may be an important system for the activation of carcinogenic aromatic amines in extrahepatic tissues.

Metabolism of aromatic amines Aromatic amines are metabolized by PHS peroxidase via one-electron oxidation to nitrogen-centered free radicals. The ease of oxidation is altered by substituents that affect the electron density of the nitrogen. For example, benzidine is an excellent cosubstrate for PHS peroxidase, but the less easily oxidized 2-aminofluorene (2-AF) (132) is a poorer cosubstrate. Acetylation of the amine reduces the ease of oxidation by PHS and other peroxidases. Acetylbenzidine, for example, is a much poorer cosubstrate for PHS than benzidine (116). The addition of electron-withdrawing groups to the aromatic ring of N-methyl anilines decreases the metabolism of these compounds by PHS peroxidase (133).

Many investigations have furnished evidence for a one-electron oxidation of aromatic amines by PHS peroxidase to free radical metabolites. Aminopyrine is oxidized by PHS peroxidase to an aminopyrine cation free radical that was detected by ESR and characterized by UV/visible spectroscopy (79, 112).

One aminopyrine cation free radical reacts with a second in a disproportionation reaction and yields an iminium cation radical (a two-electron oxidation product) and aminopyrine. The iminium cation radical is hydrolyzed to yield formaldehyde and the monomethylamine derivative. Benzidine and 3,3',5,5'-tetramethylbenzidine undergo a one-electron oxidation by PHS peroxidase or HRP to cation radicals that can undergo a second one-electron oxidation to the diimine (113, 114, 134). The one-electron oxidation products were detected by ESR techniques and the diimine or other two-electron oxidation products were characterized by spectroscopic methods. Other studies indicate that although the carcinogen 2-AF is a poor cosubstrate (135, 136), it is also oxidized by PHS peroxidase to free radical metabolites. Because the free radicals are unstable, indirect evidence was used to support the hypothesis for a one-electron oxidation. Recently, Petry et al (137) investigated the metabolism of carcinogenic heterocyclic aromatic amines derived from the pyrolysis of amino acids and proteins. Again, free radicals were not observed by ESR but the detection of a glutathionyl radical (GS·) formed by reduction of a free radical by glutathione indicated that these amines are also undergoing a one-electron oxidation. As these examples show, aromatic amines can undergo one-electron oxidation to free radical metabolites catalyzed by PHS peroxidase. In addition, these free radicals can undergo a second one-electron oxidation by several mechanisms.

The stability of isolated metabolites produced by PHS peroxidase catalyzed cooxidation of aromatic amines is dictated by the chemical nature of the free radical. Tertiary and secondary aromatic amines undergo N-dealkylation, e.g. aminopyrine and N-methylaniline are N-demethylated (133). Recent studies (115) with calcium indicator dyes such as Quin 2 indicate oxidative cleavage of the alkyl group from the nitrogen. This results in a loss of the ability of Quin 2 to sequester Ca^{+2} and consequently its indicator properties. For primary aromatic amines, the free radicals undergo nitrogen-to-nitrogen or nitrogen-to-carbon coupling reactions. The carcinogen 2-AF is oxidized by PHS peroxidase and HRP to 2,2'-azobisfluorene, 2-aminodifluorenyl amine and polymeric material (136) as shown in Figure 6. Also detected was 2-nitro-fluorene. Extensive studies with this aromatic amine indicate that N-hydroxylation is not catalyzed by PHS peroxidase since N-hydroxy-2-aminofluorene was not detected in incubation mixtures. Benzidine is also oxidized to azobenzidine by peroxidases, but a complete characterization of the stable metabolites formed by PHS peroxidase or HRP has not been published. The bladder carcinogen 2-naphthylamine (2-NA) (117) is oxidized by PHS peroxidase to 2-amino-1,4-naphthoquinone-N^4-naphthylamine presumably formed by the coupling of 2-NA to the oxygenated intermediate 2-imino-1-naphthoquinone (Figure 7). In contrast, HRP produces nitrogen-to-nitrogen and nitrogen-to-carbon coupling products indicative of free radical

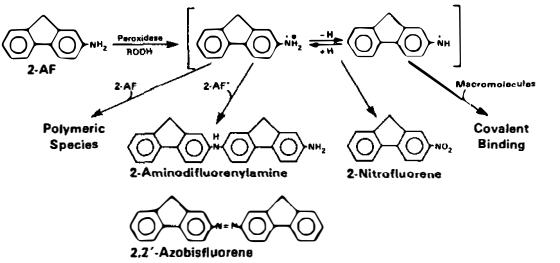

Figure 6 The oxidation of 2-aminofluorene by PHS

oxidation. The formation of oxygenated metabolites with PHS, but not with HRP, illustrates the potentially important differences between the peroxidase mechanisms for these two enzymes.

Formation of electrophiles and mutagens The formation of electrophiles by PHS peroxidase or HRP-catalyzed oxidation of aromatic amines was investigated by measuring the binding, presumably covalently, to macromolecules. The most common approach uses radiolabeled aromatic amines and measures the binding to protein, DNA, and t-RNA, in some cases. As expected, PHS peroxidase- or HRP-dependent binding to macromolecules is reported for a significant number of aromatic amines, including benzidine and its derivatives (138–141), 2-AF (117, 2-NA (117), dianisdine (141), 4-aminobiphenol (140), and a series of heterocyclic amino acid pyrolysates (142). Benzidine oxidation appears to result in the highest degree of covalent binding to DNA but the studies are difficult to fully interpret because polymer formation can interfere with the assay.

