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NAD(P)H:quinone oxidoreductase$_1$ (DT diaphorase) specifically prevents the formation of benzo[*a*]pyrene quinone-DNA adducts generated by cytochrome P4501A1 and P450 reductase

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ABSTRACT **Monkey kidney COS1 cells transiently transfected with plasmids pMT2-cytochrome P450 1A1 (CYP1A1), pMT2-cytochrome P450 reductase (P450 reductase), and pMT2-NAD(P)H:quinone oxidoreductase$_1$ (NQO_1 or DT diaphorase), individually or in combination, expressed significantly elevated levels of the respective enzyme(s). The transfected cells were homogenized to break cell membranes without affecting the nuclei and incubated with benzo[*a*]pyrene (BP) to determine the role of cDNA-encoded enzymes in metabolic activation and/or detoxification of BP. These studies were performed by measuring the capacity of the transfected cells to form DNA adducts as determined by ^{32}P postlabeling and protein adduct detection. Cotransfection of the COS1 cells with cDNAs encoding CYP1A1 and P450 reductase resulted in eight distinct BP-DNA adducts. Inclusion of cDNA encoding NQO_1 along with CYP1A1 and P450 reductase in transfection reduced the number of DNA adducts to six. The two lost DNA adducts were specifically eliminated due to the presence of cDNA-derived NQO_1 activity. Subsequent experiments with BP-1,6-quinone, BP-3,6-quinone, and BP-6,12-quinone identified these two adducts as those of BP quinones. In an *in vitro* system, BP-3,6-quinone produced two adducts with deoxyguanosine (dG) but not with dA, dC, and dT. Furthermore, the positions of BP-3,6-quinone-dG adducts on TLC plate correspond to those that are prevented by cDNA-derived NQO_1, thus identifying these adducts as BP quinones of dG. In addition, NQO_1 reduced the amount of protein-BP adducts generated by CYP1A1 and P450 reductase into transfected COS1 cells. These results show that semiquinones can directly bind to DNA and demonstrate that NQO_1 activity can specifically reduce the binding of quinone metabolites of BP generated by CYP1A1 and P450 reductase to DNA and protein.**

Benzo[*a*]pyrene [BP], a promutagenic and procarcinogenic prototype polycyclic aromatic hydrocarbon, requires metabolic activation to exert toxicity (1). Most of the metabolic and toxicity studies of BP have been focused on a particular metabolite, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), which binds to cellular macromolecules with high affinity to produce carcinogenic transformation (2). In addition to BPDE, quinones constitute a second major class of BP metabolites that assume great toxicological significance (1). Quinones are electrophilic and can interact with the cellular macromolecules (3). They can also undergo further reductive metabolism catalyzed by $1e^-$ reducing enzymes such as P450 reductase and cytochrome b_5 reductase or by the $2e^-$ reducing enzyme NQO_1 (4, 5). The $1e^-$ reduction of the quinones, in general, results in the formation of unstable semiquinones having potential to undergo redox cycling leading to formation of highly reactive oxygen species associated with oxidative stress and cytopathy (6). However, it is not known if semiquinones can directly bind to DNA resulting in mutagenicity and carcinogenicity. On the other hand, hydroquinones, the $2e^-$ reduction metabolites of quinones, are relatively more stable and are readily excreted following conjugation (7).

In the present report, monkey kidney COS1 cells transiently transfected with recombinant cDNAs encoding CYP1A1, P450 reductase, and NQO_1, individually and in various combinations, were used to investigate the role of these enzymes in metabolic activation and detoxification of BP. The highly sensitive ^{32}P postlabeling technique for detecting DNA adducts was used to determine that semiquinones generated due to catalytic activation by P450 reductase can directly bind to the DNA and that this binding is restricted to the guanine residue. We further show that NQO_1 specifically prevents the formation of BP quinone-DNA adducts generated by CYP1A1 and P450 reductase.

