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Restricted repair of aflatoxin B_1 induced damage in α DNA of monkey cells

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ABSTRACT

We have investigated the processing of adducts formed by covalent binding of aflatoxin B_1 (AFB_1) to DNA in confluent cultures of African green monkey cells. Repair synthesis elicited by AFB_1 adducts was deficient in α DNA sequences compared to that in bulk DNA, although the initial levels of modification were the same for these DNAs. The removal of the primary initial adduct, AFB_1-N^7-Guanine, was deficient in α DNA and the kinetics of its loss resembled those previously reported for removal from total DNA in xeroderma pigmentosum cells of complementation group A. Spontaneous loss of the AFB_1 moiety or the concomitant loss of the guanine to yield an apurinic site account for these results. The formation of the more chemically stable secondary product, AFB_1-triamino-Pyrimidine, occurred more rapidly and to a greater extent in α DNA than in bulk DNA, probably because of slower removal of the primary product. The excision repair patch size for AFB_1 adducts in α DNA was only 10 nucleotides compared to 20 nucleotides for repair of AFB_1 adducts in bulk DNA. Irradiation of cells with low doses of UV prior to or immediately after treatment with AFB_1 increased the rate and extent of removal of AFB_1 adducts from α DNA to the levels found in the bulk DNA, indicating that the formation of pyrimidine dimers or their repair may alter the chromatin structure of α DNA sufficiently to facilitate its repair.

INTRODUCTION

Aflatoxin B_1 (AFB_1) is a mycotoxin produced by certain strains of *Aspergillus flavus*. It is hepatoxic and hepatocarcinogenic in several animal species and epidemiological studies suggest that it may also be an important factor in the etiology of human liver cancer (1). Like many carcinogens, AFB_1 requires metabolic activation to exert its biological effects. Activated AFB_1 induces the *rec-lex* "SOS repair" system in *E. coli* (2), and unscheduled DNA synthesis (3) and repair replication (4) in normal human cells but not in cells derived from patients with xeroderma pigmentosum (XP). AFB_1 binds covalently to cellular macromolecules both *in vivo* (5,6,7) and after activation *in vitro* by liver microsomes (8,9,10). The primary AFB_1-DNA adduct is 2,3-dihdro-2-(N^7-deoxyguanosyl)-3-hydroxyaflatoxin B_1, which upon mild acid treatment is released as 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1

(AFB_1-N^7-Gua) (6,7,10). This primary adduct is chemically unstable and it decomposes by three major pathways: (1) The release of 2,3-dihydro-2,3-dihydroxyaflatoxin B_1 (AFB_1-dhd) presumably leaving the guanine residues unaltered; (2) The release of AFB_1-N^7-Gua by hydrolytic cleavage of the N-glycosylic bond, forming aguaninic sites in the DNA; and (3) The hydrolytic opening of the 5-membered ring of guanine, forming the more chemically stable secondary adduct, 2,3-dihydro-2-(N^5-formyl-2',5',6'-triamino-4'-oxo-N^5-pyrimidyl)-3-hydroxy-aflatoxin B_1 (AFB_1-triamino-Py) (6, 11).

Although AFB_1-DNA adducts in the cell are removed to a large extent by the first two spontaneous chemical reactions, active excision of AFB_1 adducts has been shown to occur in rat liver (12), mouse embryo fibroblasts $10T\frac{1}{2}$ (13), and human lung epithelial cells A549 (14) based on comparisons of the rate of loss of AFB_1 adducts from cellular DNA and from DNA incubated at neutral pH *in vitro*. In addition, active cellular repair of AFB_1 adducts has been shown by comparing the rate of removal of AFB_1 from DNA in normal human skin fibroblasts and in XP cells from complementation group A (XP-A) (15), which are deficient in the excision repair of DNA containing bulky chemical adducts.