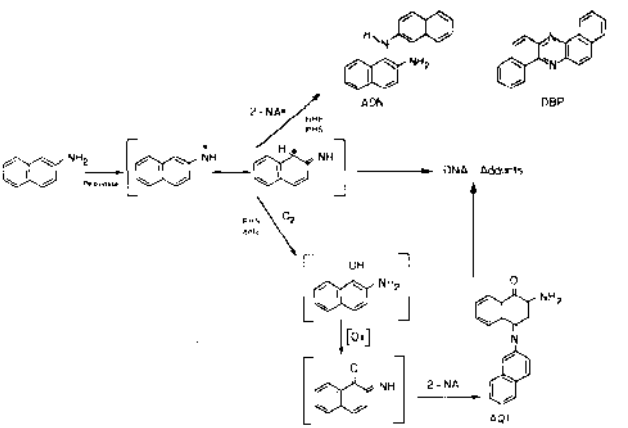

Figure 7 The oxidation of 2-naphthylamine by PHS and HRP

The formation of mutagenic metabolites of aromatic amines catalyzed by PHS peroxidase was extensively investigated by coupling ram seminal vesicle (RSV) microsomal fraction as an activating system to bacterial tester systems such as *Salmonella typhimurium* TA-98. As seen in Table 1, PHS peroxidase oxidizes many aromatic amines to products that are mutagenic. In addition to the classical aromatic amine carcinogens such as benzidine and 2-NA, heterocyclic amino acid pyrolysates such as IQ and MeIQ, which have more recently been identified as carcinogens, are likewise activated to mutagens by PHS peroxidase (143, 144).

In other studies, we have used an acetyltransferase-deficient bacterial tester strain, TA-98/1,8-DNP_6, which gave essentially no mutagenic response to benzidine or 2-AF. Acetylbenzidine was more mutagenic (141), despite being a poorer cosubstrate than benzidine for PHS peroxidase. This finding implies that bacterial acetyltransferases play a role in the formation of the ultimate mutagen. Recently, we have used a bacterial strain developed by Watanabe (145) from TA-1538 with high acetyltransferase activity. With this new strain, pYG-121, a number of aromatic amines, including benzidine, 2-AF, and the heterocyclic aromatic amino acids, exhibit enhanced mutagenic response catalyzed by PHS peroxidase (see Table 1). Studies with this new strain indicate that the bacteria supply hydrogen peroxide to PHS peroxidase, which may explain the poor sensitivity to inhibition by indomethacin and the independence from arachidonic acid reported previously. The role of bacterial acetyltransferases in mutagen formation is, at present, an enigma and may indicate potential uncharacterized PHS-dependent metabolites. Note that

Table 1 Formation of aromatic amine mutagens by PHS

Chemical	Relative mutagenicity	
	TA-98	pYG-121
2-Aminofluorene	++	++++
Benzidine	+	++
Acetylbenzidine	+++	+++++
2-Naphthylamine	++	NT
2,4-Diaminoanisole	+	NT
2,5-Diaminoanisole	++	NT
Aniline		NT
Acetylaminofluorne		--
Trp-1-P	–	--
Glu-P-1	–	+
IQ	++	++++
MeIQ	++	++++
3,3-Dimethoxybenzidine	–	–
o-Toludine	–	–
3,3',5,5'-Tetramethylbenzidine		–

NT = Not tested

HRP does not support mutagen formation in these test systems and illustrates a difference between these two peroxidases.

Characterization of aromatic amine-DNA adducts Characterization of DNA adducts could provide unique biochemical markers to measure peroxidase-catalyzed activation of aromatic amines in vivo. However, characterization is exceptionally difficult due to problems in preparing the DNA adducts in sufficient quantities and purity for structural analysis. Kadlubar and coworkers characterized several DNA adducts of benzidine (146, 147) and 2-NA (148). They reacted a chemically prepared and presumed reactive intermediate with calf thymus DNA in vitro. The resultant DNA adducts were enzymatically hydrolyzed and isolated by HPLC. For benzidine, the reaction with benzidine diimine yielded an adduct characterized as N-(deoxyguanosin-8-yl) benzidine (146). In a subsequent study (147), these workers isolated this adduct from incubations of benzidine with HRP or PHS. However, several additional uncharacterized adducts were observed. Kadlubar (148) also characterized the peroxidase-catalyzed formation of three unique 2-NA-DNA adducts. These adducts accounted for ~60% of the total DNA binding obtained by incubation of [^{3}H]2-NA with PHS in vitro and for ~20% of the [^{3}H]2-NA bound to dog urothelial DNA in vivo. The remaining adducts were identical to those previously reported as products of the reaction of N-hydroxy-2-NA with DNA.

We have also attempted to characterize the peroxidase-catalyzed DNA adducts of 2-AF (149). These studies indicate the formation of two different adducts from *N*-(deoxyguanosin-8-yl)-2-AF adducts formed by the reaction of N-hydroxy-2-AF with deoxyguanosine. An intensive effort to characterize the peroxidase-catalyzed adducts of 2-AF has not been successful, primarily because we were unable to prepare an unstable intermediate for reacting with DNA in vitro. Only small amounts of these adducts could be prepared by catalyses with HRP or PHS peroxidase.

