Binding of Benzo(a)pyrene Derivatives to Specific Proteins in Nuclei of Intact Hamster Embryo Cells

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ABSTRACT

In hamster embryo cells incubated for 24 hr with 4 μM [3H]benz(a)pyrene, a major portion of the nonextractable radioactivity in nuclear preparations copurifies with the protein fraction. When these proteins are analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, significant variations in the labeling intensities of the various proteins are seen. Control experiments demonstrate that the labeling is due to covalent binding to protein. Histones H3 and H2A are heavily labeled while the other histones of the nucleosome core, H2B and H4, are devoid of radioactivity. Large amounts of label are associated with proteins with mobilities similar to the very lysine-rich histones H1. However, the results of differential extraction experiments suggest that the labeled proteins do not belong to either the H1 or the high-mobility-group class of chromosomal proteins. During 6 hr of inhibition of protein synthesis by cycloheximide, the metabolism of [3H]-benzo(a)pyrene, as monitored by high-pressure liquid chromatography, remained normal. Patterns of labeling of nuclear proteins after 3 or 6 hr were identical in the presence and absence of cycloheximide. This finding strongly suggests that binding of benz(a)pyrene derivatives to nuclear proteins occurs in situ.

INTRODUCTION

Carcinogenic PAH’s are metabolized by mixed-function oxidases present in all mammalian cells to a wide array of oxygenated and conjugated derivatives (10, 47). The vast majority of this metabolism represents detoxification of the very hydrophobic parent compounds to more polar species that can readily be excreted (2, 7, 34). However, in this process, highly reactive intermediates are produced which covalently modify cellular macromolecules. Early work with a number of PAH’s (1, 15, 28, 39, 55) not only established generalized binding to proteins both in vivo and in cultured cells but identified specific proteins with a high affinity for PAH’s (5, 17, 18, 24), and metabolites characteristic of this pathway, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, have been demonstrated to be potent carcinogens (21, 49). Despite their prevalence, the role of PAH-protein adducts in the process of chemical carcinogenesis has remained obscure, as have the pathway(s) leading to this binding.

We have noted previously that nuclear proteins become highly labeled in HEC cultures incubated with [3H]B(a)P (34) or [3H]7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (8). In the present work, we partially define the particular species of nuclear proteins susceptible to B(a)P binding and present evidence that this binding occurs in situ.

MATERIALS AND METHODS

Chemicals. Benz(a)anthracene was purchased from Eastman Organic Chemicals (Rochester, N. Y.) and stored at −20° dissolved in dimethyl sulfoxide at 2.92 mm. Cycloheximide and PMSF were obtained from Sigma Chemical Co. (St. Louis, Mo.). [3H]B(a)P was obtained from Amersham Corp. (Arlington Heights, III.) and stored as described previously (34); specific activities used in these experiments ranged from 6.0 to 27.0 Ci/mmol. L-[4,5-3H]Leucine (1.0 Ci/mmol) was also obtained from Amersham Corp. Other chemicals were as described previously (25, 34).

Cells and Labeling Procedures. Growth and labeling of HEC cultures with [3H]B(a)P have been described (34). For protein synthesis inhibition studies, confluent tertiary cultures were incubated for 20 hr with 13 μM benz(a)anthracene to induce aryl hydrocarbon hydroxylase activity (42). The benz(a)anthracene was removed and replaced with medium containing 10 μg cycloheximide per ml. After 30 min of inhibition, medium containing cycloheximide with 4 μM [3H]B(a)P or 100 μM [3H]Leucine was added, and incubation was continued for 3 or 6 hr, at which time nuclear proteins were analyzed as described below. Control cultures were pretreated with benz(a)anthracene and labeled in the absence of cycloheximide. In the experiment shown in Chart 2 and Fig. 3, the measured depression in the rate of incorporation of [3H]Leucine into trichloroacetic acid-insoluble form was 98.7% after 3 hr of incubation with cycloheximide. Determination of total metabolism of [3H]B(a)P and analysis of organic solvent-soluble metabolites by high-pressure liquid chromatography was carried out as described previously (34, 46).

