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Modulation of Microsomal Benzo[a]pyrene Metabolism by DNA

GABRIELA M. KELLER AND COLIN R. JEFCOATE[1]

Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706

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SUMMARY

Inclusion of calf thymus DNA during microsomal benzo[a]pyrene (BP) metabolism increases product formation by decreasing the accessibility of microsomal enzymes to inhibitory BP quinones. The relief of product inhibition of BP metabolism, the stimulation of the formation of BP 7,8-dihydrodiol-9,10-oxides (DE), and the corresponding DNA adducts were all dependent to varying extents on DNA concentration. The role of BP quinones was evidenced by effects of DNA on all aspects of quinone reactivity: (a) inhibition of microsomal reduction of quinones, (b) inhibition of quinone glucuronidation, (c) inhibition of quinone monooxygenation, (d) a substantial reduction of the inhibition of BP metabolism and diol epoxide (DE) formation by added BP 6,12-quinone, and (e) a stimulation of BP metabolism even though quinoine levels were also increased. DNA inhibited the reduction of 1,6- and 3,6-quinone to a similar degree under both oxygen-depleted and aerobic conditions. Other effects of DNA were very selective; glucuronidation of added 1,6- and 6,12-quinone was inhibited less than glucuronidation of 3,6-quinone (35% and 50% versus over 80%). However, monooxygenation of 3,6-quinone was not inhibited, whereas monooxygenation of 1,6-quinone was reduced by 60–70%. There was no measurable monooxygenation of 6,12-quinone. This specificity may indicate that DNA exerts its effect not simply by sequestering BP quinones. The interaction with DNA produced a distinct 25-nm red shift in the visible spectra of 1,6- and 3,6-quinone, while the change in the 6,12-quinone spectrum was less pronounced. RNA induced a similar red shift in the 1,6-quinone spectrum. Spectral measurements indicated binding of one molecule of 1,6-quinone per 50 DNA base pairs, while binding to RNA was 10-fold less extensive. The binding of 1,6-quinone to DNA was decreased by Mg^{2+}, suggesting that 1,6-quinone binds by intercalation. DNA perturbs BP metabolism in a second way by increasing the ratio of 9-phenol to 9,10-dihydrodiol 4-fold. This is probably due to a DNA-catalyzed rearrangement of 9,10-oxide to 9-phenol. This effect contributes substantially to the greater sensitivity of the formation of 9-phenol 4,5-oxide-DNA adducts to the DNA concentration. It is evident from these data that, in addition to binding carcinogens covalently, DNA can affect the kinetics and product distribution of carcinogen metabolism. The high capacity of DNA to sequester BP quinones from cellular membranes is likely to be associated with additional DNA damage which may contribute to carcinogenesis.

INTRODUCTION

Metabolism of BP[2] by MC-induced liver microsomes leads to formation of two reactive species which predominate in the modification of DNA, (+)-anti-7,8-dihydrodiol 9,10-epoxide (DE) and 9-phenol 4,5-oxide (1–3). In a previous paper (4), we have shown that metabolism of BP to DE is accelerated at both oxidation steps by the presence of UDPGA or DNA, each of which decreases the effectiveness of BP metabolites to inhibit monooxygenation. Thus, neither UDPGA nor DNA affect the initial rate of BP metabolism and DNA has no effect in

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[2] The abbreviations used are: BP, benzo[a]pyrene; MC, 3-methylcholanthrene; DE, diol epoxide, either or both of the diastereomeric 9,10-epoxides derived from *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in which the epoxide is *cis* (syn) or *trans* (anti) to the benzylic 7-hydroxyl group; UDPGA, uridine diphosphoglucuronic acid; Me_2SO, dimethyl sulfoxide; HPLC; high-pressure liquid chromatography; 9,10-oxide, 9,10-epoxy-9,10-dihydrobenzo[a]pyrene; 4,5-dihydrodiol, *trans*-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene; 7,8-dihydrodiol, *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, 9,10 diol or 9,10-dihydrodiol, *trans*-9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene; 3-phenol, 3-hydroxybenzo[a]pyrene; 9-phenol, 9-hydroxybenzo[a]pyrene; tetrol, 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene; anti-1-tetrol or 7,10/8,9-tetrol, r7,t8-dihydroxy-t9,c10-dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene; DE-2-dG, N^2-(7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene-10-yl) deoxyguanosine; 9-PO, 9-phenol 4,5-oxide.

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the presence of UDPGA. We have established that BP quinones are major contributors to product inhibition of BP monooxygenation. Of these, 6,12-quinone is the most effective and exhibits partially noncompetitive characteristics (5). The quinones can be almost completely removed by reduction and subsequent glucuronidation of the hydroquinones. This sequence requires only NADPH-cytochrome P-450 reductase and UDP-glucuronyltransferase (5, 6).

In this paper, we show that DNA exerts two effects on BP metabolism, one on the interaction of BP quinones with microsomal enzymes and a second on the relative rates of hydration and rearrangement of 9,10-oxide. The nature of the interaction by BP quinones with polynucleotides is examined.

MATERIALS AND METHODS

Chemicals. UDPGA, calf thymus DNA, enzymes for hydrolysis of DNA, and reaction cofactors were purchased from Sigma Chemical Company (St. Louis, Mo.). [G-^{3}H]BP (65 Ci/mmole) and ACS (aqueous counting scintillant) were purchased from Amersham Radiochemicals (Arlington Heights, Ill.). Purification of [^{3}H]BP in hexane was carried out by extraction with Me_2SO and KOH according to the method of Van Cantfort *et al.* (7). Methanol and water for HPLC were purchased from Burdick and Jackson Laboratories, Inc. (Muskegan, Mich.). BP metabolite standards were obtained from the National Cancer Institute Chemical Repository. BP, Me_2SO (gold label), and 3,5-diaminobenzoic acid hydrochloride were purchased from Aldrich Chemical Company (Milwaukee, Wisc.).