MATERIALS AND METHODS

Construction of pMT2-cDNA Recombinant Plasmids, Transient Transfection, and Enzyme Assays. cDNAs encoding CYP1A1 (8), P450 reductase (9), and NQO_1 (10) were separately subcloned into the transient expression vector pMT2 (11). The vector alone and pMT2-cDNA recombinant plasmids were transfected into COS1 cells individually or in combination by the DEAE-dextran and chloroquine method (11). The nontransfected and transfected COS1 cells were harvested after 62 hr and Dounce homogenized in buffer (50 mM Tris·HCl/1.5 mM $MgCl_2$/10 mM KCl) containing protease inhibitor phenylmethylsulfonyl fluoride at a final concentration of 100 μM. The majority of nuclei were undisrupted as judged by microscopy of the homogenate.

The NQO_1 activity was assayed spectrophotometrically by following the NADH-dependent reduction of 2,6-dichloroindophenol at 600 nm (11, 12). Increase in absorbance at 550 nm due to the NAD(P)H-dependent reduction of cytochrome *c* was taken as the index of P450 reductase activity (13). CYP1A1 activity was assayed by following the hydroxylation of BP (14). Protein content of the cell extracts were estimated by Bradford's method (15).

DNA Adduct Analysis. *Isolation and quantitation of BP and BP-quinone DNA adducts.* The cellular extracts containing intact nuclei from nontransfected and transfected COS1 cells were incubated at 37°C for 30 min with BP or BP-quinones (1,6-, 3,6-, and 6,12-) in the presence of the required cofactors for their metabolism and adduct formation. The final concentrations of the various components in the reaction mixture were as follows: cell extracts equivalent to 1 mg of protein, 30 μM BP or BP-quinone, 5 μM FAD, 0.18 mg of bovine serum albumin per ml, 0.01% Tween 20, and 200 μM NAD(P)H. At the end of the incubation period, the samples were placed on ice and sonicated briefly. DNA was isolated

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Abbreviations: BP, benzo[*a*]pyrene; SSDNA, salmon sperm DNA; BPDE, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide.

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by phenolchloroform extraction and ethanol precipitation (16). One microgram of DNA was analyzed for the presence of adducts of BP and BP-quinone metabolites by the ^{32}P postlabeling procedure (17–19).

Competition between P450 reductase and NQO_1 for BP-3,6-quinone metabolism. The capacity of P450 reductase and NQO_1 to compete for BP-3,6-quinone metabolism was assessed by using COS1 cells transfected with cDNAs for P450 reductase and NQO_1 individually. The cell extracts for each enzyme were prepared separately and then mixed in varying ratios with each other before incubation with BP-3,6-quinone and the required cofactors. The total amount of cell extract protein in the reaction mixture was held constant at 1 mg by the addition of cell extracts of nontransfected COS1 cells. After incubation, the DNA was isolated and ^{32}P postlabeling analysis was performed.

In vitro *synthesis of BP-3,6-quinone-salmon sperm DNA (SSDNA) and -deoxyribonucleoside monophosphate adducts.* Five hundred micrograms of BP-3,6-quinone was incubated with 500 μg each of the SSDNA or the individual deoxyribonucleoside monophosphates (dA, dC, dG, and dT) in a total volume of 500 μl of Tris buffer (pH 7.4) containing 50 μg of microsomes from COS1 cells transfected with P450 reductase and 200 μM NAD(P)H to synthesize adduct standards by a previously described procedure (20).

Identification of BP-DNA adducts. In the present report we have attempted to identify only those BP-DNA adducts whose appearance and disappearance were specifically related to the presence of P450 reductase and NQO_1 activities. These adducts were expected to contain BP-quinones. The presence of BP-quinones in these adducts was confirmed by using *in vitro* synthesized BP-3,6-quinone-SSDNA adduct standards in mixing and comigration experiments.

Presence of dG in BP-quinone-DNA adducts. The *in vitro* synthesized BP-3,6-quinone-dG adducts and BP-3,6-quinone-DNA adducts from COS1 cells transfected with P450 reductase were ^{32}P-labeled in separate experiments. The adducts were analyzed on TLC plates individually and in combination to determine if the BP-quinone-dG adducts run along with the COS1 nuclear DNA-BP-quinone adducts.