Preparation of Nuclear Proteins. Nuclei were prepared by homogenization in hypotonic buffer containing Triton X-100 and sodium deoxycholate as described (34, 36). After dissolution in 6 M guanidine and extraction of unbound hydrocarbon with ethyl acetate, total nuclear
proteins were purified isopykically (36) and precipitated at 0°C with 2 volumes of methanol.

For preparation of mononucleosomes, detergent-treated nuclei were suspended in 10 mM Tris-HCl, pH 7.0, 1 mM CaCl₂, 0.5 mM PMSF and digested for 10 min at 37°C with micrococcal nuclease (15 units/μg). Digests were fractionated by sedimentation through linear 5 to 25% sucrose gradients prepared in 20 mM NaCl:0.2 mM EDTA, pH 7.0, at 4°C, and the mononucleosome peak was collected (26, 27).

For differential extraction experiments, nuclei were prepared by a modification of the method of Berezeny and Coffey (4). Cells were homogenized in 10 mM Tris-HCl (pH 7.4):0.5 mM PMSF (Dounce homogenizer B pestle; 15 strokes), and the crude nuclei were collected by low-speed centrifugation. The homogenization was repeated, and the final pellet was resuspended in 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.5 mM PMSF containing 2.2 M sucrose. Nuclei were pelleted by centrifugation at 21,000 rpm in a Beckman 50 Ti rotor for 90 min at 4°C (40,000 × g). The pellet of purified nuclei was washed twice with the above buffer containing 0.25 M sucrose and stored at 0°C in the same solution.

Acid Extraction. The high-mobility-group proteins and histones H1 were extracted from purified nuclei with 5% perchloric acid and differentially precipitated with acidified acetone as described by Goodwin and Johns (14). Core proteins were then extracted into 0.4 M HCl at 0–4°C for 18 hr and precipitated with acetone.

CitraterPhosphate Extraction. Purified nuclei were washed twice at 0°C for 15 min with 6.4 mM sodium citrate, 87.2 mM Na₂HPO₄, 250 mM sucrose:25 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, pH 7.15. H1 histones were then extracted at 0°C for 15 min with 89.2 mM sodium citrate, 21.6 mM Na₂HPO₄, pH 2.62 (33). The extracts were dialyzed against distilled H₂O containing 0.5 mM PMSF and lyophilized. Alternatively, the pH 7.15 washes were replaced by extraction with 0.35 M NaCl, 1.0 mM Tris-HCl, pH 7.1.

High-Salt Extraction. Purified nuclei were digested at room temperature for 15 min in 10 mM Tris-HCl, 0.2 mM CaCl₂, pH 7.4, with micrococcal nuclease (133 units/ml), and residual nuclear material was depolymerized between 770 × g for 30 min. Histones were extracted from the pellet by resuspension in 2.0 M NaCl, 10 mM Tris-HCl, pH 7.4, 0.2 mM MgCl₂ at 0°C for 10 min, and the extract was clarified by centrifugation at 770 × g for 60 min. High-salt extraction was repeated twice more, and extracted proteins were precipitated with 2 volumes of methanol.

Polyacrylamide Gel Electrophoresis. Pelleted nuclei, nuclear fractions, or precipitated proteins were dissolved in sample application buffer, sonicated, and analyzed by sodium dodecyl sulfate:polyacrylamide gel electrophoresis (30). Gels were stained with Coomassie blue, photographed, and subjected to fluorography at −70°C (32).