Methods. The induction of male Sprague-Dawley rats (60–70 g; Holtzman Company, Madison, Wisc.) with MC and the preparation of microsomes were carried out by techniques previously described (8).

BP metabolism. Standard incubations to metabolize BP contained MC-induced microsomes at a protein concentration of 0.3 mg/ml, 15 μM [G-^{3}H]BP (5.33 Ci/mmole), 50 mM potassium phosphate buffer (pH 7.5), 3 mM $MgCl_2$, an NADPH-regenerating system of NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase as previously described (8, 9), 2 mM UDPGA (10) and calf thymus DNA. UDPGA and DNA were added or omitted as indicated under Results. DNA was sonicated, dialyzed, and, except for data presented in Figs. 1, 3, and 4, heat-denatured before use as described by King *et al.* (11). Total oxidation of BP was measured by the method of Van Cantford *et al.* (7), modified for analysis of small samples as described elsewhere (4). In this method, the sample is partitioned between alkaline aqueous Me_2SO and hexane with >95% of the unmetabolized BP extracting into hexane and oxidized metabolites remaining in the aqueous phase.

HPLC analysis. Primary and secondary metabolites were extracted with ethylacetate/acetone (2:1) and analyzed by reverse-phase chromatography on Whatman ODS-2 and Zorbax-TMS columns with an aqueous methanol gradient as described in detail elsewhere (4). During the later stages of experimental work, an Altex/Beckman Ultrasphere-ODS column (5 μm, 0.46 × 25 cm) was used with methanol/water for the simultaneous determination of primary and secondary metabolites. The chromatographic conditions were as follows: temperature control at 35° and flow rate at 1.0 ml/min with a 15-min linear gradient from 40% to 50% methanol, followed by a 30-min exponential gradient (Setting −2, DuPont Model 850 controller) from 50% to 100% methanol. These conditions separate the anti-1-tetrol from other major secondary metabolites and give adequate resolution for quinones and phenols.

Determination of DNA adducts. DNA from 1-ml reaction samples was extracted with 80% aqueous phenol as described by King *et al.* (11) and hydrolyzed to nucleosides as described by Alexandrov *et al.* (12). Before hydrolysis, 25-μl samples of the DNA solution were taken to determine the amount of [^{3}H]BP bound and to measure the DNA content by the fluorometric assay described by Thomas and Farquhar (13) using 3,5-diaminobenzoic acid hydrochloride. The presence of UDPGA did not significantly increase the background fluorescence in this assay. The two major DNA adducts formed by MC-induced microsomes, DE-2-dG and 9-PO adduct, were separated from each other and the flow-through peak by elution with a step gradient of aqueous MeOH from a Sephadex LH-20 column (1 × 10 cm). The digested DNA samples (1.5 ml) were applied to the columns equilibrated with 30% aqueous methanol (MeOH) and followed by 3.5 ml of 30% MeOH. Each column was developed with 15 ml of 30% MeOH, 45 ml of 55% MeOH, and 25 ml of 80% MeOH applied in 5-ml aliquots. Manual collection of 5-ml effluent fractions into 20-ml scintillation vials was started at the time of sample application. Ten milliliters of scintillant were added to each fraction for counting. This procedure allows the simultaneous analysis of 15–20 DNA samples. The recovery of radioactivity and its distribution into the three major peaks (flow-through DE adducts, 9-PO adducts), agreed well with results from a linear gradient method reported previously (10). Some of the minor adducts derived from quinone metabolites and arene oxides (14) coelute with the DE and 9-PO adducts in the step-gradient method but contribute less than 10% to the major adduct peaks.

Spectral analyses. Visible spectra of quinones were recorded in standard microsomal incubations without the NADPH-regenerating system using an Aminco DW-2 spectrophotometer in the split-beam mode. Quinones were added in 10 μl of Me_2SO after baseline correction, and the spectrum was scanned repeatedly. Binding of 1,6-quinone to nucleic acid was determined by measuring the increase in absorbance for the wavelength pair 485 minus 600 nm in the dual wavelength mode upon addition of 2.5 μM 1,6-quinone to standard microsomal incubations containing increasing amounts of nucleic acids. Microsomal reduction of quinone was measured by monitoring absorbance changes in the dual wavelength mode (wavelength pairs 460 minus 430 nm for 1,6-quinone and 485 minus 458 nm for 3,6-quinone) as described by Capdevila *et al.* (15) and Shen *et al.* (5). Reduction was initiated by adding 250 μM NADPH after 5 min of preincubation at 37°.

RESULTS

BP conversion to DE. DNA effects a 2-fold stimulation in the conversion of BP to DE (measured as tetrols, Fig. 1A), while producing a much smaller increase in total BP metabolism (Fig. 1B). BP metabolism by MC-induced rat liver microsomes is linear for 3–5 min and then declines significantly (Fig. 1B). Calf thymus DNA does not affect the linear period of reaction but rather decreases the subsequent decline in reaction rate. In six experiments, total BP metabolism at 15 min in the presence of DNA was 120 ± 15% of control incubations without DNA, with a range of 106–142%. The variation is caused by changes in the time course of this DNA effect rather than variation in the extent of stimulation between different batches of microsomes. Although DNA exerts only a 20% effect on total metabolism after 15 min, the rate of BP metabolism between 5 and 15 min increases by 40% from 1.2 to 1.7 nmoles/min/mg). The effect is independent of whether DNA is denatured or double stranded (Fig. 1B). The increases in total BP metabolism and in tetrol formation exhibit similar hyperbolic dependences on DNA concentration which, however, are not saturated at a DNA concentration of 1 mg/ml (Fig. 1A).