PHS activation of aromatic amines in vivo Evidence to support PHS activation of carcinogens in vivo has come from several studies measuring the DNA-adducts detected in tissues from dogs fed a dose of an aromatic amine carcinogen. Dogs are used in these studies because they develop bladder cancer as do humans upon exposure to aromatic amines. These studies are exceptionally difficult and costly as the animals must be given curie amounts of the ^{3}H-labeled aromatic amine, which contributes to the high costs and causes decontamination problems. Despite the high amount of radioactivity administered, the level of ^{3}H bound to DNA is low. In spite of this difficulty, these studies detected peroxidase-catalyzed aromatic amine adduct formation. Kadlubar observed peroxidase-catalyzed benzidine (147) and 2-NA DNA

adducts (148) in the urethelium but not the liver of dogs. Krauss et al (149) recently detected peroxidase-catalyzed 2-AF-DNA adducts in the kidney and bladder epithelia of dogs fed 2-AF. Zenser et al (150, 151) characterized glutathione adducts of benzidine and ANFT catalyzed by PHS. They also observed this adduct in incubations with rabbit medullary slices. These data indicate that peroxidase-mediated activation of aromatic amines occurs in vivo, but further studies are required to fully support the hypothesis that PHS may be important in the activation of carcinogenic aromatic amines in vivo.

POLYCYCLIC AROMATIC HYDROCARBONS PAH require metabolism to reactive metabolites to elicit their carcinogenic response. BP is used extensively as a model compound for metabolism studies of PAH. Three general types of BP metabolites are detected that include quinones, phenols, and dihydrodiols. The accepted hypothesis of BP-induced carcinogenesis is that the P-450-derived BP metabolite BP-7,8-diol is epoxidized at the 9,10-position to form the ultimate carcinogen, the bay region diol epoxide (Figure 3).

PHS peroxidase oxidizes BP to a mixture of quinones (82–84) and similar results are observed with HRP (84). The cooxidation of BP results in the formation of reactive metabolites that bind covalently to protein and nucleic acids (83, 84, 152). DNA adducts isolated from these incubations indicate that the 6-position of BP is linked to the C-8 and N-7 positions of guanine (84). Though binding to DNA of BP metabolites formed by PHS peroxidase is observed, BP is not activated by PHS to a mutagenic product as measured in bacterial tester systems (153, 154).

BP-7,8-diol is epoxidized to predominantly the anti-BPDE by peroxyl radicals formed during metabolism of AA by microsomal PHS (87, 88). AA-dependent epoxidation of BP-7,8-diol is catalyzed by microsomes isolated from sources that include the target organs of BP-induced tumorigenesis such as rat lung, mouse skin, and human lung (155). BP-7,8-diol is oxidized in the presence of PHS to derivatives that bind to protein and nucleic acids (95, 155). BP-7,8-diol and other PAH dihydrodiols are mutagenic in bacterial tester systems with PHS and AA (153, 154). PHS is more selective than P-450 in activation of PAH dihydrodiols to mutagenic products. Only those dihydrodiols with double bonds adjacent to the bay region area are mutagenic with PHS (154).

BP-7,8-diol oxidation to anti-BPDE by peroxyl radicals is observed in cellular systems. Mouse embryo fibroblasts, which contain PHS and P-450, metabolize BP-7,8-diol to anti- and syn-BPDE (156). Formation of anti-BPDE and transformation of these cells is significantly enhanced by the addition of AA. The enhancement induced by AA is blocked by the cyclooxygenase inhibitor indomethacin. The basal level of BP-7,8-diol oxidation is

unaffected by indomethacin, suggesting that P-450 mediates the basal level of epoxidation of BP-7,8-diol, but with elevated concentrations of AA, PHS has a role in the epoxidation. Enriched fractions from Clara cells and alveolar type II cells isolated from rat lung also epoxidize BP-7,8-diol by an AA- and P-450-dependent mechanism (157).

AA stimulates anti-BPDE formation and binding to DNA in explant tissue cultures prepared from hamster trachea and human bronchus (158). Indomethacin inhibits the enhancement of epoxidation in the hamster trachea, but has no effect in the human bronchus. Hamster trachea metabolizes AA primarily to PHS metabolites, whereas human bronchus metabolizes AA to lipoxygenase products. Thus, two different AA-metabolizing systems may be supplying the peroxyl radical necessary for anti-BPDE formation. Recently, we observed that 15-lipoxygenase epoxidizes BP-7,8-diol by a mechanism analagous to the hematin/hydropcroxide system (103). Lipoxygenases are ubiquitous enzymes present in mammals and 15-lipoxygenase is present in human pulmonary epithelial cells in high concentrations (104). They may have an important but unrecognized role in human carcinogenesis.

Mouse skin, a target organ of BP-induced carcinogenesis, possesses P-450 (159) and PHS (160) activity. Keratinocytes freshly isolated from hairless mice were used to investigate which system was epoxidizing (+)-BP-7,8-diol (98). The anti-BPDE was the predominant product in cells isolated from control animals, indicating peroxyl radical-mediated epoxidation. Neither exogenously added AA nor indomethacin had an effect on epoxidation. Formation of the anti-BPDE appeared to be correlated with formation of peroxyl radicals within the cells, primarily by lipid peroxidation. The syn-BPDE was the major product in cells isolated from mice pretreated with β-napthoflavone, an inducer of P-450 with high specific activity towards PAH (96, 97). Thus, peroxyl radicals are responsible for epoxidation of BP-7,8-diol in skin of untreated animals, while P-450 is responsible in the skin of induced animals.

BP-7,8-diol is not the only PAH epoxidized during metabolism of AA by PHS; 3,4-dihydroxy-3,4-dihydrobenzo(a)anthracene (BA-3,4-diol) is also epoxidized to the respective anti-diol epoxide in systems containing either PHS or hematin (161). The dihydrodiol metabolites of BA and chrysene are mutagenic in the bacterial tester systems with PHS (154). Reed et al (162) recently investigated the metabolism of cyclopenteno[c,d]pyrene (CPP), a carcinogenic PAH that lacks a bay region. The activation of this compound requires a single epoxidation across the nonaromatic cyclopentene ring double bond, which is equivalent to the bay region 9,10 double bond of BP-7,8-diol. Systems that generate peroxyl radicals, including PHS and AA, hematin and 15-HPETE and the autoxidation of sulfite (see below) converted CPP to a

mutagenic species. Thus, peroxyl radicals may be involved in the formation of a number of carcinogenic epoxides.