RESULTS

Specificity of Protein Binding. We have shown previously that isopyknic centrifugation through Cs₂SO₄:guanidine-HCl density gradients can be a useful method for preparing nuclear macromolecules from cultures of HEC’s (36). In Fig. 1, nuclear proteins prepared in this way were analyzed by electrophoresis on 15% polyacrylamide gels (30) followed by staining (Lane S) and fluorography (32) (Lanes 1, 2, and 3). Derivation of nuclei was as follows: Lane 1, 24 hr before harvest, fresh medium containing 4 μM [³H]B(a)P was added to confluent cells; Lane 2, 24 hr before harvest, [³H]B(a)P was added to medium in which the cells had grown to confluency; Lane 3, [³H]B(a)P was added during homogenization of previously untreated cells.

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Binding of B(a)P to Specific Nuclear Proteins
a 24-hr incubation in culture (14 μM). After preparation of nuclei and isopyknic separation of macromolecules, the amount of nonspecifically bound B(a)P was less than 5% of the total bound radioactivity in a 24-hr incubation in culture (Table 1). When the nuclear proteins from the control were analyzed electrophoretically, no radioactivity could be detected by fluorography (Fig. 1, Lane 3). An alternative mode of labeling of macromolecules by PAH's which would be nonextractable by organic solvents could have been by tritium exchange (44). However, this would be expected to produce more nearly uniform labeling, especially in proteins as similar as the core histones. Furthermore, when DNA was extracted from [3H]-B(a)P-labeled HEC's, enzymatically hydrolyzed and analyzed by LH-20 chromatography, no radioactivity was found with the unmodified nucleosides, suggesting that tritium exchange during metabolism of B(a)P by HEC's is minimal.

In the experiments described above, nuclear proteins were purified before electrophoretic analysis to reduce any possible contribution to the fluorograms of [3H]B(a)P-derived radioactivity bound to nonproteinaceous nuclear components. However, control experiments demonstrated that whole nuclei, dissolved in sodium dodecyl sulfate-containing sample buffer and electrophoresed directly gave fluorograms which were identical to those produced with purified proteins in the region of the gel corresponding to proteins the size of histone H4 and larger (data not shown). When the nuclei were treated with proteinase K before dissolution and analysis, all protein staining and radioactivity was abolished from the relevant region of the gel; treatment with RNase, DNase, or phosphodiesterase had no discernible effect on the labeling pattern seen in the subsequent fluorographs (data not shown). We conclude that the fluorographic patterns that we have obtained are the result of covalent binding of derivatives of [3H]B(a)P to nuclear proteins.

Nucleosomal Core Histones are B(a)P Targets. The data in Fig. 1, Lane 1, suggested that histones H3 and H2A were targets for modification by B(a)P. However, since binding of different proteins exhibited widely different specific activities, it was possible that quantitatively minor nuclear proteins that were electrophoretically similar to H3 and H2A and exhibited extremely high PAH binding gave rise to the strong bands seen in the fluorograms. Accordingly, we have approached this question by 2 independent methods. In the first method, nuclei from [3H]B(a)P-treated cells were subjected to a brief digestion with micrococcal nuclease, and mononucleosomes were isolated by sucrose gradient sedimentation (Chart 1A). As expected, virtually all of the nonhistone proteins and the inter-nucleosomal H1 histones found in total nuclei were absent from the mononucleosome preparation (Chart 1B, outer lanes) which contained primarily the 4 core histones with a trace of a high-mobility-group protein (which migrates electrophoretically between the H1 region and H3). The fluorogram of the same lane showed that the radioactive bands which coelectrophoresed with histones H3 and H2A also copurified with these proteins in mononucleosomes. Longer exposure times also demonstrated significant amounts of radioactivity associated with the high-mobility-group protein (Chart 1B, arrowhead) found in the mononucleosome preparation. An alternative approach we have taken has been differential extraction of intact nuclei to remove possible nonhistone proteins which might ultimately

![Chart 1. Gel analysis of nucleosomal proteins. In A, micrococcal nuclease-digested nuclei were sedimented through 5 to 25% sucrose gradients as described in "Materials and Methods," and the gradients were fractionated with an Isco Model 640 density gradient fractionator equipped with a UV-5 absorbance monitor; the UV absorbance tracing is shown. In B, aliquots of total nuclei or the pooled fractions corresponding to mononucleosomes (Pool in A) were analyzed as in Fig. 1. The Coomassie blue-staining pattern (S) and the fluorogram (F) are shown for both lanes.](https://cancerres.aacrjournals.org/content/canres/41/8/4082/F1.large.jpg)

