MOLECULAR AND CELLULAR BIOLOGY, July 1996, p. 3714–3719 Vol. 16, No. 7
0270-7306/96/$04.00+0

Site-Specific Excision Repair of 1-Nitrosopyrene-Induced DNA Adducts at the Nucleotide Level in the *HPRT* Gene of Human Fibroblasts: Effect of Adduct Conformation on the Pattern of Site-Specific Repair

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Received 23 January 1996/Returned for modification 28 March 1996/Accepted 25 April 1996

Studies showing that different types of DNA adducts are repaired in human cells at different rates suggest that DNA adduct conformation is the major determinant of the rate of nucleotide excision repair. However, recent studies of repair of cyclobutane pyrimidine dimers or benzo[*a*]pyrene diol epoxide (BPDE)-induced adducts at the nucleotide level in DNA of normal human fibroblasts indicate that the rate of repair of the same adduct at different nucleotide positions can vary up to 10-fold, suggesting an important role for local DNA conformation. To see if site-specific DNA repair is a common phenomenon for bulky DNA adducts, we determined the rate of repair of 1-nitrosopyrene (1-NOP)-induced adducts in exon 3 of the hypoxanthine phosphoribosyltransferase gene at the nucleotide level using ligation-mediated PCR. To distinguish between the contributions of adduct conformation and local DNA conformation to the rate of repair, we compared the results obtained with 1-NOP with those we obtained previously using BPDE. The principal DNA adduct formed by either agent involves guanine. We found that rates of repair of 1-NOP-induced adducts also varied significantly at the nucleotide level, but the pattern of site-specific repair differed from that of BPDE-induced adducts at the same guanine positions in the same region of DNA. The average rate of excision repair of 1-NOP adducts in exon 3 was two to three times faster than that of BPDE adducts, but at particular nucleotides the rate was slower or faster than that of BPDE adducts or, in some cases, equal to that of BPDE adducts. These results indicate that the contribution of the local DNA conformation to the rate of repair at a particular nucleotide position depends upon the specific DNA adduct involved. However, the data also indicate that the conformation of the DNA adduct is not the only factor contributing to the rate of repair at different nucleotide positions. Instead, the rate of repair at a particular nucleotide position depends on the interaction between the specific adduct conformation and the local DNA conformation at that nucleotide.

It is well known that prokaryotic and eukaryotic cells rely on nucleotide excision repair (NER) to remove a wide range of DNA damage caused by various agents, including UV irradiation and such polycyclic carcinogens as benzo[*a*]pyrene diol epoxide (BPDE), a reactive metabolite of benzo[*a*]pyrene, and *N*-acetoxy-2-acetylaminofluorene (*N*-AcO-AAF) (9, 16, 17). Studies using loss of *Escherichia coli* UvrABC excinuclease-sensitive sites (21) or loss of radiolabeled polycyclic DNA adducts (7, 27) show that various types of DNA damage are removed from the overall genome of human cells at very different rates. To account for such differences, it has generally been hypothesized that the structure of the photoproduct or adduct is the major factor determining the efficiency with which a particular lesion is recognized and removed by DNA excision repair complex.

In 1985, Bohr et al. found that actively transcribed genes can be repaired faster than nontranscribed genes (preferential repair) (3). Shortly thereafter, Mellon and colleagues (12, 13) determined that damage in the transcribed strand of active genes is repaired faster by NER than damage in the nontranscribed strand (strand-specific or transcription-coupled repair). These observations focused attention on the contribution of the chromatin structure to the rate of NER and the role of the transcription process itself (see reference 2 for a review). Nevertheless, studies of the rate of repair of various kinds of damage in the individual strands of the hypoxanthine phosphoribosyltransferase (*HPRT*) gene of human fibroblasts in culture show that the rate of excision repair of UV-induced photoproducts (cyclobutane pyrimidine dimers [CPD] and pyrimidine-6-4-pyrimidones) (20) or adducts formed by 1-nitrosopyrene (1-NOP) (11), a partially reduced metabolite of 1-nitropyrene, is much faster than excision of BPDE adducts (4). These data suggest that the conformation of the damage itself plays a dominant role in determining the rate of repair.