ACETAMINOPHEN Acetaminophen is a commonly used analgesic/antipyretic that induces hepatotoxic and nephrotoxic responses in overdoseages (163–167). The current hypothesis of acetaminophen-induced hepatotoxicity is that acetaminophen undergoes a two-electron oxidation catalyzed by P-450 (166), forming the reactive N-acetyl-*p*-benzoquinone imine (169–171). Conversely, the mechanism of acetaminophen-induced nephrotoxicity is unclear. Acetaminophen is an excellent cosubstrate for PHS peroxidase. High levels of PHS and low levels of P-450 are found in regions of the kidney where acetaminophen toxicity occurs (172), suggesting a possible role for PHS. Microsomal PHS prepared from RSV (173) and rabbit renal medulla (174) activate acetaminophen to a species that covalently binds protein. Glutathione inhibits binding to protein by reacting with activated acetaminophen and forming the conjugate, 3-(glutathion-S-yl)acetaminophen (173).

Acetaminophen undergoes the classical one-electron oxidation catalyzed by PHS peroxidase (118, 175) and HRP (176, 177). The product thus formed is N-acetyl-p-benzosemiquinone imine (178). Acetaminophen polymers, products of a free radical-coupling reaction between N-acetyl-p-benzosemiquinone imine and acetaminophen molecules (118, 115), are isolated as stable products. The N-acetyl-p-benzoquinone imine is also formed by PHS—probably by two sequential one-electron oxidations, although some data suggest a direct two-electron oxidation of acetaminophen (118, 175).

AFLATOXIN B_1 Aflatoxin B_1 (AFB) is a potent hepatotoxin and hepatocarcinogen in several animal species (179) and is implicated in human carcinogenesis (178). Hepatic P-450 appears to be important in the epoxidation of AFB (181–183) to the reactive 2,3-dihydro-2,3-epoxy-AFB (AFB-2,3-epoxide). In extrahepatic tissue, a role for PHS activation of AFB was suggested by the finding that AFB feeding produced a 50% increase in neoplasms of rat renal papilla (184). This tissue has significant PHS activity (185) and minor P-450 activity (186). Initial studies investigating cooxidative activation of AFB during metabolism of AA were conducted with mouse embryo fibroblasts (187). Binding of AFB metabolites to DNA in these cells is decreased in the presence of inhibitors of PHS, lipoxygenase, and phospholipase A_2. The DNA adduct, AFB-N7-guanine, formed by the reaction of AFB-2,3-epoxide with guanine (188), was isolated from these cells. Additional studies (189) with AFB using microsomal PHS and AA as an activating system detected this same DNA adduct and produced a mutagenic response in bacterial tester systems. From this observation it was proposed (189) that

AFB is epoxidized to the AFB-2,3-epoxide by peroxyl radicals via a mechanism similar to that described for BP-7,8-diol. Others have proposed that AFB is membrane-active, stimulating the release and metabolism of AA to oxidizing radicals (187, 190) resulting in enhanced activation of AFB. However, AFB is unable to stimulate ^{3}H-AA release and formation of prostaglandins in prelabeled cells (191).

5-NITROFURANS A class of potent bladder carcinogens are the 5-nitrofurans (190). Several nitrofuran analogs, including N-[4-(5-nitro-2-furyl-2-thiazole]formamide (FANFT) and 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) are cosubstrates for PHS peroxidase (193–196). ANFT is oxidized by microsomal PHS prepared from RSV, rabbit renal medulla, and dog bladder epithelium to an intermediate that binds protein and DNA (194–196). ANFT is oxidized by PHS peroxidase to a reactive species (151) that can be trapped by glutathione to form the conjugate, 2-amino-4-(5-nitro-2-furyl)-5-(glutathion-S-yl)thiazole. Since PHS peroxidase-catalyzed metabolism and binding to protein of ANFT is greater than that for FANFT, carcinogenesis induced by FANFT is proposed to require deformylation to ANFT followed by PHS oxidation (196).

An important point we have attempted to emphasize in this review is the role PHS has in the in vivo activation of carcinogens. Several studies have attempted to answer this question by conducting carcinogenicity studies with FANFT and the PHS cyclooxygenase inhibitor aspirin. Many interesting and complex results have been reported. Aspirin inhibits FANFT-induced preneoplastic hyperplasia (197) and carcinoma formation (198) in the bladder of rats when coadministered with FANFT. In a two-stage carcinogenesis study (199), aspirin specifically inhibited FANFT initiation and sodium saccharin promotion of rat-bladder tumor formation. Final conclusions from these studies are problematic because of perplexing results from other studies. Forestomach tumors, normally not found in rats fed either FANFT or aspirin, form when these chemicals are coadministered (198). FANFT does induce forestomach hyperplasia, although autoradiography studies (200) show the coadministration of aspirin had no effect on this hyperplasia. Also, a recent study reported that aspirin itself had weak promoting activity in FANFT-initiation of bladder tumor formation (201). These studies suggest PHS peroxidase has a role in the in vivo activation of FANFT. However, other mechanisms, including the inhibition of prostaglandin formation by aspirin, may have a role in FANFT-induced carcinogenesis.

