# CHARACTERISTICS OF DNA DURING BINDING OF POLYCYCLIC AROMATIC HYDROCARBONS *in vitro*

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With the aid of various activation systems, polycyclic aromatic hydrocarbons (PAH) were bound covalently with DNA. The following activation systems were used: with the microsomal fraction of rat liver, with $I_2$, and with ascorbic acid and $FeSO_4$. In all three systems the appearance of breaks due to the action of the activating systems was observed in the DNA. The plateau on the curve representing PAH binding in the system with microsomal fraction is explained not by a decrease in the rate of metabolism of the hydrocarbons to zero and not by the presence of a limited number of specific combining sites for PAH in DNA, but by equality between the velocities of two opposite processes; binding of the metabolites and their removal through degradation of DNA. Against the background of breaks in DNA produced by the activation systems it was impossible to find changes introduced into the sedimentation characteristics of DNA by covalently bound PAH.

KEY WORDS: polycyclic aromatic hydrocarbons; metabolism; DNA; sedimentation.

To exert their carcinogenic action polycyclic aromatic hydrocarbons (PAH) must first undergo enzymic activation. Metabolites formed by oxidation of PAH bind with different cell macromolecules, including with DNA. To study interaction between PAH and DNA *in vitro* model systems are used — either systems containing a set of enzymes (microsomes) [1, 5, 6], or systems simulating to some degree or other enzymic oxidation [2, 3]. In a system containing microsomal fraction the maximum of binding of PAH metabolites with nucleic acids lies in the region of 15 min, but it has not proved possible to explain this kinetic behavior [6]. The effect of activation systems on DNA has not been considered.

In this investigation the action of various PAH activating systems on DNA was studied: an enzymic system (microsomes), a system with ascorbic acid + EDTA + $Fe^{++}$, and a system with $I_2$. The kinetics of binding of PAH metabolites with DNA also was studied.

## EXPERIMENTAL METHOD

DNA was incubated with labeled or unlabeled PAH in model metabolic systems. The following activation systems were investigated: with a fraction of rat liver microsomes, induced by 3-methylcholanthrene [1], with $I_2$ ($5 \times 10^{-3}$ M) [2], and with $FeSO_4$ ($1.4 \times 10^{-3}$ M) + ascorbic acid ($1.5 \times 10^{-2}$ M) + EDTA ($6.8 \times 10^{-3}$ M) [3].

DNA was isolated from the first system by Marmur's method [4] and from the rest by precipitation and washing three times with ethanol. The sedimentation characteristics of the DNA were studied in a sucrose density gradient (5-20%), containing $10^{-2}$ M Tris-HCl (pH 7.6), 0.2 M NaCl and $10^{-3}$ M EDTA (neutral) or 1 M NaCl and $10^{-3}$ M EDTA, 0.25 M NaOH. The samples were centrifuged for 135 min at 47,000 rpm in the SW-50-2 rotor. SV-40 virus DNA-$^3$H with a specific activity of 2000 cpm/μg was generously provided by M. A. Shlyankevich (Oncologic Scientific Center). Radioactivity was measured in a scintillation counter.

## EXPERIMENTAL RESULTS

Since the quantity of PAH metabolites bound with DNA ceased to increase after incubation for 10-20 min in a system containing microsomal fraction, it can be concluded that the enzyme

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TABLE 1. Binding of PAH with DNA (in cpm/mg DNA)

| First incubation | Second incubation | Incorporation of $^3H$ | Incorporation of $^{14}C$ |
|---|---|---|---|
| | | $M \pm m$ | |
| BP-$^3H$ | — | 8 200±500 | — |
| The same | BP-$^3H$ | 10 500±600 | — |
| " " | BP-$^{14}C$ | 3 900±260 | 8 900±400 |
| DMBA-$^3H$ | — | 11 900±2000 | — |
| The same | DMBA-$^3H$ | 8 400±800 | — |
| " " | BP-$^{14}C$ | 4 100±100 | 10 700±300 |
| Pyrene | BP-$^3H$ | 7 400±400 | — |
| Benzanthracene | The same | 8 800±800 | — |
| BP | " " | 7 800±400 | — |

Legend. DMBA denotes 7,12-dimethylbenzanthracene.