Recently, Zolan *et al.*, (16) dicovered a deficiency in the excision repair of α DNA containing bulky chemical adducts in African green monkey cells (α DNA is a highly repeated 172 bp sequence in monkey cells). The repair of furocoumarin photoadducts in α DNA was only about 30% as great as in the bulk of the genome, while damage by 254 nm ultraviolet (UV) light was repaired equally well in α and bulk DNA. It was postulated that some feature of the α sequence and its chromatin conformation inhibited the recognition and repair of furocoumarin damage but not UV damage. Since the primary AFB_1-DNA adducts and their secondary products can be identified chromatographically (13,15), we have examined the formation and removal of AFB_1-DNA adducts in cultured monkey cells. Our results indicate a slower loss of AFB_1 adducts from α DNA than from bulk DNA due to a deficiency in the active removal of the primary adduct, AFB_1-N^7-Gua. However, repair of aguaninic sites, produced by the spontaneous reactions of AFB_1-DNA adducts, appears normal. Treatment of the cells with UV in addition to the AFB_1 resulted in the active removal of AFB_1-DNA adducts from α DNA.

MATERIALS AND METHODS

Chemicals.

AFB_1 was purchased from Calbiochem-Behring; [^{3}H]AFB_1 (20 Ci/mmol) from Moravek Biochemicals. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase

and NADP were obtained from Sigma Chemical Co. The S9 microsomal fraction from rat liver was generously provided by Dr. A. D. Burrell (General Products Division, I.B.M. Corp., San Jose, CA).

Cell Culture Conditions and Treatment.

BS-C-1 African green monkey cells (ATCC CCL 26) were cultured as previously described (16). Confluent cultures of [^{32}P] prelabeled cells were exposed to [^{3}H]AFB_1 in the presence of S9 microsomes as follows. The medium was removed and the flasks were washed with phosphate buffered saline (PBS) (15). [^{3}H]AFB_1 (0.25 µM final concentration) in 2.5 ml activation buffer containing, in PBS, 3 mM $MgCl_2$, 5 mM glucose-6-phosphate, 0.8 mM NADP, glucose-6-phosphate dehydrogenase (0.4 unit/ml), and the S9 fraction at approximately 0.8 mg of microsomal protein per ml, was incubated with the cell culture for 30 min at 37°C. Cells were then washed with 5 ml of conditioned medium. For those cultures receiving posttreatment incubation, 15 ml of conditioned medium was added. For measurements of repair replication induced by AFB_1, the cells were incubated for 1-2 hr in 5 µM FdUrd and 50 µM BrdUrd prior to treatment with unlabeled AFB_1(0.32 mM final concentration) and S9 microsomes for 60 min at 37°C. In addition, the AFB_1 activation buffer and the conditioned medium used for the repair replication studies contained FdUrd and BrdUrd at the above concentrations. Confluent cultures of BS-C-1 cells were irradiated at 254 nm using a Westinghouse IL 782-30 germicidal lamp at an incident dose rate of 0.33 J/m^2/sec which produces 2.4 pyrimidine dimers per 10^8 daltons DNA per J/m^2 in cultured cells.

Measurement of AFB_1 Damage.

DNA from cells treated with [^{3}H]AFB_1 was isolated on 47mm polycarbonate filters (Nucleopore) as previously described (17). The solutions used for DNA isolation, restriction endonuclease digestion and electrophoresis were adjusted to pH 6.6-6.9 to minimize spontaneous decomposition of the AFB_1-DNA adducts. DNA isolated on the filters was released by incubation with 8 U Hind III endonuclease (Bethesda Research Laboratories) per µg DNA for 6 hr at 37°C in 20 mM phosphate buffer, 7 mM MgCl, 60 mM NaCl pH 6.9. The DNA digests were fractionated by electrophoresis on 2% agarose gels in 25 mM histidine, 30 mM MOPS pH 6.6 at 4°C (18). The DNA bands were visualized by staining with ethidium bromide. The 172 bp α monomer band and the bulk DNA (the brightly fluorescent region near the top of the gel) were excised, removed from the gel fragments by electroelution, and assayed for radioactivity as previously described (16). Total adduct frequencies were determined from the [^{32}P] specific activity of the DNA from each sample and the [^{3}H] specific activity

