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INHIBITION OF BINDING OF BENZO(a)PYRENE METABOLITES TO NUCLEAR DNA BY GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE B

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SUMMARY: Incubation of benzo(a)pyrene with the hepatic nuclear fraction isolated from 3-methylcholanthrene-treated rats resulted in the binding of 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 9-hydroxybenzo(a)pyrene to nuclear DNA. Addition of cytosol to the incubation decreased the level of DNA-bound metabolites especially those derived from 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene. This inhibitory effect of cytosol was related to its content of reduced glutathione and could be reproduced by the replacement of cytosol by glutathione and glutathione S-transferase B. These results suggest that the level of glutathione and glutathione S-transferases may be of prime importance in regulating the accumulation of reactive intermediates of benzo(a)pyrene in the intact cell.

INTRODUCTION: The metabolism of benzo(a)pyrene (BP)[1] to reactive electrophiles which bind to specific sites in DNA is believed to be an essential step in BP carcinogenesis (1,2). The main DNA-bound species of BP are derived from the proximate carcinogen 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (BP-7,8-diol) and from 9-hydroxybenzo(a)pyrene (9-OH-BP) which has not been implicated in carcinogenesis (3,4). The DNA-binding species formed from BP-7,8-diol have been identified as BP-7,8-diol-9,10-oxide isomers of which the (+)anti-BP-7,8-diol-9,10-oxide is considered the major ultimate carcinogenic form of BP (5-7).

The formation of these DNA-binding metabolites of BP require a sequence of reactions which can occur in both the endoplasmic reticulum and the

[1] Abbreviations: BP, benzo(a)pyrene; BP-7,8-diol, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene; 9-OH-BP, 9-hydroxybenzo(a)pyrene; GSH, glutathione, reduced form.

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nuclear envelope (8-10). Precursors of the DNA-binding metabolites may be formed in the endoplasmic reticulum and activated in the nuclear envelope. The close proximity of genetic material to the nuclear envelope may be particularly important in generating electrophilic metabolites which become bound to DNA.

Recent results from this laboratory have shown that the pattern of the binding to DNA of BP metabolites upon incubation with rat liver nuclei is markedly affected by cell constituents such as microsomes and cytosol. For example the binding of the electrophilic product(s) derived from 9-OH-BP was markedly decreased in a non-specific manner by protein, native or denatured. On the other hand, a decrease in the binding of BP-7,8-diol intermediates was observed only in the presence of native cytosol, indicating the existence there of a specific factor or factors which trap reactive species from BP-7,8-diol (11,12).

In the present study we have investigated the role of the cytosol in regulating the binding of reactive BP intermediates to DNA in isolated nuclei. Our results indicate that glutathione (GSH) and GSH S-transferases may be of particular importance in preventing BP-7,8-diol-9,10-oxides from reaction with DNA.

MATERIALS AND METHODS: |^{3}H|-BP (∿27 Ci/mmole) was obtained from the Radiochemical Center, Amersham, Bucks, England. Tritiated (±)-trans-7,8-diol (0.386 Ci/mmole) and unlabeled (±)-trans-7,8-diol were synthesized under NCI contract No. 1-CP-33387 and kindly supplied by IIT Research Institute, Chicago, Ill., USA. NADPH, 3-methylcholanthrene, unlabeled BP, protease type V, RNase type 1-A, DNase I, bacterial alkaline phosohatase and snake venom phosphodiesterase were purchased from Sigma Chemical Co., St. Louis, Mo., USA. Sephadex LH20 was obtained from Pharmacia, Uppsala, Sweden. Lumagel was obtained from Lumac Systemic AG, Basel, Switzerland. All other chemicals were of analytical grade.