DIETHYLSTILBESTROL The synthetic estrogen diethylstilbestrol (DES) is carcinogenic in experimental animals and humans (202). The mechanism of

DES carcinogenicity is most likely due to metabolism of DES to reactive intermediates, but the estrogenic properties of this chemical may have an effect. DES, a phenolic compound, is a cosubstrate for peroxidases, including PHS (203–206), and is oxidized by these enzymes to reactive derivatives that covalently bind to protein and DNA (203, 206). The final product of peroxidase-catalyzed oxidation of DES is β-dienestrol (Z,Z-DIES) (207). This metabolite is found in the urine of mice treated with DES. Z,Z-DIES is formed via the intermediacy of the DES semiquinone and DES quinone, the one- and two-electron oxidized products of DES (204). The DES quinone then tautomerizes to the final product Z,Z-DIES. The DES quinone is also a reactive species as suggested by its nonenzymatic binding to DNA (208). Despite this binding, however, DES quinone is inactive as a mutagen in bacterial tester systems.

Syrian hamster embryo (SHE) cells metabolize DES to Z,Z-DIES (209), which can be stimulated by addition of AA. The formation of Z,Z-DIES can be inhibited by indomethacin, suggesting a role for PHS in the oxidation of DES in these cells. DES and DES analogs possessing varying degrees of estrogenic potency induce aneuploidy and neoplastic transformation of SHE cells (210, 211). A good correlation exists between oxidation of the DES analogs via a peroxidase-mediated pathway and their ability to induce cell transformation. Moreover, there was no correlation between the estrogenic potency and transforming ability of the chemical, indicating the possible importance of peroxidase-mediated activation of these chemicals. Other studies indicate that DES-induced sister chromatid exchanges (SCE) in human lymphocytes are not associated with DES-derived DNA adducts (212). Indomethacin partially reduces the DES-induced SCE, suggesting a role for PHS in the activation of DES. Recent observations from Metzler and coworkers (213) indicate the peroxidative metabolism of DES results in site-specific covalent binding of DES derivatives to tubulin, a protein involved in formation of mitotic spindles. Binding to this important protein may be part of the mechanism inducing transformation of cells exposed to DES.

SULFUR DIOXIDE OR (BI)SULFITE Sulfur dioxide, the food preservative and air pollutant, exists in aqueous solution as bisulfite or sulfite (collectively referred to as (bi)sulfite). (Bi)sulfite is a suspected cocarcinogen as it increases the pulmonary carcinogenicity of PAH (214, 215). Increased formation of the ultimate PAH carcinogen, the bay region diol epoxide, via a peroxyl radical-mediated pathway may explain this carcinogenicity. (Bi)sulfite is cooxidized by PHS peroxidase and HRP (106, 107, 216, 217) to a sulfur trioxide radical anion ($\cdot SO_3^-$) that traps molecular oxygen to form a peroxyl radical, which then may oxidize a PAH-dihydrodiol and form PAH

diol epoxides. By this mechanism, the presence of (bi)sulfite could enhance the formation of PAH diol epoxides, which may contribute to the pulmonary cocarcinogenicity observed for sulfur dioxide.

MISCELLANEOUS COMPOUNDS Many other compounds are cooxidized by PHS peroxidase to reactive intermediates that may play a role in toxicity or carcinogenicity in vivo. A few recent examples of compounds for which preliminary evidence indicates that PHS peroxidase-dependent activation may be important are described below.

The phenolic antioxidant BHT has a variety of toxic, tumor-modulatory and possible carcinogenic effects in laboratory animals (218, 219). Some of the toxic effects of BHT are associated with the P-450-mediated formation of a quinone methide metabolite, a reactive two-electron oxidation product (220, 221). Recent evidence suggests that peroxidases are also capable of oxidizing BHT to quinone methide under certain conditions (111). BHT is a relatively poor reducing cosubstrate for PHS peroxidase and HRP (30, 222). The formation of a phenoxyl radical from HRP-catalyzed reactions was recently described (223). In PHS peroxidase-catalyzed reactions (111), covalent binding of ^{14}C-labeled BHT to protein was observed although only a relatively small percentage of parent BHT (23%) was metabolized. In contrast, in the prcsence of another more efficicnt cosubstrate for PHS pcroxidase, BHA, the metabolism of BHT was greatly enhanced (85% metabolized). In addition to increased covalent binding, BHT quinone methide and dimeric products of BHT were detected. The phenoxyl radical of BHA may therefore facilitate the activation of BHT via electron transfer (224). Several other phenolic and amine compounds also stimulate the oxidation of BHT by a similar mechanism, including endogenous compounds present in tissues where BHT-mediated toxicity occurs (225). This peroxidase-dependent pathway provides an alternative mechanism for the formation of BHT quinone methide, the ultimate toxic metabolic of BHT, which is distinct from the P-450 pathway. Such a peroxidase-dependent interaction may occur in vivo. Thompson & Trush (226, 227) reported that BHA enhanced the pulmonary toxicity of BHT in mice and that this may occur through the peroxidase-dependent enhanced formation of BHT quinone methide.