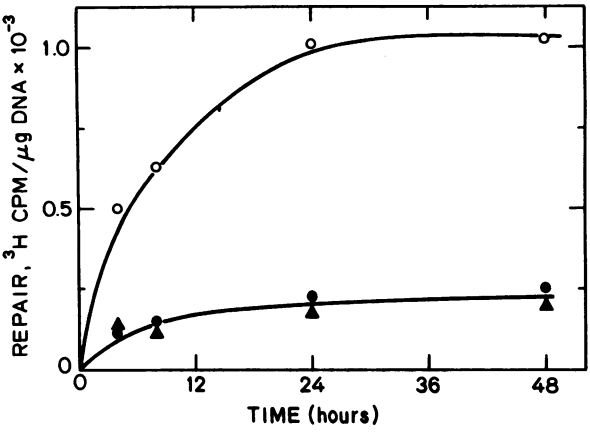

Figure 1. Time course for excision repair in α DNA and bulk DNA after treatment of cells with AFB_1. BS-C-1 cells were treated with AFB_1 (0.32 mM) activated by rat liver microsomes and incubated in medium containing 30 μCi/ml [^{3}H]thymidine, 50 μM BrdUrd, and 5 μM FdUrd. The repair synthesis was measured in the 172 bp α DNA monomer (●), 340 bp α dimer (▲) and bulk DNA (○).

of the [^{3}H]AFB_1 preparation. For analysis of individual AFB_1 adducts, the DNA was hydrolyzed in 0.15 N HCl at 100°C for 15 min (6). The hydrolysates were adjusted to pH 3-4 and then analyzed by high pressure liquid chromatography (HPLC) using a μBondapak C_{18} column (Waters) and a Beckman model 322 gradient liquid chromatograph as described by Wang and Cerutti (19).

Measurement of Repair Synthesis and Patch Size.

Repair synthesis and repair patch size were measured as described by Smith et al. (20) using the conditions of Zolan et al. (16).

RESULTS

Repair Replication induced by AFB_1

Confluent cultures of BS-C-1 cells , prelabeled with [^{32}P], were either irradiated with 50 J/m^2 UV, or exposed for 60 min at 37°C to 0.32 mM AFB_1 in the presence of a microsomal activation system derived from rat liver. After posttreatment incubations of 4 to 48 hr, the amount of repair replication per unit DNA in the 172 bp α monomer, the 340 bp α dimer and the bulk DNA was determined. The time course for repair replication after irradiation with UV was the same in α DNA and bulk DNA, with repair in α DNA being about 80% of that in bulk DNA (data not shown), in agreement with an earlier study in which efficient repair of pyrimidine dimers in both α DNA and bulk DNA was documented (16). In contrast to the results for UV the amount of repair

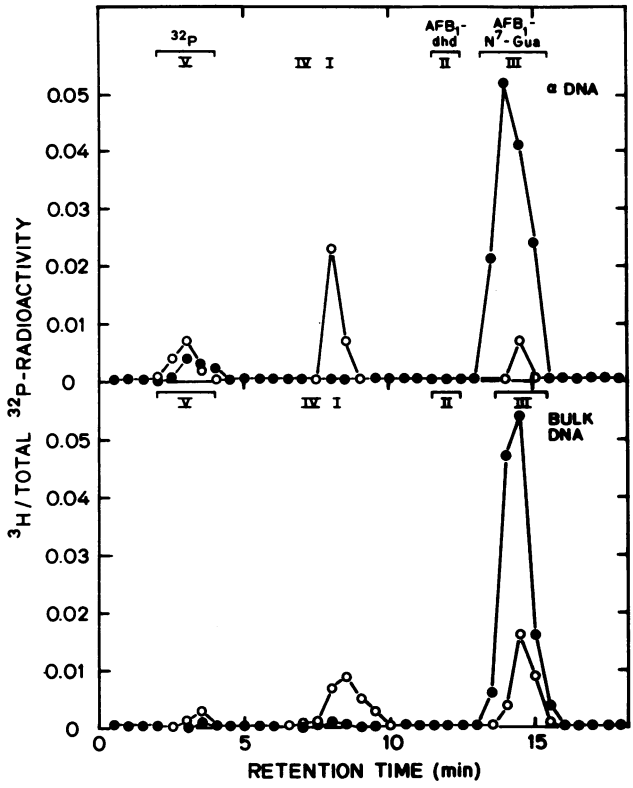

Figure 2. HPLC profiles of acid hydrolysates of DNA extracted from [^{32}P] labeled cultures of BS-C-1 cells that had been treated with [^{3}H]AFB$_1$ (0.25 μM) activated by rat liver microsomes and incubated in conditioned medium for 0 or 48 hr. The ratios of the [^{3}H] in each fraction to the total [^{32}P] contained in the sample are plotted. Brackets indicate the position of elution of the [^{32}P] and the mobilities of authentic markers of AFB$_1$-N^7-Gua and AFB$_1$-dhd. No posttreatment incubation (●) and 48 hr posttreatment incubation (○) are shown for both α DNA and bulk DNA.

replication per unit DNA induced by AFB$_1$ damage in the α DNA monomer and dimer was only 25% of that in bulk DNA over the entire time course (Fig. 1).