DNA does not affect the steady-state concentration of 7,8-dihydrodiol. However, once formation of 7,8-dihydrodiol decreases, presumably due to decline in the concentration of BP below the K_m for monooxygenation, the *net*

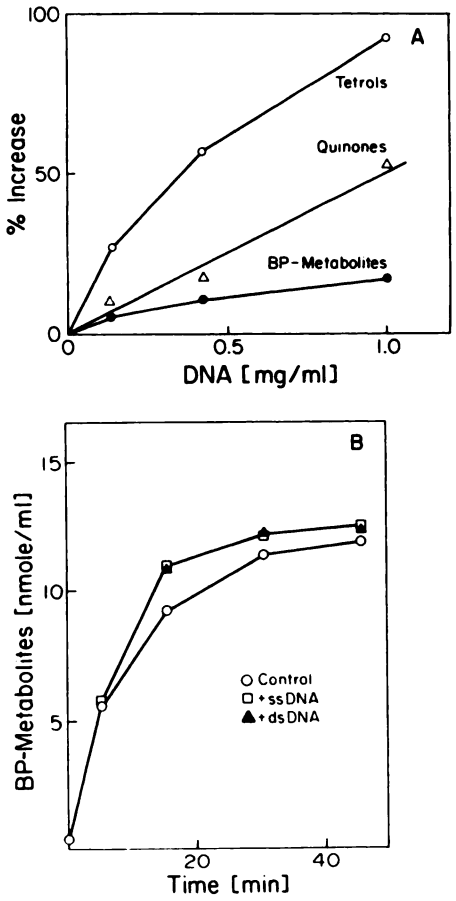

FIG. 1. *Stimulation of primary and secondary BP metabolism by calf thymus DNA*

A. Total BP metabolites were measured after 15 min of reaction and tetrol and quinone formation after 45 min, as described under Materials and Methods. Initial [BP] was 15 μM. The *Y*-axis shows the percentage increse in metabolite concentration induced by DNA. Values in the absence of DNA were 9.3 μM for total BP metabolites (●——●), 0.14 μM for tetrol (○——○), and 1.7 μM for quinone (△——△).

B. No DNA (○) or 1 mg/ml of heat-denatured (□) or double-stranded (▲) DNA was added and total BP metabolism was assayed at different times as described under Materials and Methods.

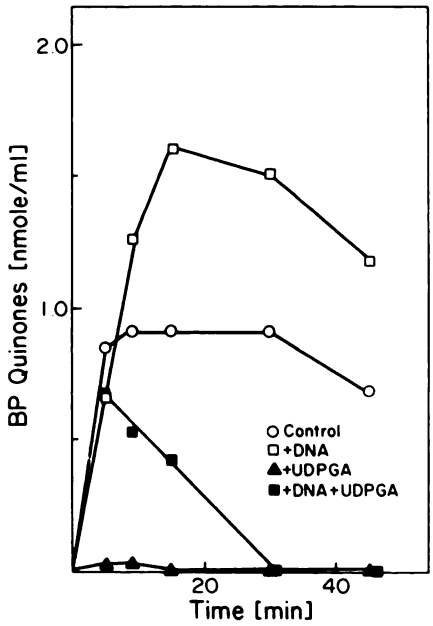

FIG. 2. *Production of quinones during microsomal BP metabolism with or without DNA and UDPGA*

Reactions contained 15 μM BP and □——□ DNA, 1 mg/ml; ▲——▲, UDPGA, 2 mM; ■——■, DNA, 1 mg/ml, and UDPGA, 2 mM; ○——○, none (control). Other conditions were as described under Materials and Methods. The quinone concentration is the sum of 1,6-, 3,6-, and 6,12-quinone.

disappearance of 7,8-dihydrodiol is increased by the presence of DNA (4). During this phase of metabolism, the effects of DNA (1 mg/ml) on both 7,8-dihydrodiol disappearance and on DE formation indicate a doubling of the conversion of 7,8-dihydrodiol to DE. Thus, DNA exerts a larger effect on the secondary metabolism of 7,8-dihydrodiol than on the primary metabolism of BP.

Effect on metabolism of endogenous quinones. Formation of quinones accounts for 10–12% of the BP metabolized in the interval of 0–5 min. DNA does not affect this proportion as measured over the initial 2 min of reaction. However, DNA increases the accumulation of quinone at later reaction times to an extent which is linearly dependent on the concentration of DNA (Fig. 1A). In the absence of DNA, quinone levels reached a steady concentration after 5 min which is maintained for 25 min (Fig. 2). DNA delays the onset of this phase but also elevates the concentration of quinone by 60–70%. We calculate that the quinone level reached after 20 min in the presence of DNA, in fact, represents the accumulation of essentially all quinone produced, i.e., 10%–12% of 12.5 μM BP metabolized; apparently there is little concomitant oxidative metabolism of quinones. The lower level of quinones found in absence of DNA, in contrast, reflects a true steady state between formation and further metabolism. In the presence of DNA, some metabolism of quinones occurs late in the reaction (30–40 min). It seems likely that at this point, the levels of preferred cytochrome P-450 substrates (e.g. BP, 7,8-dihydrodiol, 3-phenol, and 9-phenol) have declined sufficiently to permit quinone metabolism.

The ratio of 1,6- to 3,6-quinone increases by 30% from 0.9 ± 0.1 in the absence of DNA to 1.2 ± 0.05 in the presence of DNA. Since DNA does not selectively affect the formation of these quinones, this change in the ratio of accumulated quinones implies a greater inhibitory effect of DNA on the metabolism of 1,6-quinone. The 6,12-quinone comprises less than 10% of the total quinone and does not increase in the presence of DNA.

