

DNA and proteins of the nuclear matrix are the main targets of benzo[a]pyrene's action in rat hepatocytes

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The binding of [¹⁴C]benzo[a]pyrene (B[a]P) to DNA and proteins in total nuclei and subnuclear fractions of cultured rat hepatocytes was compared. The main targets of B[a]P were non-histone high molecular weight proteins of the nuclear matrix and DNA sequences attached to this structure. Following 24 h exposure to B[a]P the amounts of adducts in the nuclear matrix DNA and proteins were twice as high as in total nuclei. After withdrawal of the carcinogen containing medium the level of B[a]P-induced adducts gradually decreased but always remained the highest in the nuclear matrix proteins. Removal of adducts from the nuclear matrix DNA was more efficient than from the other DNA fractions, and 72 h after exposure to the carcinogen the level of DNA adducts in this fraction was similar to that in total nuclei.

Carcinogenic polycyclic aromatic hydrocarbons (PAHs)¹ have the ability to bind covalently to cellular macromolecules. Binding of PAHs to DNA is thought to be essential for their carcinogenic activity [1]. It was shown that carcinogens did not form adducts randomly with whole nuclear DNA. PAHs such as benzo[a]pyrene (B[a]P) or dibenzo[a,e]fluoranthene bind preferentially to the transcriptionally active chromatin and the nuclear matrix attached DNA [2 - 4]. Although DNA is considered the main target in chemical carcinogenesis, covalent binding of carcinogens to cellular proteins could be of importance for many aspects of this process. The measurement of the level of carcinogen-protein adducts is useful for assessing the degree of exposure to the carcinogen and in risk assessment [5]. Moreover, binding of carcinogen to cellular proteins may affect the target protein functions [6, 7]. Particular species of nuclear proteins were reported to be preferentially susceptible to PAH binding. In hamster

embryo cells, B[a]P derivatives bound most efficiently to histones H3 and H2A [8]. In rat hepatocytes, apart from histones, non-histone proteins of nuclear matrix were the targets of B[a]P derivatives [9].

Only activated metabolites of B[a]P, like benzo[a]pyrene diol epoxide, are able to bind covalently to macromolecules [1, 10]. It seems that liver is the main source of reactive metabolites of carcinogenic PAHs for target tissues [11]. Thus primary hepatocyte cultures are a very useful model for studying mechanisms of the carcinogen action. In the present work we were interested whether chromatin fractions of cultured rat hepatocytes differed in the ability to bind B[a]P derivatives and to undergo repair.

METHODS

Hepatocytes were isolated from male Wistar rats (2.5 month old) by collagenase perfusion

¹Abbreviations: B[a]P, benzo[a]pyrene; DMSO, dimethyl sulfoxide; PAH, polycyclic aromatic hydrocarbons.

[12]. The hepatocytes were cultured on collagen-coated 10 cm plates in Williams E medium supplemented with insulin and fetal calf serum. At 24 h after seeding (1×10^7 cells per plate) the medium was replaced and then supplemented with [7,10- 14 C]benzo[a]pyrene (58 mCi/mmol, Amersham Int. Ltd.) (final concentration 1 μ mol) dissolved in dimethyl sulfoxide (DMSO) (final concentration 0.1%) or with DMSO only for 24 h. The carcinogen-containing medium was then replaced with the carcinogen-free one (changed every 24 h). The hepatocytes were collected just after the carcinogen treatment and 24 h or 72 h after withdrawal of the carcinogen containing medium. [Me- 3 H]Thymidine (49 Ci/mmol, Amersham Int. Ltd.; 20 μ Ci per plate) was added to the medium one hour prior to collection of the cells. The hepatocytes were washed twice with medium and kept in liquid nitrogen. Five plates were used per one experiment.

The hepatocytes were homogenized in 1% Nonidet NP40 and the nuclei were washed in 1% Triton X-100. These nuclei were then treated with DNase I (10 μ g per plate; 15 min; 35°C) in 0.1 M NaCl. After addition of NaCl to the concentration of 0.5 M the nuclei were incubated in ice and centrifuged. The supernatant (fraction S1) contained about 60% of total nuclear DNA and about 45% of total nuclear proteins. The remaining nuclei were treated with 2 M NaCl and the extracted fraction S2 contained about 30% of total nuclear DNA and about 15% of

total nuclear proteins. The residual nuclear matrix fraction contained about 10% of total nuclear DNA and about 40% of total nuclear proteins.

The analysis of covalent binding of B[a]P was performed as follows. Nuclear and subnuclear fractions were extracted with ethyl acetate, precipitated with 5% trichloroacetic acid and washed with acetone and ethanol. Dried pellets were solubilized in 1 M NaOH and proteins were quantitated by the Coomassie staining method [13]. Equal parts of the samples were taken for DNA determination. DNA was purified by proteinase K/SDS/phenol treatment followed by RNase digestion [14], and quantitated spectrophotometrically. The amount of [14 C]B[a]P bound to proteins was calculated by subtracting the 14 C radioactivity of purified DNA from the radioactivity of the entire sample. The 3 H and 14 C radioactivity were counted in a scintillation counter. Electrophoretic analysis of proteins was performed on 10% and 15% polyacrylamide/SDS gels [14].