AFB$_1$-DNA Adduct Formation.

The smaller amount of repair replication observed in α DNA following AFB$_1$ damage could be a consequence of a lower frequency of adducts in α DNA than in the bulk of the DNA. AFB$_1$-DNA adducts were therefore quantitated in isolated DNA from [^{32}P] labeled cells that had been exposed to [^{3}H]AFB$_1$ activated by microsomes. Exposure of BS-C-1 cells to AFB$_1$ under the conditions used for repair replication experiments (0.32 mM AFB$_1$ for 60 min at 37°C) produced

approximately 1360 adducts per 10^8 daltons for both α DNA and bulk DNA. When cells were treated with much lower doses of [^{3}H]AFB_1 (0.25 μM for 30 min at 37°C), the modification level was 0.8 to 1.2 AFB_1 adducts per 10^8 daltons DNA for both α DNA and bulk DNA. AFB_1-DNA adducts from cells treated in this manner were further analyzed by HPLC and a typical profile is shown in Figure 2. The chromatographic properties of the [^{3}H] labeled species were identical to those obtained from metabolically active cells (13,14) and from human fibroblasts in culture (15). Peak I consists of AFB_1-triamino-Py, Peak II consists of AFB_1-dhd, and Peak III consists of AFB_1-N^7-Gua. These products correspond respectively to the peaks with the same notation of Lin et al. (6). It has been suggested that Peak IV contains 2,3-dihydro-2-(8,9-dihydro-8-hydroxy-guan-7-yl)-3-hydroxyaflatoxin B_1 (6), while Peak V remains unidentified. The distribution of [^{3}H] radioactivity immediately after treatment in both α DNA and bulk DNA in different experiments was: Peak I, 0 to 3%, Peak II, not detectable; Peak III, 92-96%; Peak IV, 0 to 1%; Peak V, 1 to 5%. Thus, the precursor to the AFB_1-N^7-Gua is the major primary adduct formed in both α DNA and bulk DNA by microsome-activated AFB_1, and the lower repair replication in α DNA after AFB_1 damage can not be due to the production of fewer or different AFB_1 adducts in α DNA compared to those in bulk DNA.

AFB_1-DNA Adduct Repair.

Since the initial distribution of AFB_1-DNA adducts was the same for both α DNA and bulk DNA, the smaller amount of repair replication in α DNA may be due to the removal of fewer adducts from α than from the bulk DNA. To examine this possibility, BS-C-1 cells were exposed to [^{3}H]AFB_1 followed by 0 to 48 hr incubation. DNA samples were then acid hydrolyzed and the frequency of the individual adducts determined by HPLC. Total adduct removal occurred more rapidly in the bulk DNA than in α DNA (Fig. 3). By 24 hr after treatment, 65% of the initial adducts had disappeared from bulk DNA while only 35% had been lost from α DNA. The rate of AFB_1 loss from α DNA was similar to that previously reported for repair-deficient XP-A cells (dashed line) (15), consistent with the view that the release of the adducts occurs through a similar process.

The kinetics for removal of AFB_1-N^7-Gua, based on HPLC analysis, is presented in Fig. 4. The rate of loss of this adduct from α DNA is clearly slower than from bulk DNA. After a 24 hr posttreatment incubation, 85% of AFB_1-N^7-Gua has disappeared from bulk DNA while only 55% was lost from α DNA. Therefore, the difference in the kinetics of total adduct removal between bulk DNA and α DNA (Fig. 3) can be explained by the more rapid removal of AFB_1-N^7-