Phenytoin (diphenylhydantoin, Dilantin) is a widely used anticonvulsant drug with teratogenic effects in mice, rats, and rabbits, and associated in humans with a variety of fetal defects, characterized as the fetal hydantoin syndrome (228). The P-450-derived reactive arene oxide metabolite is presumed responsible for the toxicity of phenytoin (229). However, the teratogenicity of phenytoin analogs such as trimethadione, which lack phenyl rings necessary for the formation of arene oxides, suggests that alternate

pathways of activation may be important. The peroxidase-dependent formation of reactive intermediates from phenytoin was recently demonstrated by Kubow & Wells (230). PHS peroxidase-mediated cooxidation of phenytoin resulted in the formation of a free radical metabolite and the covalent binding of ^{3}H-phenytoin to protein. Aspirin inhibits the in vivo teratogenicity of phenytoin in mice as does the antioxidant caffeic acid and the spin trap α-phenyl-N-*t*-butylnitrone (231). Aspirin likewise inhibits teratogenicity caused by trimethadione and dimethadione (232). In addition, the administration of the tumor promoter 12-0-tetradecanoylphorbol-13-acetate, which stimulates AA release, enhances the teratogenic effects of phenytoin (233). These results suggest a significant role for PHS in the teratogenicity of phenytoin.

Chronic exposure to benzene is associated with hematopoetic toxicity (234). The bone marrow effects of benzene are thought to be due to metabolites rather than the parent compound (235). Recent attention has focussed on the peroxidase-mediated oxidation of the phenolic metabolites of benzene. Hydroquinone, an hepatic metabolite, accumulates in bone marrow following exposure of rats to benzene and has toxic effects similar to benzene (235, 236). After exposure to benzene, PHS activity increases in bone marrow and the suppression of bone-marrow cell function by benzene can be blocked by PHS inhibitors (237). Hydroquinone is converted by PHS to reactive intermediates that covalently bind to DNA (238). Eastmond et al (239) demonstrated that phenol, another benzene metabolite, increases the peroxidase-dependent oxidation of hydroquinone to covalent-binding species. They also demonstrated that phenol and hydroquinone administered together to mice can reproduce bone-marrow toxicity caused by benzene. Thus, one possible explanation of benzene myelotoxicity is the phenol-enhanced peroxidative oxidation of hydroquinone to reactive intermediates that covalently bind to macromolecules. In addition to PHS, myeloperoxidase present in bone marrow leukocytes may also contribute towards the activation of hydroquinone.

The nephrotoxin 2-bromohydroquinone may exert its toxicity through the P-450-mediated formation of glutathione conjugates in liver (240). Lau & Monks (241) reported that renal PHS oxidizes 2-bromohydroquinone to a reactive intermediate that can also form the same nephrotoxic glutathione conjugates. The relative contribution of the hepatic and renal pathways to the in vivo formation of these glutathione conjugates and subsequent toxicity remains to be established.

Nicotine may contribute to the carcinogenic effect of tobacco. The primary P-450-mediated metabolite of nicotine is cotinine, which is used as an indicator of exposure to tobacco smoke. Mattammal et al (242) recently identified a new PHS-dependent metabolite, 3-(2',3'-dihydro-1'-methyl-2'-py-

rollyl)pyridine, in the urine of rabbits administered nicotine and in the urine of a male cigarette smoker. The amount of peroxidatic product present in urine from these two species was 15% and 6%, respectively, of the levels of cotinine. It is not known what role, if any, the PHS-dependent metabolite(s) have on the carcinogenic effects of nicotine and tobacco.

Cattle and goats (243) sustain acute pulmonary edema from 3-methylindole. The toxicity of 3-methylindole may require the P-450-dependent formation of a reactive imine methide metabolite, a two-electron oxidation product, since deuteration of the methyl group decreases its pulmonary toxicity (244). However, Formosa et al (109) demonstrated that 3-methylindole is a cosubstrate for PHS peroxidase and suggested that cooxidation by PHS peroxidase present in lung may also represent an important pathway for the formation of a reactive intermediate. HRP-mediated oxidation of 3-methylindole yields a carbon-centered free radical (245). In addition, 3-methylindole stimulates the formation of prostaglandins from AA in goat-lung microsomes and forms a metabolite that covalently binds to protein (109). Therefore, PHS peroxidase and P-450 may both contribute to the formation of reactive intermediates from 3-methylindole in lung tissue.

Ellipticine is a natural antitumor alkaloid from which potent antitumor drugs were developed. These compounds are potent cytotoxins and are mutagenic and form adducts with DNA. HRP catalyzes the oxidation of ellipticine analogs to quinone imine metabolites that are electrophilic, arylating species capable of binding to the 2'–O position of the ribose moiety of guanosine and adenosine (246, 247). The ability of other peroxidases to oxidize these compounds to quinone imines has not been addressed, but it is likely that mammalian peroxidases, such as PHS peroxidase, may catalyze a similar reaction and thus play a role in the cytotoxic and mutagenic effects of these drugs.

Eugenol (4-allyl-2-methoxyphenol) exerts toxic effects on several cell types but little is presently known about its mechanism of toxicity (248). Peroxidase enzymes, including PHS, cooxidize eugenol to a phenoxyl radical and a reactive quinone methide-like metabolite, both of which are cytotoxic (119, 249). P-450 also catalyzes the formation of the quinone methide-like metabolite (D. Thompson, D. Constantin-Teodosiu, B. Egestad, et al, unpublished observation). This two-electron oxidation product covalently binds to protein and forms conjugates with glutathione. The toxicity of eugenol may therefore be related to the formation of this quinone methide intermediate, with the relative contributions of peroxidase versus monooxygenase systems being dependent on the tissue. PHS peroxidase-dependent cooxidation of eugenol is limited to the availability of hydroperoxide substrates, however, since eugenol is a potent inhibitor of PHS cyclooxygenase activity (250).

Amitrole (3-amino-1,2,4-triazole) is a thyroid carcinogen and goitrogen. It gives negative results in a wide variety of short-term mutagenicity assays, but induces gene mutations and morphological transformations in SHE cells containing PHS. Krauss & Eling (251) demonstrated that PHS activates amitrole to a reactive intermediate that binds to protein and tRNA. These results suggest that PHS may play a role in the mutagenic and carcinogenic effects of amitrole.