Liver fractions nuclei and cytosol were isolated from rats pretreated once with 3-methylcholanthrene (50 mg/kg given i.p. 48 hours before sacrifice) as described previously (11). To deplete GSH from the cytosol, rats were pretreated with diethylmaleate (400 mg/kg) 1 hour before sacrifice. The cytosol was then dialyzed for one hour against 50 mM Tris HCl buffer, pH 7,5, containing 25 mM KCl and 5 mM $MgCl_2$ (TKM buffer). Incubations contained $250x10^6$ nuclei in 10 ml of TKM buffer (see above), 1 mM NADPH and 20 µM |^{3}H|-BP (2.7 Ci/mmole) or 4 µM |^{3}H|-BP-7,8-diol (0.6 Ci/mmole). Incubations were for 30 min. Isolation, purification and hydrolysis of nuclear DNA, and analysis of modified deoxyribonucleosides, were performed as previously described (11). GSH S-transferase B was prepared either according to Tipping et al (13) or Guthenberg and Mannervik (14).

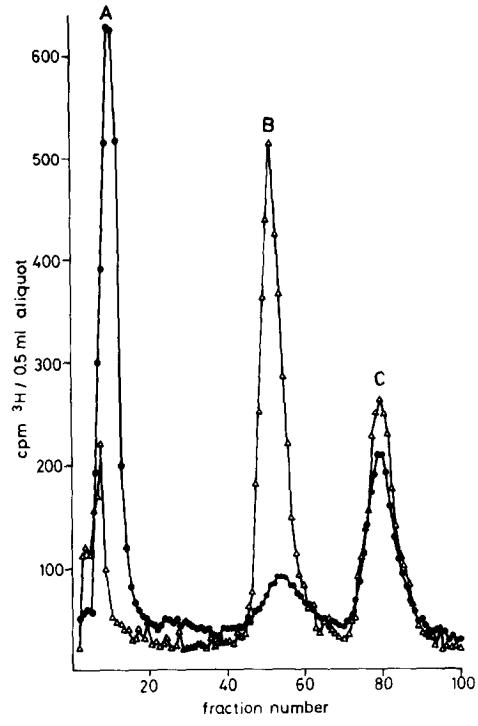

Fig. 1. Binding of benzo(a)pyrene metabolites to rat liver nuclei (a,Δ) and effect of cytosol on the binding (b,●). BP-modified DNA was hydrolyzed and deoxyribonucleosides were separated by Sephadex LH20 chromatography. The total amount of binding was calculated by integrating the corresponding peaks and correcting for the total volume of elution, the amount of DNA added to the column, and the specific activity of the $|^3H|$-BP substrate. However, no correction was made for ^3H loss during metabolism which has been estimated to affect the measure of bound products from 9-OH-BP (20-30%) but not that of products from BP-7,8-diol (16). Under these circumstances binding in the absence of cytosol corresponded to 4.5±1.3 (n=8) pmol/mg DNA/30 min of BP-7,8-diol metabolites (Peak "B") and 3.5±1.9 (n=8) pmol/mg DNA/30 min of 9-OH-BP-metabolites (Peak "C").

RESULTS AND DISCUSSION: Rat liver nuclei were incubated with $|^3H|$-BP in the presence of NADPH and DNA was isolated and hydrolyzed to deoxyribonucleosides which were analyzed by LH-20 chromatography. The main part of covalently bound radioactivity was found to be localized in two peaks, designated B and C (Fig. 1a). As previously shown, these peaks represent the binding of products of BP-7,8-diol and 9-OH-BP, respectively (6,15). Binding to nuclear DNA corresponded to 4.5 pmol/mg DNA of products from BP-7,8-diol and 3,5 pmol/mg DNA of products from 9-OH-BP.

Addition of the cytosol fraction (corresponding to 100 mg soluble protein) to the incubation resulted in a marked decrease in DNA-binding, espe-

cially of BP-7,8-diol-derived products (Fig. 1b). Thus cytosol appeared to possess potent nucleophiles which were effective in trapping reactive metabolites formed from BP. To investigate this "trapping effect" further cytosol from normal rats was replaced by cytosol from diethylmaleate-treated rats. This treatment lowered the level of reduced glutathione in the cytosol from about 1.5 mM to about 0.1 mM. In the presence of GSH-depleted cytosol, the binding of BP-7,8-diol metabolites was increased almost 4-fold and that of 9-OH-BP products more than 2-fold (Table 1). When GSH was added back to the incubation medium, DNA-binding of BP products decreased in inverse proportion to the increased levels of GSH.