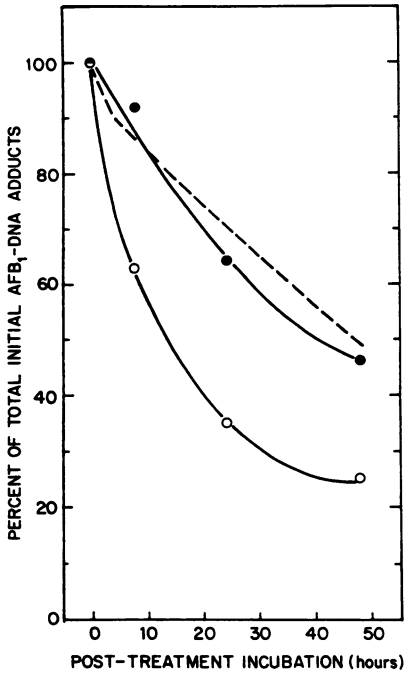

Figure 3. Kinetics of the disappearance of total AFB_1 adducts from α DNA (●) and bulk DNA (O) during posttreatment incubation. The cells were prelabeled with [^{32}P] and treated with activated [^{3}H]AFB_1 (0.25 μM). AFB_1 adduct frequencies were derived from the [^{3}H]/[^{32}P] ratios in purified DNA. Kinetics of the disappearance of AFB_1 adducts from XPA cells (----) are taken from Leadon et al.,(15).

Gua from the bulk.

In contrast to the disappearance of Peak III (Fig. 2 and 4), the level of the minor species AFB_1-triamino-Py (Peak I) increases with posttreatment incubation (Fig. 2 and 5), accumulating in both α DNA and bulk DNA. However, the rate and extent of formation of AFB_1-triamino-Py is higher in α DNA. The amount of this secondary product reaches a maximum in both α and bulk DNA by 24 hr posttreatment. No evidence was obtained for the loss of this product from the cellular DNA.

The early-eluting Peak V in the HPLC profiles (Fig. 2) initially contained 1 to 5% of the [^{3}H] radioactivity in both α DNA and bulk DNA and it varied between 1 and 8% during the posttreatment incubation. These results suggest that Peak V material is formed by a spontaneous secondary reaction. The structural identity of Peak V has not been established but it probably

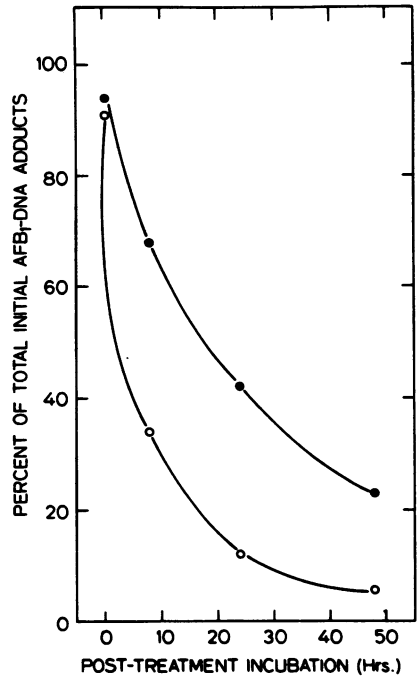

Figure 4. Kinetics of the disappearance of AFB_1-N^7-Gua (HPLC Peak III), from α DNA (●) and bulk DNA (○) during posttreatment incubation of BS-C-1 cells. The relative adduct frequencies were determined by HPLC analysis as described in Fig. 2.

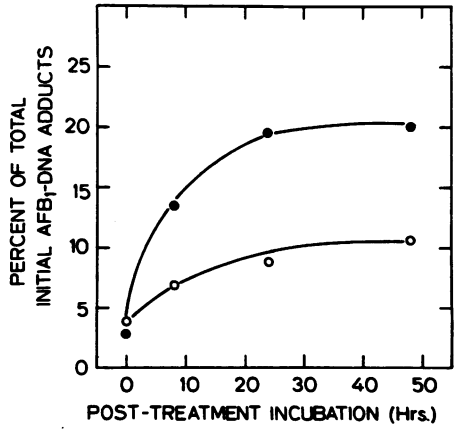

Figure 5. Kinetics of the formation of AFB_1-triamino-Py (HPLC Peak I), in α DNA (●) and bulk (○) during posttreatment incubation of BS-C-1 cells. The relative adduct frequencies were determined as described in Fig. 2.