Reduced quinones are conjugated by microsomal UDP glucuronyl transferase when UDPGA is provided (4–6). The near-elimination of quinones after addition of UDPGA (Fig. 2) requires a rate of glucuronidation that is 10–50 times faster than oxidative metabolism at these low quinone concentrations. Addition of DNA in the presence of UDPGA produces a transient peak of quinones which is comparable to the level of quinones produced after the same period of reaction without UDPGA. The quinone levels then decay linearly, disappearing by 30 min. This large elevation of the quinone concentration can only be attributed to decreased qui-

none metabolism, since DNA has no effect on BP metabolism to quinone in the presence of UDPGA. The change requires at least a 20-fold inhibition of quinone glucuronidation by DNA between 0 and 5 min.

DNA-induced changes in visible spectra of quinones. The above experiments suggest a diminished reactivity of quinones in the presence of DNA, possibly through sequestration. Such an interaction is expected to induce a bathochromic shift in the quinone spectrum analogous to those reported for the binding of BP and BPDE to DNA (16–19). A spectral red shift of 25 nm was observed for both 1,6- and 3,6-quinone in the presence of double-stranded DNA (Fig. 3A) which was not duplicated by any of the solvents tested (Fig. 3B). This is in contrast to BP, for which pyridine effects a major spectral shift compared to ethanol (16). High concentrations of bovine serum albumin (10 mg/ml) also failed to produce a red shift, even though interaction of the 1,6-quinone with the protein was indicated by increased absorbance at 440 nm and decreased the absorbance at 460 nm. RNA and heat-denatured DNA induced a red shift similar to double-stranded DNA. The nucleic acid concentration required to bind 50% of 2.5 μM 1,6-quinone was about 10-fold higher for RNA than for double-stranded DNA (Fig. 4), whereas there was no detectable difference in the binding between heat-denatured and double-stranded DNA at 0.5 mg/ml. At 50% binding, the molar ratio of bases to 1,6-quinone is about 100 for DNA and 1300 for RNA. The binding of 1,6-quinone to DNA was decreased by Mg^{2+} in the presence of lipid and absence of phosphate. Polynucleotides had no effect on the spectra of 3-phenol, 9-phenol, or 7-8-dihydrodiol in accord with the insensitivity to DNA of the metabolism of these BP derivatives.

When DNA was added to microsomal suspensions containing dispersed quinone, the spectral shift was established very rapidly (within 5 sec), followed by a slow gradual increase in absorbance. The proportion of this slow increase decreased with the concentration of quinone and became insignificant at 2.5 μM or less. It seems likely that the rapid spectral shift is due to the transfer of microsomally bound quinone to DNA, whereas the slow increase in absorbance is due to uptake of the aqueous dispersed quinone by DNA. The extinction coefficients for both 1,6- or 3,6-quinone (at 460 and 485 nm) in microsomal suspensions decreased with increasing quinone concentrations in the range of 1–10 μM. This decrease probably arises from the much lower absorbance of quinone dispersed in the aqueous phase.

Effect on microsomal metabolism of exogenous quinones. To test the hypothesis that DNA binding of quinones is responsible for DNA effects on BP metabolism, we examined the effect of DNA on various metabolic reactions of BP quinones. DNA (1 mg/ml) substantially decreased microsomal reduction of 1,6- and 3,6-quinone under aerobic conditions as shown for 1,6-quinone in Fig. 5. A nearly steady state of reduced quinone was established in about 10 sec after addition of NADPH, both with and without DNA. The extent of this reduction after 10 sec and its inhibition by DNA is shown in Table 1. The steady state for quinone reduction under aerobic conditions represents a complex balance between reduction, disproportionation, and O_2-oxidation reactions (6, 20, 21). When oxygen was depleted by addition of glucose and glucose oxidase (5, 15), the extent of reduction was