**Table 1**

<table>
<thead>
<tr>
<th>Labeling conditions</th>
<th>RNA</th>
<th>DNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hr incubation with [3H]-B(a)P</td>
<td>116.5</td>
<td>103.2</td>
<td>257.8</td>
</tr>
</tbody>
</table>
| Addition of [3H]B(a)P to cell homogenate | 1.97 (1.7)

**Numbers in parentheses, specific activities of the controls as a percentage of the experimental.**

5 B. K. Mansfield and M. C. MacLeod, unpublished observations.
of histone H1. However, the possibility that the observed binding was to a minor variant of H1 with different solubility properties or that the covalent association of B(a)P with H1 in some manner prevented efficient extraction by dilute acid could not be ruled out.

**Binding is Independent of Cell Growth and Protein Synthesis.** The experiments presented above were performed by replacing the medium on "confluent" cultures of HEC's with fresh medium containing [3H]B(a)P. It is well known that such treatment induces macromolecular synthesis and cell proliferation in quiescent fibroblast cultures. Control experiments demonstrated a dramatic increase in DNA synthesis in HEC cultures given fresh medium. However, when [3H]B(a)P was added directly to the cultures without replacing the medium, the same levels of binding to nuclear macromolecules were found as with the standard procedure (data not shown), and the same distribution of radioactivity among nuclear proteins was seen (Fig. 1, Lane 2). Thus, the labeling patterns observed were not dependent on the overall growth state of the cultures.

However, in preliminary experiments, we found that in both quiescent and stimulated cultures most or all nuclear proteins were continuously synthesized as monitored by incorporation.

### Table 2

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Radioactivity recovered in H1 region of gels</th>
<th>Radioactivity recovered in H3-H2A region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm</td>
<td>% of total</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7.15 extract</td>
<td>3,144</td>
<td>7.1</td>
</tr>
<tr>
<td>pH 7.62 extract</td>
<td>1,709</td>
<td>3.8</td>
</tr>
<tr>
<td>Residual protein</td>
<td>39,728</td>
<td>89.1</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.35 M NaCl extract</td>
<td>4,306</td>
<td>10.1</td>
</tr>
<tr>
<td>pH 2.62 extract</td>
<td>1,736</td>
<td>4.1</td>
</tr>
<tr>
<td>Residual protein</td>
<td>36,578</td>
<td>85.8</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% perchloric acid extract</td>
<td>258</td>
<td></td>
</tr>
</tbody>
</table>

* ND, not done.