Protein Adduct Measurements. The nontransfected and transfected COS1 cell extracts were incubated under experimental conditions essentially similar to those for the DNA adduct analysis, except that 0.02 μM [^3H]BP was mixed with 29.98 μM nonradiolabeled BP before incubation to monitor protein binding due to BP metabolites. The reaction was stopped by the addition of 1 ml of ice-cold acetone. The precipitated protein (DNA-free) was isolated and washed several times to remove noncovalently bound radioactivity. The protein precipitate was dissolved in 1 M NaOH and neutralized with 1 M HCl. An aliquot was used for estimation of protein (A_{280}) and the remainder was used for determining radioactivity associated with the covalently bound radiolabeled metabolites of BP using a liquid scintillation counter.

RESULTS

Activities of CYP1A1, P450 Reductase, and NQO_1 in COS1 Cells. Table 1 summarizes data from COS1 cells transfected with pMT2 and pMT2-cDNA recombinant plasmids. The nontransfected COS1 cells possessed little or no endogenous CYP1A1 and NQO_1 activities but contained substantial amounts of P450 reductase activity. The COS1 cells, upon transfection with pMT2-cDNA recombinant plasmids individually or in combination, produced significantly elevated levels of the respective enzymes as compared to nontransfected cells. The increase in enzyme activities due to expression of cDNA-derived protein(s) varied from 68-fold in the case of P450 reductase to 1130-fold in the case of NQO_1. The CYP1A1 transfected COS1 cells expressed high levels of CYP1A1 activity, although the fold expression could not be determined because of undetectable levels of endogenous CYP1A1 in COS1 cells. The enzyme activities in the transfected cells were adequate for the BP metabolism and related studies.

BP-DNA Adducts. Upon treatment with BP, the nontransfected COS1 cells and COS1 cells transfected with P450 reductase or NQO_1 did not reveal adduct formation (Fig. 1 *A–C*). This is presumably because these cells did not contain sufficient CYP1A1 activity to metabolize BP to produce metabolites that bind to DNA. Transfection of COS1 cells with the cDNA encoding CYP1A1 produced six BP-DNA adducts (Fig. 1*D*). Cotransfection of the COS1 cells with cDNAs encoding CYP1A1 and P450 reductase increased the number of BP-DNA adducts to eight (Fig. 1*E*). The new adducts (because of P450 reductase) are labeled as 5 and 6 in Fig. 1*E*. Interestingly, inclusion of the cDNA encoding NQO_1 along with CYP1A1 and P450 reductase in transfection reduced the number of DNA adducts back to the original pattern of six (compare Fig. 1 *D*, *E*, and *F*). The two adducts induced by P450 reductase (labeled as 5 and 6 in Fig. 1*E*) were eliminated due to the presence of NQO_1 activity (Fig. 1*F*). The disappearance of the two adducts was specifically related to the presence of NQO_1 activity, since these adducts did not disappear either in the presence of dicoumarol (a potent inhibitor of NQO_1 activity) (Fig. 1*G*) or when pMT2 plasmid expressing antisense NQO_1 cDNA replaced the plasmid containing the sense NQO_1 cDNA (Fig. 1*H*). Subsequent studies focused on identification of adducts 5 and 6, the appearance and disappearance of which specifically related to the presence of P450 reductase and NQO_1 activities, respectively.

BP-3,6-Quinone-DNA Adducts. The extracts of COS1 cells transfected with P450 reductase revealed two BP-3,6-quinone DNA adducts (Fig. 2*A1*). In a similar experiment the replacement of P450 reductase with NQO_1 in transfection showed no adduct formation (Fig. 2*B1*). The cotransfection of NQO_1 with P450 reductase significantly reduced the amount of the two adducts (compare Fig. 2 *A1* and *C1*).