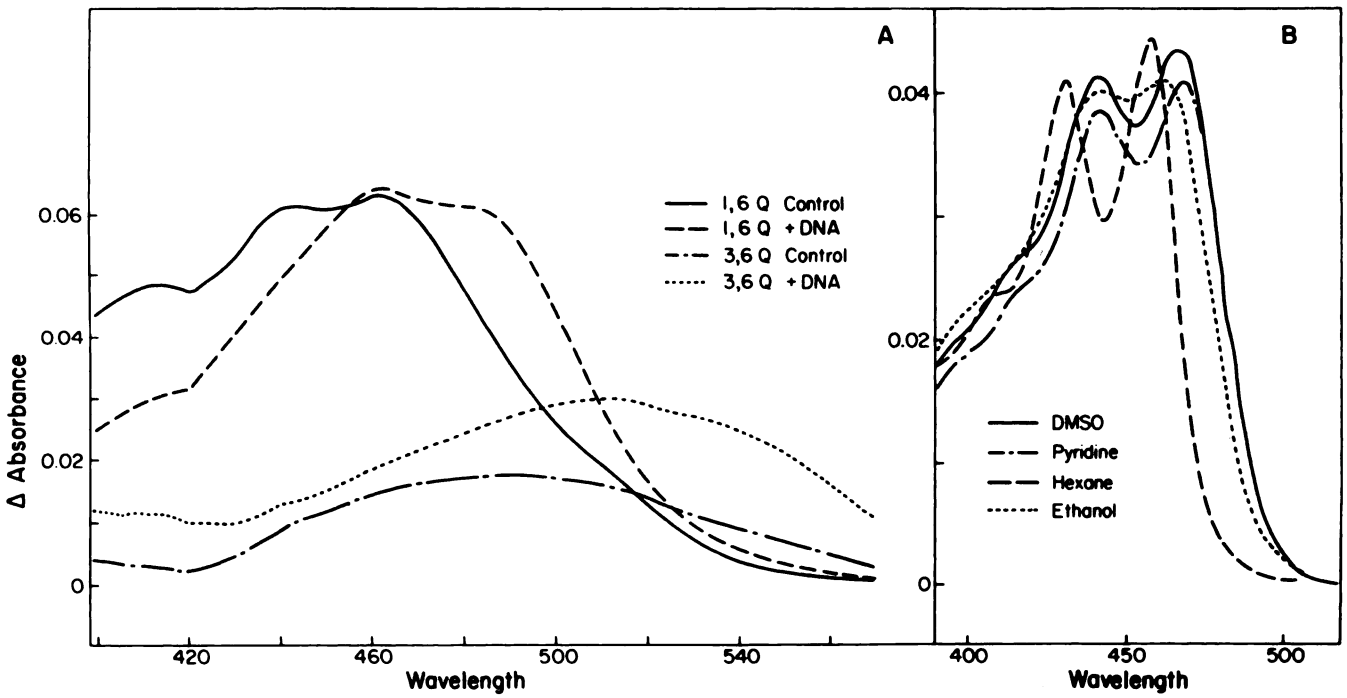

FIG. 3. *Effect of DNA on the visible spectrum of 1,6- and 3,6-quinone in microsomal suspensions*

A. The spectra of 1,6-quinone (10 μM) or 3,6-quinone (5 μM) in standard microsomal incubation mixtures, but without the NADPH-regenerating system. ——, 1,6-quinone control; - - -, 1,6-quinone with 1.0 mg/ml DNA; —·—, 3,6-quinone control; -----, 3,6-quinone with 1.0 mg/ml DNA.

B. Spectra of 2.5 μM 1,6-quinone in different solvents. ——, Me_2SO (*DMSO*); -----, ethanol; —·—·, pyridine; - - -, hexane.

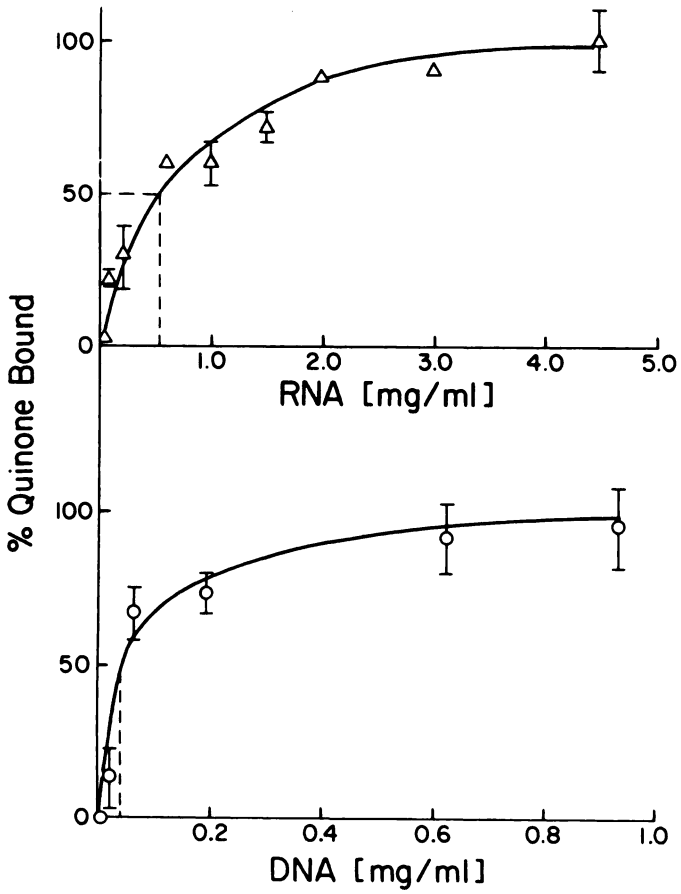

FIG. 4. *Adsorption isotherms of 1,6-quinone binding to DNA and RNA*

1,6-Quinone (2.5 μM) was added to standard microsome incubations without NADP or NADPH which contained increasing amounts of DNA or RNA. The increase in the absorbance difference between 486 nm and 600 nm was monitored at 37°. The percentage of quinone partitioning into nucleic acid was calculated assuming that the quinone was entirely bound to nucleic acid-associated quinone at 2.0 mg/ml of DNA and 4.5 mg/ml of RNA. *Bars* indicate the range of duplicates when it exceeded the *symbol* size.

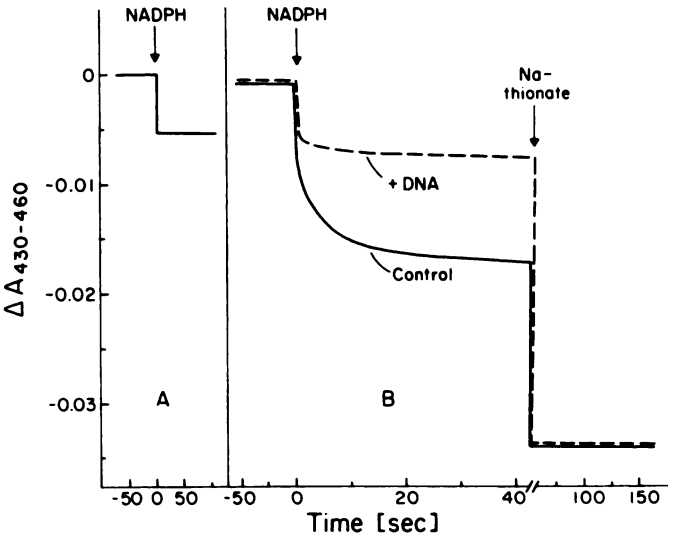

FIG. 5. *Time course of microsomal reduction of 1,6-quinone*

The time-dependent reduction of 1,6-quinone in the presence or absence of DNA was monitored by the change in absorbance in the wavelength pair 430 nm and 460 nm as described under Materials and Methods. Concentrations were 0.3 mg/ml microsomal protein, 2.5 μM quinone (B) or no quinone (A), and 250 μM NADPH. Reduction was initiated with NADPH after 5 min of preincubation at 37°. Full reduction to the hydroquinone was obtained by addition of a few crystals of sodium dithionite to the cuvette (15).

higher but DNA inhibition was comparable to that under aerobic conditions. Although quinones are probably reduced to hydroquinones via an intermediate semiquinone (15), spectra during reduction were predominantly those of the hydroquinone (5, 15).