RESULTS

We compared the binding of the carcinogen to total nuclear DNA and DNAs of particular chromatin fractions. Figure 1A shows the amount of DNA-adducts as the number of carcinogen molecules per 1 μ g of DNA. We found that just after the carcinogen treatment the

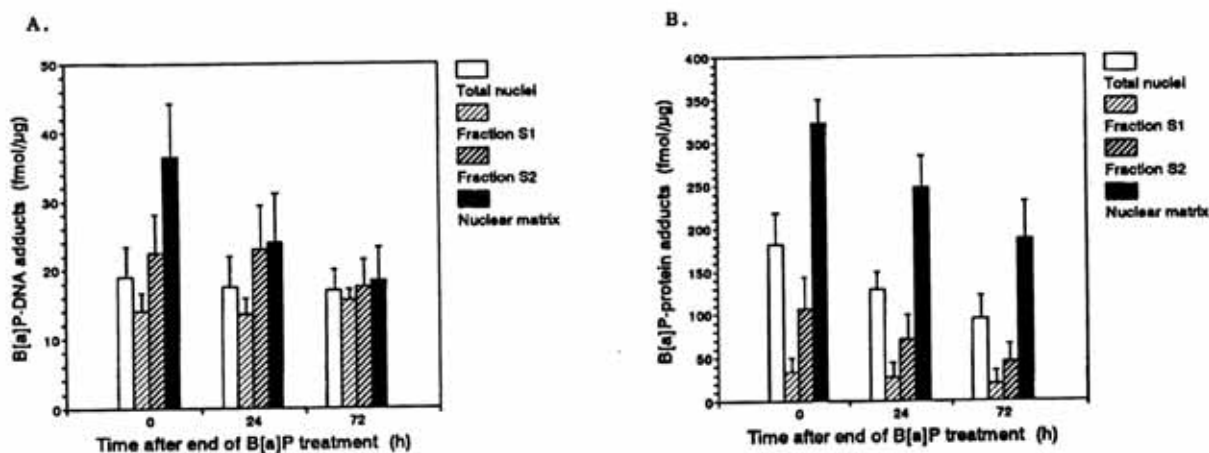


Fig. 1. The amounts of DNA-adducts (A) and protein-adducts (B) in total nuclei and in chromatin fractions of cultured hepatocytes exposed to B[a]P. The results are the mean \pm S.D. of five replicate culture plates.

amount of DNA-adducts was markedly higher in the nuclear matrix than in total nuclei and in non-matrix fractions. Later, 24 h and 72 h after the withdrawal the B[a]P containing medium the amount of adducts was similar in all DNA fractions. The amount of adducts in total DNA was about 20 fmol/ μg DNA right after the exposure to B[a]P and decreased only slightly over the next 72 h.

The electrophoretic analysis of proteins of subnuclear fractions is shown in Fig. 2. Fraction S1 (lane B) contained mainly the core histones

and histone H1, both present in similar amounts (suggesting that fraction S1 contained a compact form of chromatin). Fraction S2 (lane C) contained a few high molecular weight non-histone proteins. The nuclear matrix fraction (lane D) contained mainly lamins and other high molecular weight proteins. The amounts of the carcinogen molecules bound to the proteins of whole nuclei and of subnuclear fractions are shown in Fig. 1B. Just after exposure of the hepatocytes to B[a]P the amount of protein-adducts in total nuclei was about 180