Table 1. Activities of drug-metabolizing enzymes in nontransfected and transfected COS1 cells

cDNA transfected	Enzyme activity		
	CYP1A1*	P450 red.†	NQO_1‡
None	UD	14.41 ± 2.62	0.031 ± 0.001
CYP1A1	34.66 ± 1.76	13.86 ± 1.24	0.024 ± 0.001
P450 reductase	UD	973.87 ± 41.99	0.032 ± 0.001
NQO_1	UD	14.78 ± 1.91	35.01 ± 1.53
CYP1A1			
+ P450 reductase	30.00 ± 2.07	893.66 ± 46.32	0.028 ± 0.001
+ P450 reductase + NQO_1	21.33 ± 1.45	862.33 ± 38.21	18.88 ± 1.41

Values are mean ± SE of three separate experiments. UD, undetectable.
*Arbitrary units of 3-OH-BP generated per min per mg of protein.
†nmol of cytochrome *c* reduced per min per mg of protein.
‡μmol of 2,6-dichloroindophenol reduced per min per mg of protein.

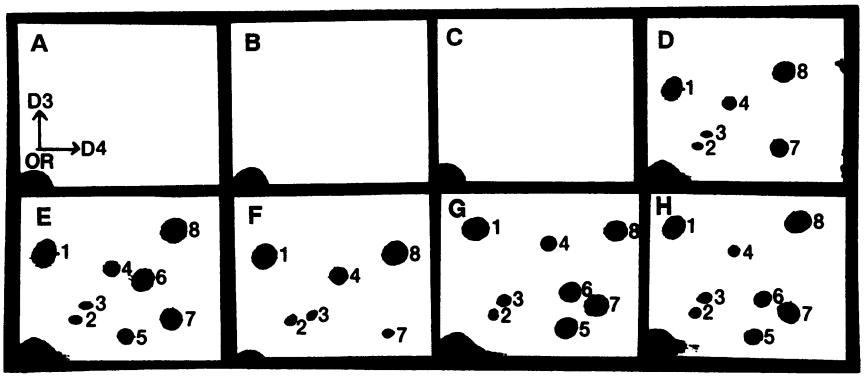


FIG. 1. ^{32}P adduct maps of DNA from nontransfected and pMT2-cDNA transfected COS1 cell extracts incubated with BP. The COS1 cells were transfected with pMT2 (vector alone), pMT2-CYP1A1, pMT2-P450 reductase, and pMT2-NQO_1 individually and in combination. The cell extracts containing intact nuclei were incubated with BP and the ^{32}P-labeled DNA adducts were analyzed by multidirectional PEI-cellulose TLC. The adduct numbering is based on Fig. 1*E*, which contained the maximum number of DNA adducts. (*A*) Nontransfected COS1 cells. (*B*) COS1 cells plus P450 reductase. (*C*) COS1 cells plus NQO_1. (*D*) COS1 cells plus CYP1A1. (*E*) COS1 cells plus CYP1A1 plus P450 reductase. (*F*) COS1 cells plus CYP1A1 plus P450 reductase plus NQO_1. (*G*) COS1 cells plus CYP1A1 plus P450 reductase plus NQO_1 plus 10 μM dicoumarol. (*H*) COS1 cells plus CYP1A1 plus P450 reductase plus NQO_1 (3′ → 5′). Results with nontransfected and COS1 cells transfected with pMT2 (vector control) did not reveal any adduct formation. Therefore, results are shown with nontransfected COS1 cells as control.

However, addition of the specific NQO_1 inhibitor, dicoumarol, to the reaction mixture containing extracts from cotransfected COS1 cells, as well as the use of NQO_1 cDNA in 3′ → 5′ orientation in the cotransfection of COS1 cells had no effect on the amount of the two adducts (Fig. 2 *D1* and *E1*), consistent with the specific involvement of NQO_1 activity. The competition between P450 reductase and NQO_1 for reductive metabolism of BP-3,6-quinone was also determined (Fig. 2 middle and bottom panels). Upon incubation with BP-3,6-quinone, 500-μg protein equivalents each of COS1 cells transfected individually with P450 reductase and NQO_1 produced low levels of two DNA adducts that were hardly visible (Fig. 2 *A2* and *A3*). Decreasing the amount of NQO_1 protein and maintaining that of P450 reductase constant resulted in an increased DNA adduct formation (Fig. 2 *B2–E2*). On the other hand, keeping the amount of NQO_1 protein constant at 500 μg and reducing that of P450 reductase from 500 to 32.5 μg resulted in the disappearance of the two adduct spots (Fig. 2 *B3–E3*). Similar results as described above were obtained with BP-1,6- and -6,12-quinones (data not shown).