Microsomes catalyze the removal of 1,6- and 3,6-quinone (Fig. 6) and produce more polar products when supplied with NADPH under aerobic conditions (22). The spectra, however, change very little with time (up to 20 min) once an apparent steady state between reduced and oxidized quinone had been established. Surprisingly, during this period essentially all of the initial 3,6- or 1,6-quinone is metabolized. This metabolism, which apparently has little effect on the quinone chromophore, has been attributed to monooxygenation in aromatic rings of these polycyclic quinones (22). This disappearance of 1,6-quinone (Fig. 6) was inhibited 60–70% by the addition of DNA (1.4 versus 0.5 nmoles min^{-1} mg^{-1}). The rates of glucuronidation of the quinones have been derived from the enhanced disappearance of quinones in the presence of UDPGA. On this basis, rates of glucuronidation of 1,6-quinone in absence and presence of DNA were 4.3 and 2.8 nmoles min^{-1} mg^{-1}, respectively. These rates were several times faster than monooxygenation and were less inhibited by DNA. Monooxygenation of 3,6-quinone was slower than that of 1,6-quinone (1.0 nmole min^{-1} mg^{-1}) but, surprisingly, was not inhibited by DNA. This difference in the effect of DNA on monooxygenation of exogenous 1,6- and 3,6-quinone was consistent with the DNA-induced increase in the ratio of these metabolites during BP metabolism which has been described above. The initial rate of glucuronidation of 3,6-quinone occurs at a rate (3.9 nmoles min^{-1} mg^{-1}), comparable to the glucuronidation of 1,6-quinone. However, inhibition by DNA increases with time from 35% at

TABLE 1

Inhibition of microsomal reduction of 1,6- and 3,6-quinone by DNA

Reduction of quinones (2.5 μM) was monitored spectrophotometrically at a 0.3 mg/ml concentration of microsomal protein and 37° as described under Materials and Methods and for Fig. 5. The same wavelength pairs were used with or without DNA (1 mg/ml), since DNA-induced changes in absorption coefficients were compensated for by basing calculations on full quinone reduction obtained with sodium dithionite. Reduction at 10 sec after addition of NADPH is shown. This represents a nearby steady state for aerobic conditions.

Substrate	Treatment	Reduced quinone	Inhibition
		μM	%
1,6-Quinone	Control	0.77	—
	+ DNA	0.05	94
3,6-Quinone	Control	1.14	—
	+ DNA	0.15	87

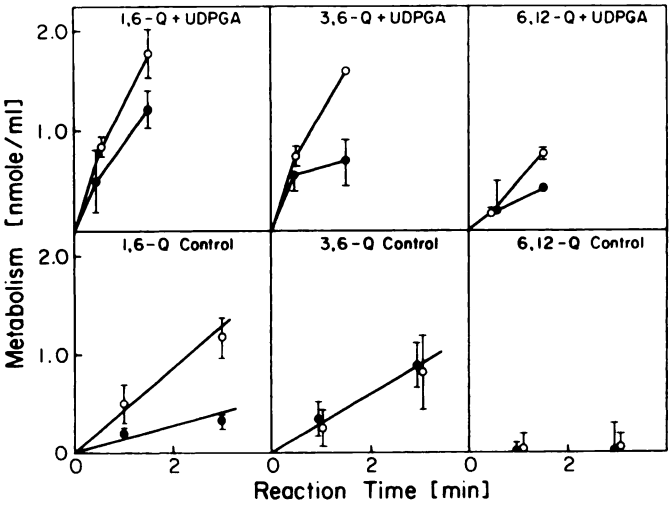

FIG. 6. *Effect of DNA on the glucuronidation and monooxygenation of BP quinones in microsomal incubations*

Quinones (2.5 μM) were metabolized in standard microsomal incubations in the presence or absence of UDPGA and DNA. Samples were extracted with ethylacetate/acetone (2:1) containing 0.3 μM 7,8-dihydrodiol as an internal standard, and residual quinones were determined by HPLC as described under Materials and Methods. The *bars* represent the standard deviation of four determinations when it exceeded the size of the *symbol*. ○——○, No DNA; ●——●, 1.0 mg/ml DNA.

30 sec to essentially complete inhibition at 90 sec. Monooxygenation of 6,12-quinone is negligible, while glucuronidation was 2 times slower (1.7 nmoles min^{-1} mg^{-1}) than for the other BP quinones and was inhibited 50% by DNA.

Reversal of inhibition by 6,12-quinone. Addition of DNA diminished the inhibition of BP metabolism and DE formation by 6,12-quinone (Table 2). Thus, 1.5 μM 6,12-quinone decreased BP metabolism by one-half in the absence of DNA but had no significant effect in the presence of DNA (1.0 mg/ml). Exogenous 6,12-quinone was more effective in inhibiting the conversion of BP to both major classes of DNA adducts than in inhibiting primary BP metabolism.