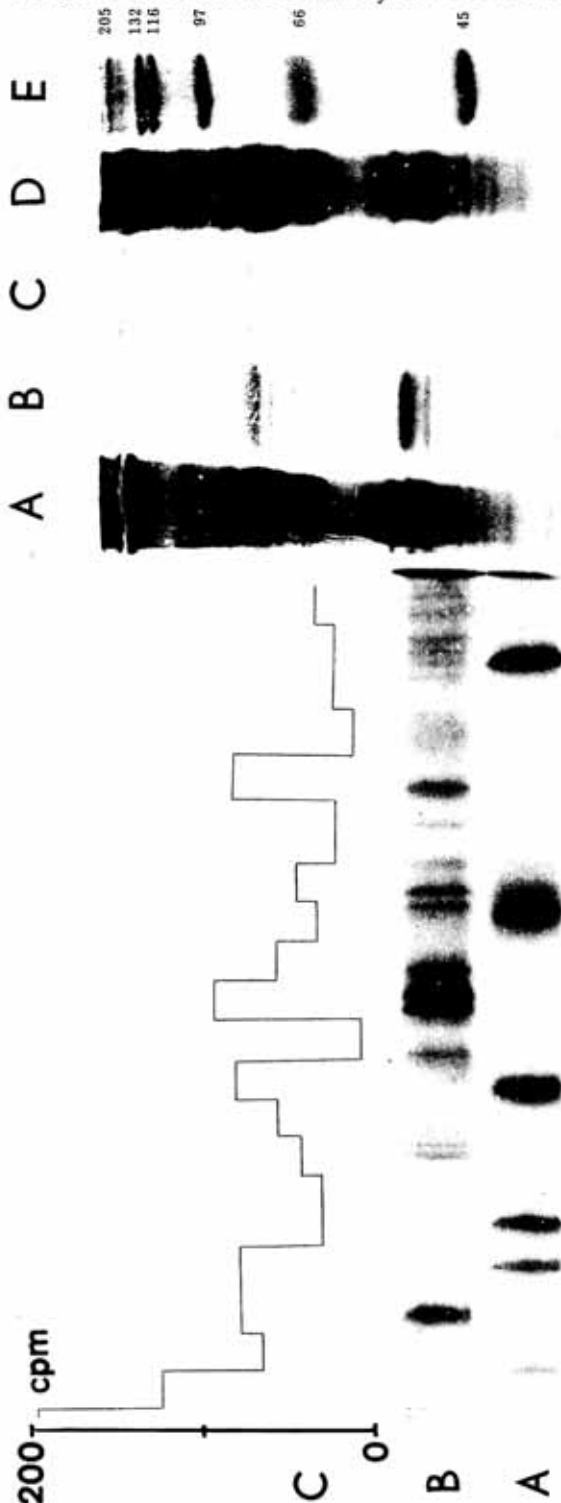


Fig. 2. The electrophoretic analysis of proteins in rat hepatocyte nuclear fractions. Lane A: total nuclear proteins; lane B: fraction S1; lane C: fraction S2; lane D: nuclear matrix fraction; lane E: molecular mass markers - 205, 132, 116, 97, 66 and 45 kDa. Proteins were run on 15% polyacrylamide gel.

fmol/ μg protein and gradually decreased to about 100 fmol/ μg protein at the 72th h after withdrawal of the carcinogen containing medium. The amount of protein-adducts was in the nuclear matrix about twice as high as in the total nuclei and about four times lower in proteins of fraction S1 than in total nuclei, at any time of measurement. The nuclear matrix proteins of the cells collected just after the carcinogen treatment were run on 10% polyacrylamide gel and the ^{14}C radioactivity was counted in gel slices. We found that the radioactive carcinogen molecules bound mainly to proteins of about 50, 70 - 80, 90 - 105 and more than 130 kDa (Fig. 3).

Fig. 3. The electrophoretic analysis of the nuclear matrix [^{14}C]B[a]P-modified proteins of B[a]P treated hepatocytes. Proteins of the hepatocytes collected just after the exposure were run on 10% polyacrylamide gel and ^{14}C radioactivity of dried gel slices was counted. Lane A: molecular mass markers (the same as on Fig. 2); lane B: proteins of the nuclear matrix; C: ^{14}C radioactivity of the lane B proteins [c.p.m. per slice].

The number of cells and the rate of DNA synthesis in hepatocytes were similar in both DMSO- and B[a]P-treated cultures. In the course of 5 day-long culture the number of the hepatocytes decreased (from 1×10^7 to about 0.6×10^7 per plate) and the rate of DNA synthesis (measured as the rate of [3 H]thymidine incorporation) gradually increased (not shown).

DISCUSSION

It was previously reported that the UV-induced photoadducts were preferentially removed from the nuclear matrix DNA of human fibroblasts [15]. It is also well known that PAHs, like B[a]P, bind more efficiently to DNA sequences attached to the matrix than to the non-attached ones [2 - 4]. We tried to check whether B[a]P-induced DNA-damages were preferentially repaired in this nuclear fraction (as it was observed in the case of UV-induced damages). We found that during the 72 h period following the carcinogen withdrawal there was no significant decline of the amount of DNA-adducts in total DNA and in DNAs of non-matrix fractions. Nevertheless, we observed a significant decrease in the amount of DNA-adducts in the nuclear matrix DNA (where the initial adduct level was higher than in the other fractions). This suggests that DNA sequences attached to the nuclear matrix were preferentially repaired.

We were interested whether proteins of the nuclear matrix were also preferentially damaged by the carcinogen. We found that B[a]P metabolites were bound mainly to the nuclear matrix proteins of high molecular weight. However, we did not observe any differences in the rate of removal of B[a]P-protein adducts among the subnuclear fractions. It seems that removal of protein-adducts from the cell is due to molecular turnover and that there are no specific mechanisms of repair of chemically damaged proteins (unlike in the case of repair of DNA-adducts) [16]. Our results show that in all subnuclear fractions the gradual removal of the protein-adducts proceeded at the same rate. Moreover, the B[a]P-modified proteins persisted at a high level in the nuclear matrix. Many important nuclear functions, e.g. DNA replication and transcription, take place in this nuclear

structure (reviewed in [17]). We cannot exclude that chemical damage to proteins of the matrix may affect these nuclear functions and can be of importance in the carcinogenic processes.

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