Identification of BP-Quinone-DNA Adducts. In Fig. 3 we show experiments that establish that the two SSDNA adducts of BP-3,6-quinone (Fig. 3*A*) have similar chromatographic

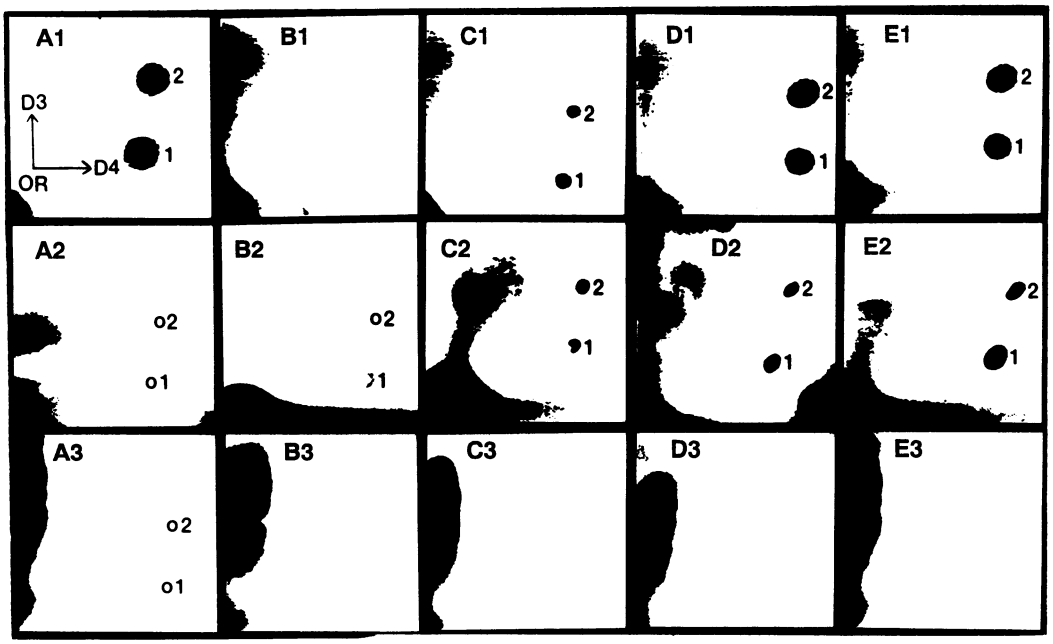
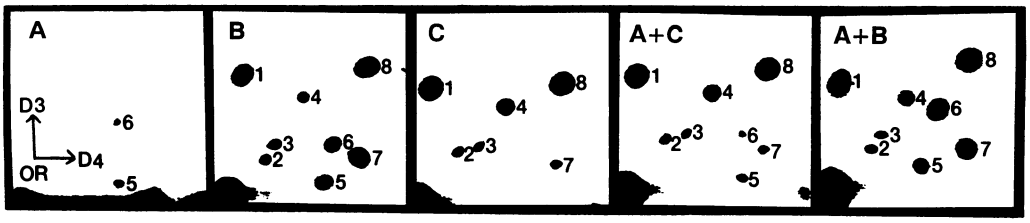