The data summarized above provide ample evidence of the importance of peroxidase and peroxyl radical activation of chemicals, particularly carcinogens. It is important to remember that activation occurs in cells, tissues, and organs and depends on the relative contribution of the peroxidase or peroxyl radicals versus the monooxygenase enzyme system.

PHS AND SULFUR-CENTERED RADICALS

MECHANISMS OF FORMATION OF THIYL RADICALS The formation of nitrogen-, carbon-, and oxygen-centered free radical metabolites by PHS peroxidase and in some cases the eventual formation of peroxyl radicals can ultimately lead to a toxic response. The activation process caused by these reactions has been the major focus of studies on cooxidation. The presence in living systems of the ubiquitous tripeptide, glutathione, acts to compromise or alter this activation process (252). For example, glutathione is a cofactor for the detoxifying enzymes glutathione transferases, which act to form water-soluble conjugates of potentially carcinogenic epoxides (253). Glutathione also participates in a number of reductive reactions and in many cases can reduce nitrogen- and oxygen-centered free radicals. As a consequence of this reduction, glutathione is oxidized to a glutathionyl free radical (GS·) as described below (254). Other thiols such as cysteine and N-acetyl cysteine can reduce cosubstrate-derived free radicals back to the ground state that occurs for a number of free radicals generated by PHS peroxidase (79, 254–256). This reducing ability of thiols served as the basis for a study of the mechanism by which methimazole inhibits PHS peroxidase-catalyzed reactions (54). We showed that this sulfur-containing drug inhibits peroxidase-catalyzed oxidations by reducing the free radical metabolites back to the parent cosubstrate rather than by inhibiting PHS peroxidase directly. This results in the net inhibition of product formation and the apparent enzyme inhibition.

Two mechanisms by which PHS peroxidase forms GS· are shown in Figure 8. Glutathione also serves as a cosubstrate for PHS peroxidase and is oxidized to GS· (121). The upper part of the scheme represents the direct electron donation by GSH (i.e. where GSH acts as a reducing cosubstrate) to PHS peroxidase. Using both RSV microsomes and purified PHS, we detected GS·

by ESR, which was dependent upon a functioning enzyme, GSH, and a peroxide source (either AA or 15-HPETE) (121). In the cycle of reactions shown in the lower portion of Figure 8, the peroxidase first oxidizes a compound that is a more efficient reducing cosubstrate than GSH (e.g. phenols, aromatic amines) (30). The cosubstrate-derived radical produced by this oxidation is then reduced by GSH and forms both GS· and the parent-reducing cosubstrate. With efficient reducing cosubstrates such as aminopyrine (79), phenol (122), and acetaminophen (254), this process leads to markedly enhanced levels of GS·. Recent studies suggest that GS· may be formed intracellularly. In incubation mixtures containing microsomes prepared from mouse keratinocytes, GSH, and AA, the reducing cosubstrates phenol and aminopyrine both enhanced GS· formation and also stimulated threefold the metabolism of AA to prostaglandins. Likewise, mixtures containing intact mouse keratinocytes and phenol enhanced the formation of GS· and increased AA metabolism threefold. However, this enhancement was not seen with aminopyrine. As phenol is expected to penetrate cell membranes more readily than aminopyrine, one interpretation of this result is the cellular ingress of phenol and intracellular production of GS· (257).

FATE AND ROLE IN DETOXIFICATION PROCESSES Glutathione disulfide is the eventual fate of GS· in systems providing no alternate electron-donating species even though direct coupling is not a major pathway (256). Evidence of

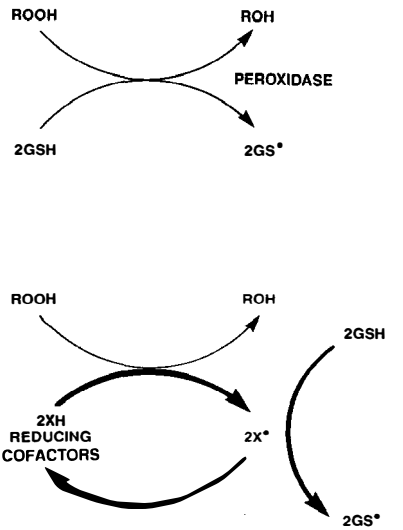

Figure 8 The formation of thiyl free radicals by PHS

the reaction of GS· with molecular oxygen to form the glutathione peroxyl radical, GSOO·, has been presented by Tamba et al (258). Formerly considered limited by diffusion, this reaction is apparently several orders of magnitude slower (259, 260), indicating a longer existence of GS·.

GS· may also add directly to compounds, thereby forming GSH conjugates. This new route for formation of GSH conjugates, mediated by peroxidases through the production of GS·, was first demonstrated by Stock et al (123) and is compared to the glutathione transferase (GST)/monooxygenase pathway in Figure 9. In the presence of GSH, PHS, and AA, styrene was directly converted to two isomeric glutathione conjugates in contrast to the four isomeric GSH conjugates formed by GST-catalyzed addition of GSH to styrene oxide. A potential mechanism for the formation of glutathione conjugates via the peroxidase-mediated pathway is illustrated in Figure 10. GS· initiates the reaction by adding to a double bond proximal to a highly conjugated system such as an aromatic ring. This results in the formation of a carbon-centered radical that reacts with molecular oxygen to yield a peroxyl radical. Recently, Kanofsky (261) showed that two styrene GSH conjugate-derived peroxyl radicals undergo a Russell reaction and yield singlet oxygen and the styrene GSH conjugate. Other studies (262) showed that an exocyclic double bond, as found in styrene, is required for a compound to undergo GS· addition. With the precocenes, antijuvenile hormones that possess this structural arrangement, up to 94% conversion to GSH-precocene conjugates was observed in the presence of PHS peroxidase when supplemented with a GS· enhancing agent, phenol (262).

