BINDING OF POLYCYCLIC HYDROCARBONS TO NUCLEAR COMPONENTS IN VITRO[1]

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SUMMARY: Rat liver nuclei were incubated with microsomes, a NADPH-generating system, microsomes and ^{3}H-benzo(a)pyrene. Binding of polycyclic hydrocarbon was noted to nuclear DNA, nuclear proteins and microsomal proteins. When nuclei or microsomes from 3-methylcholanthrene treated animals were used, binding to nuclear DNA and microsomal protein was increased. These data confirm the presence of a nuclear aryl hydrocarbon hydroxylase, extend previous studies on macromolecular acceptors to include nuclear proteins and demonstrate reduced binding to nuclear proteins and DNA when microsomes are included in the incubation system with nuclei.

Carcinogenic polycyclic aromatic hydrocarbons bind covalently to nucleic acids both in vivo and in vitro (1-7). This binding is preceded by activation of the polycyclic hydrocarbons by the NADPH-dependent mixed function oxidases (2,3,6). In addition, enhanced complexation is observed when microsomes are employed which have been obtained from animals pretreated with 3-methylcholanthrene (3MC); this results from the induction of the microsomal aryl hydrocarbon hydroxylase (AHH) system (8-11).

Although AHH activity was believed to be present only in microsomes, recent studies by Khandwala and Kasper (12) and Rogan and Cavalieri (7) have suggested such activity within nuclei. Furthermore, this enzyme is inducible by 3MC and increased binding of polycyclic hydrocarbons to nuclear DNA is also observed in vitro (7). In view of the possible significance of these observations to the problem of chemical carcinogenesis, we attempted to confirm these studies. Toward this end, we wished to employ in vitro systems which closely approximate the in situ conditions. We utilized an incubation medium which contained ^{3}H-benzo(a)pyrene (^{3}H-BP), purified liver nuclei and microsomes.

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The results of these investigations a) confirm the observations of Rogan and Cavalieri (7) and Khandwala and Kasper (12); b) demonstrate, in addition, significant complexation of polycyclic hydrocarbons to nuclear proteins and c) show a reduction of binding to both nuclear proteins and DNA when microsomes are included in the nuclear incubation system.

MATERIALS AND METHODS

^{3}H-benzo(a)pyrene (5 Ci/mmole) (^{3}H-BP), purchased from Amersham-Searle Co., was purified by high pressure liquid chromatography. Liver nuclei and microsomes were prepared from 50-70 g male Sprague-Dawley rats as described previously (6). In some experiments, male C3H/Ki mice served as the source for the microsomal preparation. When indicated, rats (or mice) were injected i.p. with 3MC, 20 mg/kg, or with the vehicle alone, corn oil, and were used 24 hr later for the isolation of the subcellular components.

Polycyclic hydrocarbon binding was determined in an incubation medium which consisted of the following components: Na phosphate buffer pH 7.4, 100 µmoles; EDTA, 100 µmoles, glucose-6-phosphate dehydrogenase (Sigma Chemical Co., 2000 U/mg), 6 units; glucose-6-phosphate, 18 µmoles; NADPH 3 µmoles; $MgCl_2$, 5 µmoles; rat liver nuclei; liver microsomes; and ^{3}H-BP, 50 µCi. The total volume was 3 ml and the incubation was conducted for 30 min at 37° with shaking. Thereafter, the tubes were immersed in a cold bath at 4° and the nuclei were sedimented at 1000 x g for 10 min. The supernatant fraction was saved for analysis of binding to microsomal proteins. The nuclei which were still intact after the incubation were pelleted by centrifugation for 10 min at 1000 x g, were washed three times with 5 ml of 0.25 M sucrose- 5 mM MgCl -0.05 M tris pH 7.4. The washed nuclear pellet was lysed in 0.03 M NaCl-3 mM Na citrate-2% Na dodecyl sulfate (SCS) and the solution was extracted with buffer-saturated phenol. The phenol phase was saved for the determination of protein binding while the nucleic acids were precipitated from the aqueous fraction with ethanol. The nucleic acid precipitate was redissolved in buffer, 100 µg preheated RNase (80° for 10 min) was added and digestion of the RNA allowed to continue for 60 min at 37°. The DNA was then precipitated by ethanol and washed twice with ether. The DNA pellet was hydrolyzed by heating in 1.5 ml 0.6N perchloric acid for 30 min at 85°, and aliquots were utilized for the determination of radioactivity and DNA concentration (13).