However, very recent studies of the rates of excision repair of UV-induced CPD (6, 19) or BPDE-induced adducts (24) at the nucleotide level (site-specific repair) in particular genes in human fibroblasts by using ligation-mediated PCR (LMPCR) indicate that the conformation of the photoproduct or adduct cannot be the sole determinant. This is because the rate of excision of a specific type of CPD, e.g., a cytosine-cytosine dimer, or of the guanine-BPDE adduct varies significantly from one position in the gene of interest to another position in that gene, sometimes by more than 10-fold. No consensus DNA sequence could be found to account for this difference. Gao and colleagues (6) proposed that the presence of transcription factors at specific sites interfered with excision repair because they found out that the region in the *PGK1* gene where the repair of CPD is the slowest is also the region where

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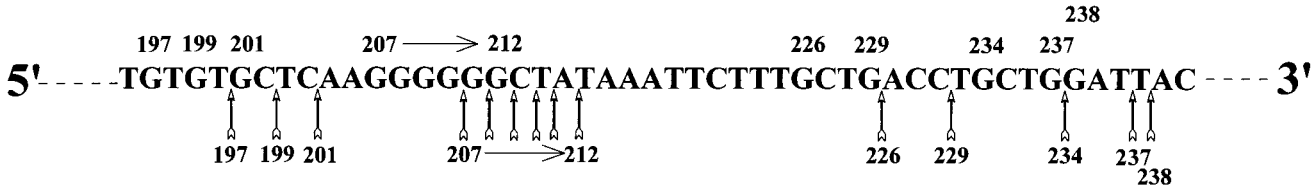

FIG. 1. DNA sequence of the region of interest in the nontranscribed strand of exon 3 of the *HPRT* gene. The positions of guanines where adducts form are shown. The sites of incision by UvrABC that are located 3′ of the corresponding adducts are indicated by arrows and numbers below the sequence.

transcription factors bind. Since most of the human DNA is considered to be wrapped around histone octamers to form nucleosomes, Tornaletti and Pfeifer (19) hypothesized that the nucleosomal structure causes steric hindrance to the repair enzymes, making the rate of repair of dimers vary at different sites. Wei et al. (24) also hypothesized that site-specific repair of BPDE adducts reflects the specific local DNA conformation at a particular nucleotide position.

Although evidence suggests that both DNA adduct conformation and local DNA conformation affect the rate of repair at a particular nucleotide position, it was not known whether these factors act independently or interactively. Previously we determined rates of repair of BPDE-induced adducts in the nontranscribed strand of exon 3 of the *HPRT* gene and found that they are repaired in a site-specific manner (24). In the present study, we investigated the rates of excision at the nucleotide level of a structurally related bulky DNA adduct induced by 1-NOP in that same region (Fig. 1). To examine the relative contribution of the adduct conformation versus that of the local conformation of the DNA, including nucleosome pattern effects and possible transcription factors, etc., we then compared the pattern of site-specific repair of 1-NOP-induced DNA adducts with that of BPDE-induced adducts in the same region under the same conditions. The principal adducts of both polycyclic compounds involve guanine, with BPDE binding primarily at the N^2 position (25) and 1-NOP at the C8 position of guanine (1). If site-specific excision repair is the result of steric hindrance to the action of the repair enzyme complex at specific positions in the region of interest, caused by various forms of protein-DNA interaction or by local DNA conformation, the contribution of these factors to the rate of repair at a specific position in the gene should be relatively constant. Then, substituting one structurally related adduct for another at that specific position ought not to affect the pattern of site-specific repair. Our results show that in the region of interest, the average rate of repair of 1-NOP adducts was two to three times faster than that of BPDE adducts. However, at some sites repair of 1-NOP adducts was almost as slow as that that of BPDE adducts or even slower, indicating that both the adduct conformation and the local DNA conformation affect the rate of repair at a particular site and that their effects depend on each other.

MATERIALS AND METHODS

Cell culture and synchronization. Diploid human male fibroblasts from neonatal foreskin, designated SL68, were cultured in Eagle's minimal essential medium modified as described previously (22) and containing 10% supplemented bovine calf serum (culture medium). Cells were synchronized by being driven into the G_0 state by density inhibition and mitogen deprivation and then being released (23). To stimulate the cells to reenter the cell cycle, they were trypsinized and plated at 1.7×10^4 cells per cm^2 in fresh culture medium.

Carcinogen treatment and posttreatment incubation. 1-NOP, kindly provided in solid form by Frederick Beland of the National Center for Toxicology Research (Jefferson, Ark.), was dissolved in anhydrous dimethyl sulfoxide, and stored in a −80°C freezer under nitrogen gas until just prior to use. Five h after the cells were released from G_0, the medium was removed, the cells were rinsed twice with phosphate-buffered saline, and serum-free medium was added. The cells were treated with 1-NOP by adding an appropriate volume of stock solution and incubating for 1 h at 37°C. The cells were rinsed and lysed immediately or incubated in fresh culture medium for the times indicated below before being lysed.