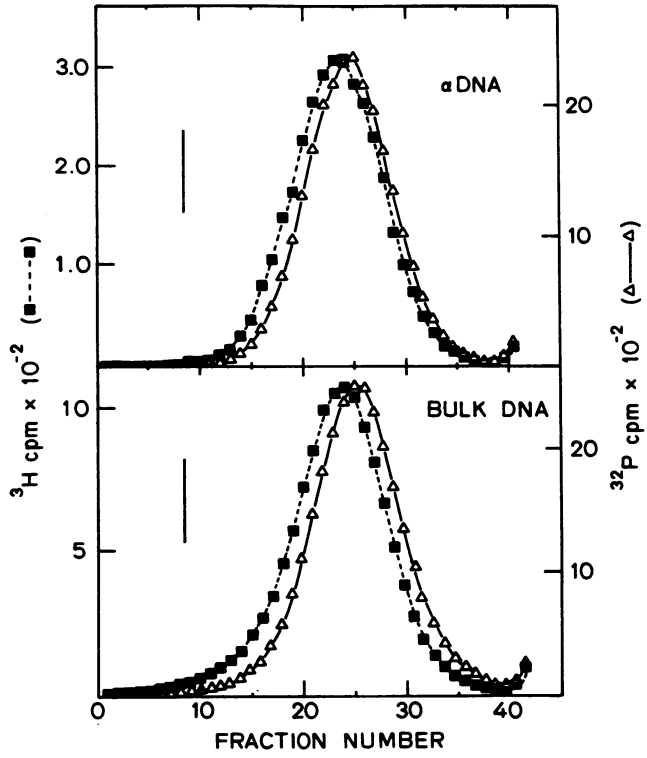

Figure 6. Distribution of repair patch size in α DNA and bulk DNA after treatment of the cells with AFB_1. The cells were prelabeled with [^{32}P], treated with activated AFB_1 (0.32 mM) and incubated for 24 hr in medium containing 30 μCi/ml [^{3}H]thymidine, 100 μM BrdUrd, and 10 μM FdUrd. Parental density α DNA and bulk DNA were isolated and centrifuged to equilibrium in alkaline CsCl gradients after the bulk DNA had been sonicated to a number-average molecular weight of about 160 nucleotides. Vertical lines represent the position of fully BrdUrd-substituted DNA.

corresponds to the peak with the same numerical designation of Lin et al. (6) and to Peak E of Croy and Wogan (21). The radioactivity in Peaks II and IV was not sufficient in our experiments for kinetic analysis.

Repair Patch Size.

Correlating the deficient removal of AFB_1 adducts from α DNA with its lower level of repair replication is made difficult because loss of the adducts can occur by multiple pathways, namely: (1) The spontaneous release of the adducts leaving an unaltered guanine, which would require no repair synthesis; (2) The spontaneous release of the AFB_1-guanine residue, which would lead to repair synthesis initiated by an apurinic endonuclease; and (3)

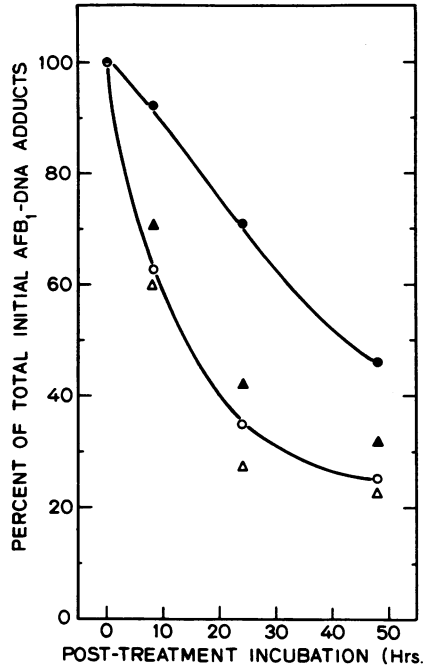

Figure 7. Kinetics of disappearance of total AFB_1 adducts from α DNA and bulk DNA in UV irradiated BS-C-1 cells. The cells were prelabeled with [^{32}P] and irradiated with 5 J/m^2 UV immediately after treatment with [^{3}H]AFB_1. Total AFB_1 adducts were determined from [^{3}H]/[^{32}P]: α DNA without UV (●); α DNA with UV (▲); bulk DNA without UV (○); bulk DNA with UV (△).