FIG. 2. Analysis of ^{32}P postlabeled BP-3,6-quinone-DNA adducts in pMT2-cDNA transfected COS1 cells. The COS1 cells transfected with pMT2-cDNA plasmids individually and in combination were incubated with BP-3,6-quinone and DNA adducts were analyzed by ^{32}P postlabeling. Top panel: (*A1*) COS1 cells plus P450 reductase. (*B1*) COS1 cells plus NQO_1. (*C1*) COS1 cells plus P450 reductase plus NQO_1. (*D1*) COS1 cells plus P450 reductase plus NQO_1 plus 10 μM dicoumarol. (*E1*) COS1 cells plus P450 reductase plus NQO_1 (3′ → 5′). Middle panel: The extracts from COS1 cells transfected with P450 reductase (500-μg protein equivalent) were mixed with varying amounts of extract from COS1 cells transfected with NQO_1 [(μg protein equivalents) 500 (*A2*), 250 (*B2*), 125 (*C2*), 62.5 (*D2*), and 31.25 (*E2*)] and incubated with BP-3,6-quinone before analyzing the DNA adducts. Lower panel: The extracts from COS1 cells transfected with NQO_1 (500-μg protein equivalent) were mixed with varying amounts of extract from COS1 cells transfected with P450 reductase [(μg protein equivalents) 500 (*A3*), 250 (*B3*), 125 (*C3*), 62.5 (*D3*), and 31.25 (*E3*)] and incubated with BP-3,6-quinone before analysis of DNA adducts. Adducts that required a prolonged exposure time (>4 hr) are marked with circles in *A2* and *B2* of the middle panel and *A3* of the bottom panel.



Adduct	A	B	C	A+C	A+B
5	2.12	2.86	0	2.04	3.98
6	1.84	3.14	0	1.95	4.47

FIG. 3. BP-3,6-quinone adducts comigrate along with adducts 5 and 6 of BP activated by CYP1A1 and P450 reductase. The extracts from COS1 cells transfected with individual or combinations of pMT2-cDNA plasmids were incubated with BP to produce BP-DNA adducts. The BP-3,6-quinone SSDNA adducts were prepared as described in the text. The BP metabolites-COS1 DNA adducts and the BP-3,6-quinone-SSDNA adducts were ^{32}P-labeled in separate experiments. The ^{32}P-labeled adducts were run individually or mixed with each other in various combinations before running on multidirectional TLC. (*A*) SSDNA plus COS1 cell microsomes containing P450 reductase plus BP-3,6-quinone. (*B*) COS1 cells plus CYP1A1 plus P450 reductase plus BP. (*C*) COS1 cells plus CYP1A1 plus P450 reductase plus NQO_1 plus BP. (*A+C*) One microgram each of DNA from *A* and *C* were separately ^{32}P-labeled, mixed, and analyzed. (*A+B*) One microgram each of DNA from *A* and *B* were separately ^{32}P-labeled, mixed, and analyzed. Radioactivity (cpm × 1000) associated with adducts 5 and 6 is given below the adduct maps.

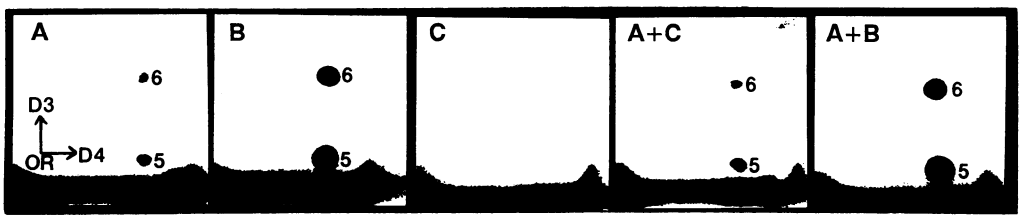
mobilities as COS1 DNA adducts 5 and 6 (Fig. 3*B*) generated from BP by CYP1A1 and P450 reductase. It is for this reason that the two BP-3,6-quinone-DNA adducts are labeled as 5 and 6 in Fig. 3*A*. Fig. 3*B* is essentially the same as described earlier for Fig. 1*E* and shows eight BP-DNA adducts in the presence of CYP1A1 and P450 reductase. Inclusion of NQO_1 reduced the number of BP-DNA adducts to six (Fig. 3*C*) as described earlier (Fig. 1*F*). Cochromatography of BP-3,6-quinone adducts (Fig. 3*A*) with DNA adducts obtained from COS1 cells transfected with CYP1A1 plus P450 reductase plus NQO_1 (Fig. 3*C*) showed eight adducts with a pattern similar to that in Fig. 3*B* (Fig. 3 *A+C*). The positions of BP-3,6-quinone adducts were very similar to adducts 5 and 6 in Fig. 3*B*. This indicated that BP-quinone adducts comigrated with the disappearing adducts 5 and 6. In a similar and more conclusive mixing experiment, the BP-3,6-quinone-SSDNA adducts comigrated along with adducts 5 and 6 (compare Fig. 3 *A*, *B*, and *A+B*) as evident from increased radioactivity associated with spots 5 and 6. No new adducts appeared in the mixing experiment, confirming that adducts 5 and 6 are due to BP-quinones.