An important point in this peroxidase mechanism (Figure 9) is that formation of the electrophilic epoxide is not required for GSH-conjugate formation as with GST (lower portion of Figure 9). This peroxidase-mediated pathway of glutathione-conjugate formation could then be viewed as an "epoxide shunt". Such a shunt would be of considerable toxicological relevance in compounds such as the PAH dihydrodiols whose epoxidation forms the ultimate carcinogen, the PAH diol epoxides (263). Peroxidase-mediated

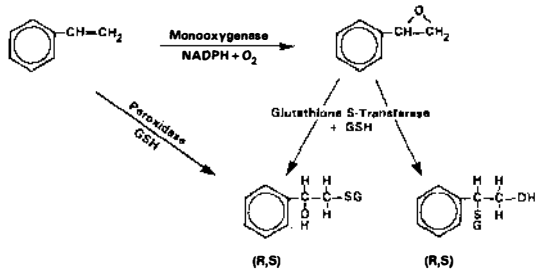

Figure 9 The mechanisms for formation of glutathione conjugates.

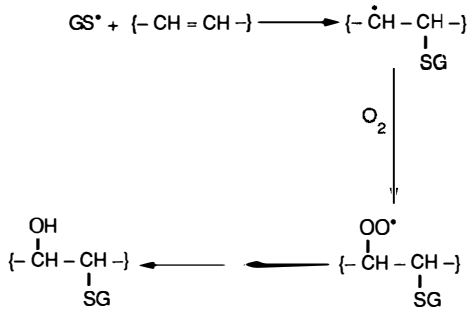

Figure 10 Reaction mechanism for peroxidase-catalyzed glutathione conjugate formation

formation of PAH dihydrodiol-GSH conjugates would shunt the dihydrodiols away from further activation. Several PAH dihydrodiols, structurally similar to styrene, were tested for their ability to undergo GS· addition catalyzed by PHS-peroxidase (262). The amount of dihydrodiol converted into GSH conjugates varied from 24% of the amount present with BP-9,10-diol, 29% with BP-7,8-diol, 38% with BA-3,4-diol, to 77% with BA-1,2-diol. From these results, neither the position of the double bond nor the extent of the conjugating system (either 3 or 4 rings) appears to prevent the conversion of the parent dihydrodiol to GSH conjugates by the PHS peroxidase-mediated pathway. Peroxidase-catalyzed formation of GSH conjugates is therefore general and may represent an alternative route to detoxication of a wide variety of compounds.

GSH is not the only compound that can form peroxidase-catalyzed addition products from sulfure-centered free radicals. During investigation of the bisulfite-derived peroxyl radical and its interaction with BP-7,8-diol, Curtis et al (107) observed the formation of a product that was characterized as a BP-7,8-diol-sulfonate. Formation of this sulfonate product may potentially be a detoxification reaction by removing the precursor of an ultimate carcinogen.

INTERACTIONS WITH P-450 METABOLITES Phenols are excellent reducing cosubstrates for PHS peroxidase (30) and can greatly enhance the formation of GS· (Figure 11) (123, 262). A principal metabolic capacity of P-450 is hydroxylation, which, in the case of aromatic compounds, forms phenols. P-450 metabolism of PAHs such as BP produce principally phenols but also dihydrodiols and quinones (264). Thus, during the metabolism of PAH, both dihydrodiols, which can undergo GS· addition as described above, and phenols, which may enhance the formation of GS· in accordance with the lower portion of Figure 8, are formed. Several isomeric phenols of BP as well as a phenolic BA-derivative enhanced the formation of peroxidase-mediated GSH

conjugation with BP-7,8-diol (Foureman & Eling, unpublished observation). The phenol most efficient at this process was 9-hydroxybenzo(a)pyrene, which enhanced conjugate formation fivefold. These results indicate that P-450 metabolites and peroxidases could interact with each other in the metabolism of xenobiotics.

SUMMARY

We have attempted in this article to summarize and review cooxidation reactions that occur during the metabolism of AA and potential roles that these reactions can play in the activation and detoxification of chemicals. This review summarizes approximately 15 years of intensive investigation by a number of laboratories, and as such not all studies are cited, and in some cases data are not discussed with the emphasis that the original investigators may have intended. The major focus of many of these studies has been toward carcinogenesis. In the future, emphasis may shift to the formation of metabolites that will lead to other toxic effects.

The cooxidation reactions that occur during AA metabolism are dependent upon the peroxidase activity of PHS. For some chemicals that are not cosubstrates, the epoxidation reactions that occur are dependent upon the subsequent formation of peroxyl radicals. A large and diverse number of chemicals are metabolized by an equally large and diverse number of chemical reactions. The unifying theme is the free radical nature of these oxidations. The subsequent reactions that these chemicals undergo is dictated by the nature of the free radical and the environment in which it is generated.

Ample evidence now exists for the contribution of these free radical-mediated reactions not only in the formation of toxic metabolites, but also in some cases in the detoxification of chemicals. The overriding factor for this type of metabolism to occur is the relative concentrations in the specific tissue of PHS and peroxyl radicals with respect to other activating systems, particularly the monooxygenase system. In vivo investigations support the importance of the peroxidase and peroxyl radical systems in both activation and detoxification of chemicals in extrahepatic tissues.

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