The enzymatic recognition of the AFB_1 adduct in DNA, leading to excision repair similar to that operating on pyrimidine dimers and other bulky adducts. To gain further information on the nature of the observed repair synthesis, we measured the size of repair patches produced in both α and bulk DNA during repair of AFB_1 adducts by the method previously described (20). We analyzed in alkaline CsCl gradients α DNA and bulk DNA reduced in size by sonication to roughly the same average molecular weight as that of α DNA. Under these conditions, the increase in density of DNA fragments that contain repair patches (synthesized in the presence of BrdUrd) is large enough to be measured and can be compared to the increase in density of DNA completely substituted with BrdUrd. A knowledge of the molecular weights of the fragments then allows the calculation of the average repair patch size. Comparison of the results for α DNA and bulk DNA shows, in cells treated with AFB_1, a smaller increase in the density of the α DNA than in the bulk DNA fragments (Fig. 6).

The patch size in α DNA was calculated to be about 10 nucleotides while the patch size in bulk DNA was about 20 nucleotides. In parallel experiments in which a UV dose of 50 J/m^2 was used, the patch size in both α DNA and bulk DNA was about 20 nucleotides (data not shown).

Combined AFB_1 and UV Treatment.

Since UV induced damage was efficiently repaired in α DNA but AFB_1-DNA adducts were not, we examined the effect of a UV exposure on AFB_1 adduct removal. When BS-C-1 cells were exposed to 5 J/m^2 UV immediately after treatment with activated [^{3}H]AFB_1, an increase in the rate of adduct loss from α DNA was observed, with little or no change in the kinetics of adduct removal from bulk DNA (Fig. 7). By 24 hr posttreatment, 60% of the initial adducts had disappeared from α DNA compared with 70% from bulk DNA. Thus, treatment of the cells with UV actually increased the rate and extent of removal of AFB_1-DNA adducts from α DNA to the levels found in the bulk of the DNA.

DISCUSSION

Excision repair of AFB_1-DNA adducts in the highly repeated α sequence of cultured monkey cells was found to be deficient when compared with that in the bulk of the genome. This finding extends previous observations (16) in which decreased repair in α DNA was found for furocoumarin photoadducts and for N-acetoxy-2-acetylaminofluorene but not for pyrimidine dimers. In cells treated with microsome-activiated AFB_1, a reduced amount of repair replication was found in α DNA compared with bulk DNA over the entire time course studied. In studies using activated [^{3}H]AFB_1, the initial adduct frequency and composition were found to be the same for both α DNA and bulk DNA, with the primary adduct being AFB_1-N^7-Gua. Therefore, both α DNA and the bulk of the DNA were equally accessible to activated AFB_1. The sequence-specific binding of AFB_1 reported by Muench et al. (22) would be predicted to occur with equal frequency in both α DNA and bulk DNA based on the known sequence of α DNA (23). The smaller amount of repair synthesis observed in α DNA can be explained in part by the slower removal of the initial AFB_1 adducts. The kinetics of AFB_1-N^7-Gua loss from α DNA (Fig. 4) were similar to those for this adduct in excision repair-deficient human XP-A cells (15). Therefore, it is likely that the loss of AFB_1-N^7-Gua from α DNA represents simply the spontaneous loss of AFB_1 from the DNA leaving either intact guanines or aguaninic sites (24). Besides the slower removal of the primary adduct, the formation of the secondary product, AFB_1-triamino-Py, was more rapid and occurred to a greater extent in α DNA than in the bulk DNA. This effect was also observed in XP-A cells and

probably reflects the slower removal of the initial AFB_1 adduct (15). This represents the formation of a persistent lesion since it is apparently not removed from DNA.