BP-3,6-Quinone Deoxyribonucleoside Binding. BP-3,6-quinone bound to deoxyguanosine and produced two dG adducts (Fig. 4*A*). However, no binding was detected with dA, dC, and dT (data not shown). The BP-3,6-quinone-dG adducts were indistinguishable with BP-3,6-quinone-DNA adducts obtained from COS1 cells transfected with P450 reductase as determined by their comigration on TLC plates (compare Fig. 4 *A*, *B*, and *A+B*). This indicated that BP-quinone adducts observed in COS1 cells are likely to be due to BP-quinones binding to the dG residue of DNA.

Protein Binding. Nontransfected COS1 cells as well as those transfected with cDNAs for NQO_1 or P450 reductase did not result in any significant protein binding of [^{3}H]BP (Table 2). The protein binding of [^{3}H]BP was significantly increased in COS1 cells transfected with CYP1A1. Cotransfection of COS1 cells with CYP1A1 and P450 reductase further increased protein binding. The inclusion of NQO_1 in cotransfection with CYP1A1 and P450 reductase reduced protein binding of [^{3}H]BP metabolites generated by CYP1A1 and P450 reductase. The specific role of the NQO_1 in reducing the protein binding due to BP was confirmed by experiments involving 10 μM dicoumarol and NQO_1 cDNA in the 3′ → 5′ orientation where the binding was unaffected.

DISCUSSION

Chemoprevention has attracted much attention recently, primarily because of an increase in the incidence of chemical carcinogenesis due to the almost unavoidable exposure to the many potential carcinogenic chemicals present in food, water, and the environment. A critical analysis of the recent advances in our understanding of the mechanism of carcinogenesis has prompted much interest on the role of the individual enzymes responsible for processing carcinogens within the body. Enhanced detoxification and consequent elimination of the carcinogens from the body by way of modulating the activities of the drug-metabolizing enzymes is considered a feasible approach for chemoprevention (21).



Adduct	A	B	C	A+C	A+B
5	0.41	3.86	0	0.35	4.14
6	0.68	4.21	0	0.69	4.57

FIG. 4. BP-3,6-quinone-DNA adducts bound to deoxyguanosine comigrated with BP-3,6-quinone-DNA adducts. The BP-3,6-quinone-COS1 DNA and BP-3,6-quinone-dG adducts were ^{32}P-labeled in separate experiments. The ^{32}P-labeled adducts were separated on multidirectional TLC either individually or after mixing with each other. Results are shown only for dG as the binding was not detected with dA, dT, and dC. (*A*) dG + COS1 cell microsomes containing P450 reductase plus BP-3,6-quinone. (*B*) COS1 cells plus P450 reductase plus BP-3,6-quinone. (*C*) COS1 cells plus NQO_1 plus BP-3,6-quinone. (*A+C*) One microgram of DNA from *A* and 1 μg of dG from *C* were separately ^{32}P-labeled, mixed, and analyzed. Radioactivity (cpm × 10^4) associated with adducts 5 and 6 is given below the maps.

Table 2. Covalent binding of ^{3}[H]BP metabolites to cellular proteins of transfected COS1 cells

cDNA	[^{3}H]BP binding, pmol/min per mg of protein
CYP1A1	324.00 ± 31.08
P450 reductase	69.55 ± 14.83
NQO_1	8.36 ± 1.56
CYP1A1	
+ P450 reductase	479.55 ± 55.30
+ P450 reductase + NQO_1	332.11 ± 38.26
+ P450 reductase + NQO_1 (3′ → 5′)	469.99 ± 40.63
+ P450 reductase + NQO_1 + dicoumarol	478.89 ± 39.55

Values are mean ± SE of three separate experiments.