The high capacity of intact cytosol to trap reactive metabolites of BP was thought to be due to the presence of GSH S-transferase activity. This was tested by incubating nuclei in the presence of GSH and purified GSH S-transferases. The effect of three preparations of GSH S-transferase B on the binding of BP-7,8-diol metabolites to DNA during nuclear metabolism of [^{3}H]-BP is shown in Fig. 2. Preparation I was devoid of GSH, while preparations II and III contained GSH, which is added to stabilize the activity of the purified transferase. Accordingly, the control experiments with preparations II and III contained 0.2 and 0.02 mM GSH, respectively. When preparation I was added in the absence of GSH, the decrease in DNA-binding

TABLE 1. EFFECT OF GLUTATHIONE ON DNA-BINDING OF BENZO(a)PYRENE METABOLITES FORMED BY NUCLEI IN THE PRESENCE OF CYTOSOL

Additions	Metabolites bound (pmol/mg DNA)	
	BP-7,8-diol metabolites	9-OH-BP metabolites
Nuclei plus cytosol A	0.4	1.4
Nuclei plus cytosol B	1.5	3.2
Nuclei plus cytosol B plus 0.1 mM GSH	1.0	3.1
Nuclei plus cytosol B plus 0.2 mM GSH	0.5	2.2

Cytosol A was obtained from liver of 3-methylcholanthrene-treated rat whereas cytosol B was obtained from liver of 3-methylcholanthrene- and diethylmaleate-treated rat.

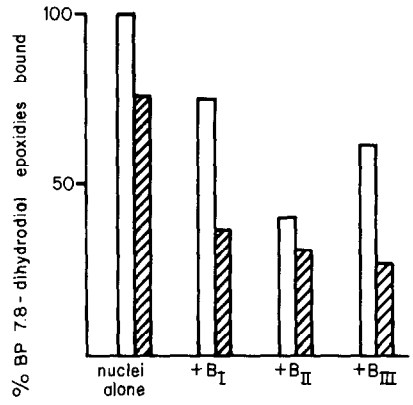

Fig. 2. Effect of three different preparations of purified glutathione S-transferase B (called B_I, B_{II} and B_{III}) on the binding of BP-7,8-diol metabolites in the presence (hatched bars) and in the absence (open bars) of 0.2 mM GSH. 4 mg of each preparation was incubated with rat liver nuclei and $|^3H|$-BP as described in Materials and Methods. B_{II} contained 2 µmoles GSH/4 mg protein, and B_{III} 0.2 µmoles GSH/4 mg protein whereas preparation B_I contained no GSH.

due to the presence of protein was about 20%. Addition of GSH to all preparations caused a decrease in DNA-binding by approximately 70-80% compared to the binding obtained with nuclei alone. Thus, it appears that GSH S-transferase B plus GSH had a similar capacity to trap reactive metabolites of BP to that observed with normal cytosol.

This is further illustrated in Table 2. Preliminary findings indicate that GSH conjugates are formed and that the extent of their formation is

TABLE 2. EFFECT OF GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE B ON THE BINDING OF PRODUCTS FORMED DURING NUCLEAR METABOLISM OF BENZO(a)PYRENE-7,8-DIHYDRODIOL

Addition	Metabolites bound (pmol/mg DNA)	
	- Glutathione S-transferase B	+ Glutathione S-transferase B
Nuclei	51.8	50.7
Nuclei plus 0.1 mM GSH	55.7	19.2
Nuclei plus 0.2 mM GSH	55.7	17.8
Nuclei plus 1.0 mM GSH	53.0	13.5

Incubation was performed in the presence of 4 µM $|^3H|$-BP-7,8-diol. 4 mg of purified glutathione S-transferase B were added.

correlated to the extent of formation of reactive metabolites that otherwise would be bound to DNA.

In conclusion the present study has shown that hepatic cytosol interferes with the accumulation of DNA-bound metabolites derived from both BP-7,8-diol and 9-OH-BP in isolated rat liver nuclei. This effect is particularly marked with BP-7,8-diol which gives rise to the ultimate carcinogen BP-7,8-diol-9,10-oxide and in this case can be reproduced by a mixture of GSH S-transferase B and GSH.

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