Following the initial centrifugation to separate nuclei (see above), 3 ml of SCS was added to the supernatant fraction which contained the microsomes. The resultant dispersion was extracted with buffer-saturated phenol. To the phenol phases containing either the microsomal or nuclear proteins, ethanol was added and the resultant protein precipitate was washed 5 times with cold ethanol. The final pellet was dissolved in 1N NaOH and aliquots were utilized for the determination of radioactivity and protein concentration (14).

RESULTS AND DISCUSSION

The kinetics of complexation of polycyclic hydrocarbon to nuclear protein, nuclear DNA and microsomal protein are presented in Figure 1a-c. Binding to all three components was linear for at least 30 min under our assay conditions. Accordingly, the latter time was utilized for all subsequent studies.

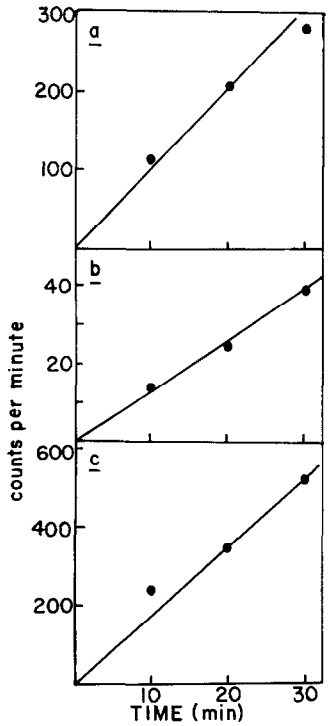

Figure 1: Complexation of polycyclic hydrocarbon to nuclear protein (a) nuclear DNA (b) and microsomal protein (c). ^{3}H-BP was incubated for varying periods of time with nuclei and microsomes as indicated in the text.

The results presented in Table 1 indicate substantial complexation of polycyclic hydrocarbon to both nuclear and microsomal proteins in the complete system (containing both nuclei and microsomes). In the all control system the specific activity of nuclear protein binding, however, was 1.5 fold that of microsomal protein. In confirmation of the report of Rogan and Cavalieri (7), increased binding of polycyclic hydrocarbon was observed to nuclear DNA after administration of 3MC. However, 3MC administration reduced the complexation to nuclear protein when the nuclei from drug-treated animals were employed. In the system consisting of control nuclei and 3MC microsomes, nuclear protein binding was unaffected (when compared to the all control system). Nuclei obtained from the livers of 3MC-treated rats exhibited increased complexation to DNA and microsomal protein when compared to control nuclei

TABLE 1

Binding of Polycyclic Hydrocarbon to Microsomal and Nuclear Components In Vitro After Induction with 3MC

Source of Microsomes	Source of Nuclei	Nuclear DNA (cpm/μg DNA)	Nuclear Protein (cpm/μg)	Microsomal Protein (cpm/μg)
C	C	10	267	175
3MC	C	16 (+60%)	249	330 (+88%)
C	3MC	15 (+50%)	139 (-48%)	280 (+60%)
3MC	3MC	30 (+300%)	142 (-47%)	501 (+186%)
-	C	17 (+70%)	147 (-46%)	-
-	3MC	30 (+200%)	226 (-16%)	-

^{3}H-BP was incubated with the above components as described in the text for 30 min at 37°. The microsomes and nuclei were obtained from the livers of corn oil-treated (C) or 3MC-injected (3MC) rats at 24 hr after the treatment. Comparable quantities of microsomes (2-3 mg) and nuclei (3 mg) were used in all the experiments. The data are representative of 4 series of experiments; the counting efficiency was 40%. The numbers within the parentheses indicate the % increase over the value seen in the incubation system consisting of control nuclei and microsomes.

and the increased binding to microsomal protein in the complete 3MC system was additive. Of particular interest was the reduction of binding to nuclear DNA upon the addition of microsomes to the nuclear system.

The interaction of polycyclic hydrocarbons to macromolecular components is probably related to the event (or events) leading to the neoplastic transformation. The data presented in this manuscript point out the very significant complexation to nuclear proteins. It is interesting to speculate that such binding may in some manner influence the subsequent activities of the genomic material, i.e., transcription and replication, and thus may lead to altered cellular activities.

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