The reduced repair synthesis in α DNA following treatment with AFB_1 can also be ascribed in part to a shorter repair patch in α DNA than in bulk DNA. This smaller patch size was not observed with other damaging agents that were deficiently repaired in α DNA (16) and therefore probably reflects the repair of the aguaninic sites produced in the DNA by the spontaneous hydrolysis of AFB_1 adducts. The repair of apurinic and apyrimidinic (AP) sites, resulting from either spontaneous loss of a base or from the action of a DNA glycosylase, is initiated by endonucleases which recognize these sites in DNA (25). This pathway is operative in XP-A cells, and may be responsible for the small amount of repair synthesis observed in these cells after AFB_1 damage (4). The proficient repair of some types of damage in α DNA, through a glycosylase/AP endonuclease pathway, is further supported by recent work in our laboratory in which damage induced by methylmethane sulfonate, dimethylsulfate, and ionizing radiation are efficiently repaired in α DNA (Leadon et al., manuscript in preparation). These agents all produce lesions in DNA that are subject to repair via a glycosylase/AP endonuclease pathway and that are proficiently repaired in XP-A cells (26). Our observation that the average patch size for repair of AFB_1 damage in α DNA is smaller than that for repair in the bulk of the DNA is also consistent with the notion that different repair pathways are functioning in the two DNA species. Shorter repair patches are not mandated by the structure of α DNA, since repair of UV and furocoumarin damage in α DNA produces the same patch size as in bulk DNA (16). Early measurements, using the BrdUrd photolysis method, of patch sizes produced during repair in cells treated with methylmethane sulfonate suggested that the glycosylase/AP endonuclease initiated repair pathway resulted in very short patches, i.e.,approximately 4 nucleotides long (27). However, more recent experiments with this technique (28,29) as well as with the density shift technique that we have used (30) have indicated that a repair pathway producing a larger patch size component may also be involved. The average patch size of about 10 nucleotides we measured in α DNA could represent the contributions of two or more size classes. In our experiments with methylmethane sulfonate, a value of about 8 nucleotides was obtained for the repair patch size in both α DNA and bulk DNA (Leadon et al., manuscript in preparation).

In contrast with the results for methylating agents and ionizing radia-

tion, chemical agents that produce bulky adducts in DNA are deficiently repaired in cultured XP-A cells, and deficiently repaired in α DNA of monkey cells. In addition to AFB_1, photoadducts of the furocoumarins angelicin, aminomethyl trioxsalen (16) and hydroxymethyl trioxsalen (Zolan et al., manuscript submitted) and N-acetoxy-2-acetylaminofluorene adducts (16) are deficiently repaired in α DNA and in XP-A cells. Although XP-A cells are believed to be deficient in the appropriate incision activity for repair of bulky lesions in DNA, the situation for α DNA appears to be one of accessibility of α chromatin to these incision enzymes, since there is differential repair within the same cells. Evidence that the chromatin structure of α DNA is different from that of the bulk of the genome comes from finding that the conversion of monofunctional psoralen photoadducts to interstrand crosslinks is inhibited in α DNA in cells, but not in purified α DNA (Zolan et al., manuscript submitted). However, the sensitivity of α chromatin to micrococcal nuclease, DNase II and certain restriction enzymes appears to be indistinguishable from that of bulk chromatin (31,32,33). Thus, although the nucleotide sequence of α DNA is not unusual, the unique chromatin structure of α may only allow accessibility to relatively low molecular weight enzymes, such as nucleases and glycosylases, while excluding the larger repair enzyme complexes, such as those that might be involved in the repair of bulky chemical adducts.

Pyrimidine dimers are not repaired in cultured XP-A cells (34) but they are proficiently repaired in α DNA of monkey cells. If chemical adducts are not repaired in α DNA because the chromatin structure of α inhibits the accessibility of incision enzymes to the lesions, then pyrimidine dimers may be proficiently repaired in α DNA because their formation disrupts the special chromatin structure of α . This is plausible when one considers the fact that pyrimidine dimers form with equal frequencies in all regions of chromatin (35,36) whereas those of AFB_1 (37,38) and furocoumarins (36,39,40) form primarily in linker regions between nucleosome cores, where their effect on chromatin structure may be minimal. Our finding that AFB_1 adducts in α DNA are repaired when the cells are also exposed to UV indicates that the formation of pyrimidine dimers or their repair may alter the chromatin structure of α DNA.

The deficient repair in α DNA of bulky chemical adducts may represent an extreme in a spectrum of repairability among different regions of the genome. Other cellular DNA sequences in the bulk of the DNA, such as genes that are actively transcribed and their adjacent regions, also possess altered

chromatin conformation (41). It will be of interest to determine whether these sequences exhibit differential rates of repair for bulky DNA adducts.

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