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**Major Species of Histones H1 Are Probably Not B(a)P Targets.** We next investigated the association of B(a)P-derived radioactivity with proteins which migrate in the H1 histone region of the gel. As shown in Fig. 1 and Chart 2, the bulk of radioactivity migrates at and just below the position of the major H1 histone with the highest electrophoretic mobility. Since H1 can be selectively extracted from nuclei or chromatin by dilute acid (14, 33), we asked whether the H1 region radioactivity was similarly extracted. The 3 major H1 bands were selectively extracted with citrate:phosphate buffer, pH 2.62 (Fig. 2A, Lanes 2 and 5), or with 5% perchloric acid (not shown). However, very little radioactivity was associated with the extracted proteins (Fig. 2B, Lanes 2 and 5). When the H1 and core histone regions of a similar gel (Fig. 2, brace) were sliced and dissolved and their associated radioactivity was determined by liquid scintillation counting (Table 2), more than 85% of the total radioactivity in the H1 region was recovered in the residual nuclei, not in the pH 2.62 extract. It thus appeared that the radioactivity found in the H1 region of the gels was associated with proteins other than the major species of histone H1. However, the possibility that the observed bind-
of tritiated amino acids. Since the major site of metabolism of PAH's (10) has been found to be the microsomal fraction, it seemed possible that a major portion of the labeling of nuclear proteins by [3H]B(a)P could have occurred during or soon after biosynthesis and before the newly synthesized protein had reached the nucleus. To test this, we studied labeling patterns in cells treated with cycloheximide to inhibit protein synthesis. Aryl hydrocarbon hydroxylase activity was preinduced by treating the cells with benz(a)anthracene prior to cycloheximide treatment. After a 3- or 6-hr incubation with [3H]B(a)P or [3H]-leucine, cells were harvested and nuclear proteins were prepared. To control for possible changes in aryl hydrocarbon hydroxylase activity, aliquots of the medium were analyzed for B(a)P metabolism. As shown in Chart 2, the high-pressure liquid chromatographic distribution of metabolites after 6 hr of incubation was independent of the presence or absence of cycloheximide. In fact, at both 3 and 6 hr, the total extent of metabolism was slightly higher in the presence than in the absence of cycloheximide. This was in spite of the fact that at both time points incorporation of [3H]leucine into total cellular protein was inhibited 96.7% by cycloheximide. Thus, interpretation of the labeling results was not complicated by a loss of aryl hydrocarbon hydroxylase activity or a shift in the specificity of the enzyme.

Proteins from nuclei labeled 3 or 6 hr with [3H]B(a)P or

\[ ^{[3H]} \text{B(a)P} \]

were separated by electrophoresis. Comparison of Lanes 1 and 2 or of Lanes 3 and 4 in Fig. 3B revealed that many, possibly all, nuclear proteins were being synthesized and that labeling of all nuclear proteins with [3H]leucine was blocked by cycloheximide. Covalent binding of [3H]B(a)P (Lanes 5 to 8) was apparently not altered by the presence of cycloheximide. The labeling pattern, which differed somewhat from that obtained in 24-hr incubations (Fig. 1), was the same in all lanes and the relative intensities were similar. When a replicate set of gel lanes was cut into 1-mm slices and the radioactivity in each slice was determined, it was found that, after normalization to protein mass, there was at most a 10% inhibition of covalent binding to nuclear proteins caused by cycloheximide (data not shown). There data provided strong evidence that this binding occurred in situ, not in the cytoplasm before transport to the nucleus.

**DISCUSSION**

Abundant proteins such as the histones, high-mobility-group proteins, and nuclear lamins play an important role in determining the structure of nuclei in eukaryotic cells, and changes in nuclear protein content, polymerization state, and degree of posttranslational modification have been linked with cell division (13, 37, 38, 43) and differences in transcriptional activity
Structural proteins have also been implicated in the altered conformational state of chromatin found in active genes (52) and in genes destined to be active at later stages of development (50). Thus, modification of these proteins may lead to alterations in the processes which control cell growth, determination, and differentiation. Since disruption of these processes may well be involved in the carcinogenic effect of B(a)P, we believed that it was important to study the interactions of B(a)P derivatives with nuclear proteins.

Binding of PAH’s to cellular nucleophiles depends on prior metabolism, generating reactive electrophilic derivatives (40). In HEC’s, binding of B(a)P to DNA (3, 17) and to nuclear proteins (35) is mediated by diol-epoxide derivatives which are produced by the enzymes aryl hydrocarbon hydroxylase and epoxide hydrase. Although in rat liver these enzymes are primarily microsomal, small amounts of activity have been found to be associated with nuclear envelopes (5, 23, 41). Thus, our finding that labeling of nuclear proteins is independent of protein synthesis and thus occurs in situ (Fig. 3) may be due to synthesis of the highly reactive diol-epoxides by enzymes located in the nuclear membrane. Alternatively, the derivatives may be made in the microsomes and then be transported into the nucleus. Since nuclear adducts represent a small fraction of the total products derived from the diol-epoxides (approximately 5 to 10%), the latter possibility is not inconsistent with the high reactivity of the diol-epoxides.