BP is a procarcinogen and metabolic activation of the parent compound to the ultimate mutagenic and carcinogenic metabolite(s) is a prerequisite for its carcinogenic effect. Cytochromes P450 are the principal enzyme system that catalyze the metabolic activation of BP (1). In the present report, nontransfected COS1 cells and the COS1 cells transfected with P450 reductase or NQO_1 gene constructs did not exhibit detectable levels of CYP1A1 activity and therefore failed to bioactivate BP as evidenced by the absence of DNA adducts. These results fortify the earlier observation (1) that CYP1A1 is indeed an important enzyme responsible for metabolic activation of BP. The metabolic activation of BP by CYP1A1 and many other enzymes leads to the generation of several metabolites including BPDE and BP-quinones (1). The mutagenic and carcinogenic properties of BPDE are well known. However, very little is known about the quinones. Quinones may enter into redox cycling and produce highly reactive oxygen species, the toxicity of which is well characterized (22). The binding of activated quinones (semiquinones) to macromolecules is speculated because of their highly reactive nature. However, there is no experimental evidence to support this.

The data presented in Fig. 1 indicate that the number and intensity of DNA adducts due to BP metabolites depend on the kind of enzyme(s) expressed by cDNA transfections. The COS1 cells cotransfected with cDNAs for CYP1A1 and P450 reductase lead to the formation of a greater number of BP adducts as compared to those transfected with cDNAs for CYP1A1 alone. The appearance of two of the BP adducts (nos. 5 and 6) required the presence of P450 reductase activity besides CYP1A1 activity. Interestingly, NQO_1 specifically prevented formation of these two adducts. Subsequent experiments (Fig. 2) with BP-1,6-, 3,6-, and 6,12-quinones identified BP-DNA adducts 5 and 6 as those of BP-quinones generated due to the metabolism of BP by CYP1A1 and P450 reductase. Results (Fig. 2) also suggest that the ultimate toxicological fate of BP-3,6-quinone within the cells depends on the relative amount of P450 reductase and NQO_1 present. Thus, increasing the relative quantity of P450 reductase resulted in increased bioactivation of the BP-3,6-quinone as evidenced by the appearance of the more intense fingerprints of the DNA adducts. On the other hand, an increase in the relative amount of NQO_1 resulted in the disappearance of the DNA adducts due to BP-3,6-quinone, suggesting that NQO_1 was in fact facilitating the detoxification of 3,6-quinone and thereby preventing its binding with cellular macromolecules. All of these results demonstrate that P450 reductase-catalyzed products of BP-quinones (semiquinones) can directly bind to DNA and that NQO_1 competes with P450 reductase and specifically prevents the binding of quinone metabolites of BP generated by CYP1A1 and P450 reductase to DNA. The binding of BP-quinones seems to be specific to dG residues in the DNA. At present, it is not clear if BP-quinone-DNA(dG) adducts are mutagenic or carcinogenic and this is a subject of future interest. In addition to reduced DNA binding, NQO_1 also reduced the amount of BP protein adducts generated by CYP1A1 and P450 reductase in transfected COS1 cells.

It is noteworthy that results on BP-quinones may be applicable as such to the other quinones, which are widely distributed in nature and human exposure to them is extensive (3). Results of the experiments in the present report suggest a possible chemopreventive role for NQO_1 due to exposure to quinones and its derivatives, as evidenced by the decreased DNA and protein binding of BP-quinones. Potential for such chemopreventive capacity was attributed to NQO_1 indirectly following induction of the enzyme activity by identified and unidentified constituents of vegetables (23), green tea (24), and several other inducers and inhibitors of the NQO_1 enzyme activity (25). Since DNA adduct formation is a prerequisite for initiation of carcinogenesis by chemicals, the results presented here are of obvious toxicological significance. However, the potentially genotoxic adducts have to overcome protective events such as DNA repair to exert the ultimate mutagenic and carcinogenic effect. Therefore, it is imperative that further studies be carried out to determine the mutagenicity of the BP-quinones-dG adducts prior to attributing a definite chemopreventive role for NQO_1.

Our studies also suggest that COS1 cell expression system is a powerful *in vitro* technique that can be used to study the specific role of individual enzymes in drug metabolism.

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