DNA adduct formation. The diversion of BP metabolites to adduct formation rose with DNA concentration. Although the modification of DNA by DE increased linearly with DNA concentration (Fig. 7A), this represents an increase in the proportion of DNA adducts

TABLE 2

Quinone inhibition of BP metabolism and DNA adduct formation is reduced by DNA

Incubations for microsomal BP metabolism were set up with additions of DNA and BP 6,12-quinone as indicated. After a 30-min reaction, total BP metabolism and DNA adduct formation were measured by Me_2SO/hexane extraction and Sephadex LH-20 chromatography as described under Materials and Methods.

DNA	6,12-Quinone	BP metabolism	DE adducts	PO adduct
mg/ml	*μM*	%	%	%
1.0	0	100	100	100
1.0	1.5	93	78	89
1.0	10	72	29	22
0	1.5	53	—	—

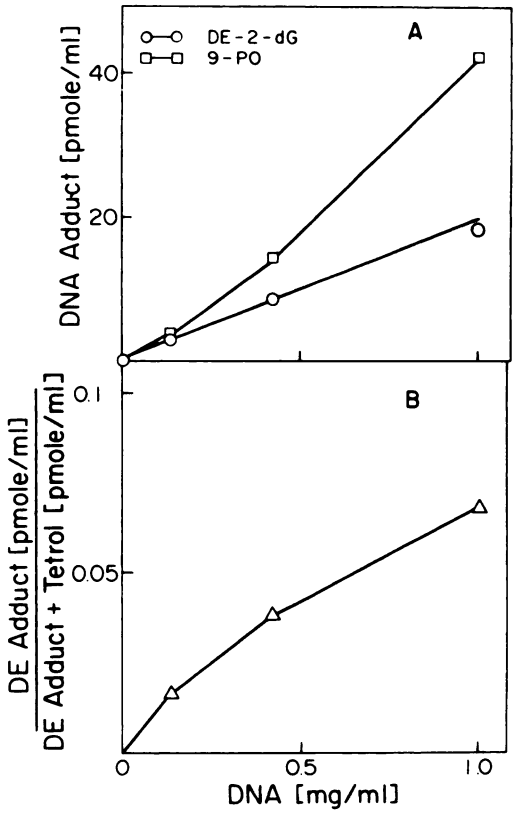

FIG. 7. *Effect of DNA concentration on adduct formation in microsomal BP incubations*

After 45 min of reaction in the presence of heat-denatured DNA, tetrol and adduct formation was measured as described under Materials and Methods. A, ○——○, DE adduct; □——□, 9-PO adduct; B, △——△, DE adduct to tetrol ratio.

formed from each DE produced (tetrol + DNA adducts); i.e., the ratio of adduct to DE depends hyperbolically on the DNA concentration (Fig. 7B). In contrast, formation of the 9-PO adduct exhibited an exponential dependence on DNA concentration. This probably derives from an unexpected effect of DNA on the competition between the hydration and the non-enzymatic rearrangement of the 9,10-oxide to 9,10-dihydrodiol or 9-phenol, respectively. DNA increased the ratio of 9-phenol to 9,10-dihydrodiol by 4-fold (Fig. 8) but had no apparent effect on the formation of 9,10-oxide, as the combined rate of formation of these two products does not change. This increase in 9-phenol was lost in the later stages of the reaction, owing to increased metabolism of 9-phenol in the presence of DNA, only part of which resulted in adduct formation. The levels of 3-phenol, 4,5-dihydrodiol, and 7,8-dihydrodiol (4) were little affected by DNA.

DISCUSSION

The stimulation of primary and secondary monooxygenation of BP by UDPGA has been attributed to the removal of inhibitory BP quinones (4, 10). The absence of an effect of DNA on either BP metabolism or DE formation in the presence of UDPGA implies that a product which is removed by conjugation is also the source of DNA-induced stimulation, i.e., a quinone or a

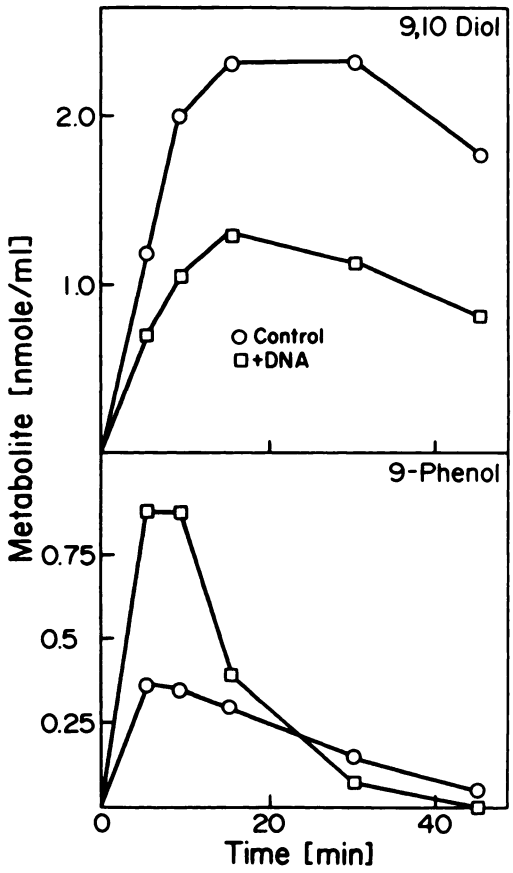

FIG. 8. *Effect of DNA on the production of 9,10-diol and 9-phenol during microsomal BP metabolism*

Symbols and conditions are as described for Fig. 2.

phenol. DNA renders quinones less accessible to microsomal enzymes, both as inhibitors and substrates. This is directly demonstrated in the decreased effectiveness of 6,12-quinone as an inhibitor of BP monooxygenation in the presence of DNA. Similarly, quinone reduction, monooxygenation, and glucuronidation are, for the most part, decreased by DNA. Thus, higher levels of these products accumulate when BP is metabolized in the presence of DNA. It seems most likely that the effect of DNA, both on the reactivity of BP quinones and on their effectiveness as feedback inhibitors of BP metabolism, is due to their uptake into DNA.