The basic subunit structure of chromatin is determined by the interaction of 2 molecules each of histones H3, H4, H2A, and H2B with 145 base pairs of DNA to form a nucleosome (11), and this structural element occurs in both active and inactive chromatin (11). As can be seen in Figs. 1, 2, and 3 and Chart 1, we find that histones H3 and H2A are strongly labeled by B(a)P while H2B and H4 are essentially unlabeled. This labeling is due primarily to 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (35). Such a result would not be predicted on the basis of the amino acid compositions of these histones, and in fact incubation of denatured histones with (±)-trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene results in binding to all 4 histones (53, 54). This implies that potential nucleophilic sites in H2B and H4 are blocked by some element of chromatin structure and that this structural constraint is maintained during relatively long periods of exposure to [3H]B(a)P. Interestingly, a different specificity was observed in total chromatin from chick erythrocytes, where histones H3 and H2B were labeled by (±)-trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (26). However, when the DNA was dissociated from the histone core of chick erythrocyte mononucleosomes by treatment with 2.0 M NaCl before treatment with the diol-epoxide, the labeling pattern shifted to the H3 and H2A pattern (26), and this labeling pattern was also found in a subclass of nucleosomes which lack the very lysine-rich histones but contain certain of the high-mobility-group proteins (25). The significance of this binding as a perturbant of chromatin structure and function is not clear. It should be pointed out that histone binding is a relatively rare event, occurring in these cells an average of about once/100 nucleosomes.7 However, given the high reactivity of the diol-epoxides, it is possible that regions of chromatin which are closer to the periphery of the nucleus and hence to the metabolic source of the diol-epoxides may be exposed to significantly higher levels of modification.

The very lysine-rich histones H1 are thought to be important determinants of higher-order structure in chromatin (11), and it has been suggested that accumulation of a particular species of H1, H10, is important in density-dependent inhibition of cell growth (43). The fluorograms demonstrate an intense band of radioactivity in the H1 region of the polyacrylamide gels. The failure to extract significant amounts of this radioactivity from nuclei with dilute acid (Fig. 2; Table 2) argues against binding of B(a)P to the known species of H1, including H10, or to any of the high-mobility-group proteins. Furthermore, the interphase-specific protein A24 (37, 38), which is derived from histone H2A, migrates considerably faster than the intensely labeled band in the H1 region. This band, therefore, represents a minor nuclear protein which is more highly labeled by B(a)P than the more abundant proteins. In recent experiments, we have noted a variable release of this highly labeled protein during washing of nuclei, which occurs without significant release of histone proteins.6 A number of proteins have been described which have acid solubility and electrophoretic properties similar to this protein (19, 22, 31), but further experimentation will be necessary to define its identity.

The effects of the binding of derivatives of B(a)P to nuclear proteins are undoubtedly rather complex and could conceivably either enhance or inhibit B(a)P-induced malignant transformation. The multiplicity of nucleophilic sites on proteins may act as a “sink” for reactive electrophiles and thereby protect more important target molecules. Since over 70% of the nuclear adducts formed in HEC’s are with protein (34), this is certainly true in a quantitative sense. On the other hand, binding to particular nuclear proteins may contribute directly to either the initiation of transformation or subsequent processes leading to the expression of malignancy. Bulky hydrocarbon-protein adducts might be expected to significantly perturb the structure of chromatin, possibly altering the ability of local regions to recognize and respond to normal regulatory signals. Although the average level of modification is low, as pointed out previously, the spatial distribution of modifications in intact nuclei may be inhomogeneous, leading to high levels of modification in relatively localized regions of chromatin. Further study of the spatial and temporal distributions of B(a)P-nuclear protein adduct formation in HEC cultures is needed to define the role of these interactions in the overall balance of activation and detoxification processes in cultured cells.

REFERENCES


M. C. MacLeod et al.
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