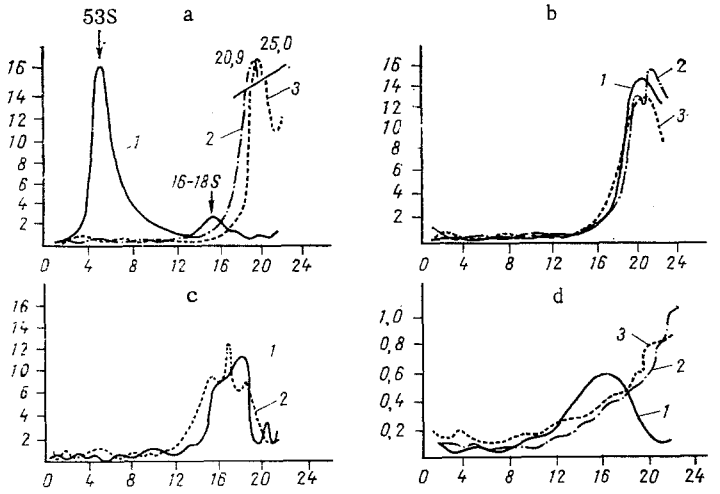


Fig. 1. Sedimentation of DNA in sucrose density gradient. a-c) Sedimentation of SV-40 DNA-$^3H$ (10,000-15,000 cpm per gradient) in alkaline gradient (abscissa — No. of fraction; ordinate — radioactivity, in %). a) Metabolic system with rat liver microsomal fraction; 1) Original DNA; 2) DNA after incubation in metabolic system without PAH; 3) DNA after incubation in metabolic system with BP. b) Metabolic system with $I_2$; 1) incubation without PAH; 2) with pyrene; 3) with BP. c) Incubation in system with ascorbic acid, $FeSO_4$, and EDTA: 1) incubation without PAH; 2) with BP. d) Metabolic system with microsomal fraction. Sedimentation of DNA (not more than 100 μg per gradient) in a neutral gradient; 1) Original DNA; 2) incubation without PAH; 3) incubation with BP. Abscissa, No. of fraction; ordinate, optical density ($A_{260}$ at 260 nm)

system was inactivated. To verify this, DNA was isolated from the incubation mixture, washed to remove unbound PAH, and reincubated in the system with microsomal fraction. As Table 1 shows, if the first incubation was carried out with tritiated hydrocarbon, after the second incubation with the same or another (also tritiated) PAH, no increase in incorporation of the label into DNA was observed.

Consequently, either there are specific combining sites in DNA which are occupied already after the first incubation, or simultaneously with binding, PAH metabolites are removed from DNA; moreover, the velocity of the forward and reverse reactions become equalized after about 15 min. However, if unlabeled hydrocarbon was used for the first incubation, after the second incubation with tritiated 3,4-benz(a)pyrene (BP-$^3H$) the same incorporation of label into DNA was observed as after incubation with labeled BP alone. This result cannot be explained by the existence of specific combining sites in DNA for PAH residues.

To test the possibility of removal of PAH metabolites from DNA, the first incubation was carried out with BP-$^{3}$H or with 7,12-dimethylbenzanthracene-$^{3}$H, and the second with BP-$^{14}$C. It will be clear from Table 1 that in both cases incorporation of $^{3}$H was reduced and labeling with $^{14}$C appeared. Simultaneously with the formation of a covalent bond between PAH metabolites and DNA, removal of the bound compounds thus also took place. To study the causes of removal of the label, the possibility of degradation of DNA was tested in model systems by the appearance of single- and double-strand breaks in it. The appearance of single-strand breaks was tested by changes in the shape of the sedimentation characteristic curve of SV-40 virus DNA in an alkaline gradient. Molecules of this DNA, with no single-strand breaks, sediment in the region with sedimentation constant 53S, whereas those having one single-strand break do so in the region of 16-18S. The data given in Fig. 1a-c show that after incubation of DNA in all the systems studied the 53S fraction disappeared completely (practically no molecules without single-strand breaks remained) and most of the DNA material sedimented to the right of the peak with single-strand breaks. The sedimentation constant of the DNA also was sharply reduced in a neutral gradient (Fig. 1d). Degradation of DNA in a system with microsomal fraction was inhibited by the addition of polyvinyl sulfate and can evidently be explained by the nuclease activity of the microsomes. However, polyvinyl sulfate at the same time inhibited PAH metabolism also.

Changes in the sedimentation properties of DNA caused by the activation systems were so great that they made it impossible to detect deviations from the control due to interaction between PAH metabolites and DNA. When studying changes in the template, physicochemical, and other properties of DNA bound with carcinogens, it is thus necessary not only to differentiate them from disturbances introduced by the activation systems, but also to bear in mind the possibility of their canceling out against the general background of injuries.

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