The DNA-induced increase in the K_i for inhibition of BP metabolism suggests that the effective concentration of quinones in the microsomal phase is decreased about 10-fold when DNA is added. Almost complete removal of BP quinones from the microsomal phase by addition of DNA has been directly demonstrated spectrophotometrically. The 25-nm red shift in the spectrum on BP 1,6- and 3,6-quinones, upon binding to DNA, is analogous to shifts observed upon intercalative binding of BP (16) and DE in aqueous media (17–19). However, no such shift was observed for BP dihydrodiols or BP phenols (3- and 9-). Reversible binding of BP quinones to DNA has previously been demonstrated (21). Binding was sufficiently strong that only extraction with phenol completely dissociated the complex. BP quinones bind to DNA over 10 times more extensively (1 quinone:100 bases) than reported for BP (16), while both compounds show comparable binding to RNA (1 to 2 PAH:2000 bases). Preliminary data indicate that the binding for 1,6-quinone is at least 5- to 10-fold stronger than for DE as reported by MacLeod and Selkirk (17) under similar experimental conditions. The inhibitory effect of $MgCl_2$ and the greatly diminished uptake by RNA suggest intercalative binding of BP quinones to double-stranded DNA. The effective binding of BP quinones to heat-denatured DNA is not inconsistent with intercalative binding, since helical regions remain in heat-denatured DNA (23).

DNA exerts highly selective effects on the microsomal metabolism of BP quinones. Monooxygenation of 1,6-quinone is inhibited by DNA, whereas monooxygenation of 3,6-quinone is unaffected. In contrast, glucuronidation of 3,6-quinone is more sensitive to DNA than glucuronidation of 1,6-quinone. DNA increases the ratio of 1,6,- to 3,6-quinone generated from metabolism of BP, indicating that the selective effect of DNA on monooxygenation of added 1,6-quinone also applies to metabolism of the endogenous quinone. This selectivity may derive from differences in the respective K_m values for quinone (monooxygenation) or hydroquinone (glucuronidation). The magnitude of the increase in quinone levels also indicates that the inhibitory effect of DNA must be greater on the metabolism of microsomally generated quinones than we have observed for metabolism of added quinones.

The second effect of DNA on BP metabolism derives from an alteration in the fate of 9,10-oxide. An increase in the 9-phenol to 9,10-dihydrodiol ratio, with no change in the sum of these products derived from 9,10-oxide, indicates that DNA either enhances the rate of rearrangement of 9,10-oxide to 9-phenol or decreases enzymatic hydration. Participation of DNA phosphate could result in enhanced rearrangement, analogous to the solvolysis of diol epoxides catalyzed by phosphate ions (24, 25), riboflavin phosphate (26), and DNA (27, 28). In addition, DNA may also sequester 9,10-oxide, leading to an apparent increase in the K_m for hydration.

The stimulation of BP metabolism and DE formation by DNA suggests that, without DNA, primary product quinones inhibit BP metabolism and DE formation to an extent that is at least equal to this stimulation. This is probably an underestimate because of residual inhibition by the high level of quinones which are bound to DNA. We have previously shown that inhibition of epoxide hydratase with low levels of 3,3,3-trichloropropene oxide elevates 9-phenol 3-fold without affecting quinone formation and with only marginal effects on total product formation (9). This suggests that 9-phenol contributes much less than quinones to the product inhibition of BP metabolism and DE formation.

The effect of DNA concentration on adduct formation derives both from an increase in formation of DE and an increase in the rate of reaction between DE and DNA. The ratio of adduct to tetrol exhibits a trend toward a limiting value of 0.1 at higher levels of DNA. DE intercalates rapidly into DNA with a subsequent acceleration of tetrol formation (27). Consequently, the limiting adduct to tetrol ratio probably reflects the proportion of adduct to tetrol obtained when all DE partitions from

the aqueous or microsomal phase into DNA without prior solvolysis. The formation of 9-PO adducts saturates less readily at higher levels of DNA. This must, in part, derive from the large increase effected by DNA in the formation of the precursor, 9-phenol.

BP quinones are readily conjugated in hepatocytes following reduction at cytochrome P-450 reductase (6) or cytosolic DT-diaphorase (22). However, salicylamide, an inhibitor of UDP-glucuronyl transferases and of sulfotransferases, increases BP quinone levels in MC-induced hepatocytes by 4-fold to 1.9 μM without apparent effect on total BP metabolism (28). One explanation of this insensitivity is provided by the binding of BP quinones to DNA. At the cell concentration used, the cellular DNA (50 μg/ml) may bind about 50% of the 1.9 μM quinone, as the ratio of DNA/microsomes in heptocytes (1/10) is similar to the ratio in Fig. 4 at a DNA concentration of 50 μg/ml (1/6). In addition, RNA and protein probably also contribute to lower the effective concentration of BP quinones.

The reduction and conjugation of BP quinones is remarkably effective in hepatocytes. This provides an important protective mechanism for the cell, since prolonged binding of quinones to DNA may be expected to lead to single-strand breaks as a result of radical generation at the binding site (21). DNA damage of this type may contribute a promotional effect to the oncogenic transformation of cells initiated by BP 7,8-dihydrodiol 9,10 oxide (29). The high-affinity binding of polycyclic quinones to polynucleotides is also likely to interfere with DNA transcription and replication as has been observed for other intercalating drugs (30). The further characterization of quinone formation and detoxication in extrahepatic tissues is clearly of importance.

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Send reprint requests to: Dr. Colin R. Jefcoate, Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisc. 53706.