Determination of extent of DNA synthesis. After 1-NOP treatment, cells were incubated in fresh culture medium for 10 h and [^{3}H]TdR (6.7 Ci/mmol; DuPont) was added. Cells were incubated for an additional 10 h and then lysed. DNA was extracted and purified as described previously (24). The specific activity of DNA (in counts per minute per microgram of DNA) was determined, and the amount of the newly synthesized DNA was calculated as described (24).

Determination of the rates of excision repair of 1-NOP adducts at the nucleotide level. DNA was isolated from the cell lysates and purified (with phenol-chloroform) as previously described (24). Unless otherwise specified, 12 μg of purified DNA was cut with *Spe*I and *Bgl*I and then digested as described previously (4) with 18 pmol of UvrABC excinuclease, kindly provided by Pieter van de Putte at Leiden University (Leiden, The Netherlands), to excise the 1-NOP adducts. The frequency of UvrABC cuts at each site was then measured by using LMPCR as described by Wei et al. (24), except a [^{3}H]dCTP-labeled DNA fragment was included in each sample as an internal control (see below). Briefly, about 10^5 cpm of the internal control was added to each UvrABC-digested DNA sample, and the samples were purified and redissolved in 11 μl of H_2O. Then DNA was denatured and a gene-specific primer, designated primer 2 (24), containing a biotin at the 5′ end was allowed to anneal and was extended by Sequenase 2.0. A specially designed linker was ligated to the 5′ cut end of the DNA fragment of interest, and the desired fragments were isolated with 75 μg of Dynabeads M-280 Streptavidin by following the manufacturer's instructions. The purified fragments were then amplified with 22 cycles of PCR by using the longer oligomer of the linker and a second gene-specific primer (primer 3), located 3′ of primer 2. Amplified DNA was purified with phenol-chloroform, precipitated in ethanol along with 10 μg of yeast tRNA, and redissolved in 7 μl of solution containing 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. One microliter of the final product was counted on a scintillation counter, and the final products with equal counts of the internal control were labeled by using a ^{32}P-labeled gene-specific sequencing primer as described below, precipitated with ethanol, redissolved in 3 μl of loading buffer (U.S. Biochemicals), and separated on a sequencing gel. The intensities of the bands were analyzed by using a PhosphorImager (Molecular Dynamics).

Determination of the efficiency of UvrABC excision of 1-NOP-induced DNA adducts. A genomic DNA fragment containing the region of interest was amplified with an intron primer and gene-specific primer 2 as described previously (24). DNA was purified and digested with *Dde*I to generate a fragment of 354-bp, which was then gel purified and treated with 8 μM 1-NOP as previously described (26). DNA was extracted with phenol-chloroform three times and precipitated with ethanol. An appropriate amount of DNA was treated with UvrABC as described by Chen et al. (4) and then was purified and denatured. Gene-specific primer 2 was labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase according to the manufacturer's suggestions, annealed to the denatured DNA, and extended with Sequenase 2.0 as described previously (24). The sample was then separated on a sequencing gel and exposed to Kodak X-Omat film.

Generation of the radiolabeled DNA fragment as the internal standard. A DNA fragment, with no homology to the region of interest, was amplified for 30 cycles with *Taq* DNA polymerase in the presence of 60 mM KCl, 2.75 mM $MgCl_2$, 15 mM Tris-HCl (pH 8.55), 25 μM deoxynucleoside triphosphates, 2 μM [^{3}H]dCTP (New England Nuclear), 0.3 μM of a biotinylated primer, and another primer. The unincorporated [^{3}H]dCTP was removed by centrifugation with a Centricon-30 microconcentrator by following the manufacturer's suggestion, and 5 μl of the purified sample was counted on a scintillation counter to obtain the specific activity of the final internal standard.

RESULTS

Measuring site-specific rates of repair of 1-NOP-induced adducts. To determine the rates of repair of 1-NOP-induced adducts at the nucleotide level in exon 3 of the *HPRT* gene, we synchronized populations of excision repair-proficient diploid

human fibroblasts and treated them for 1 h in early G_1 phase with 0.8 μM 1-NOP, a dose that introduces about one DNA adduct per 2×10^4 nucleotides, i.e., ~0.01 adducts in the nontranscribed strand of exon 3. At that level of adduct formation, the cells are fully capable of NER (11). The cells were lysed immediately or after allowing 5, 10, or 20 h for repair. Cellular DNA was extracted and purified, and *E. coli* UvrABC excinuclease was used to excise the 1-NOP-induced adducts. Frequencies of UvrABC excision in various DNA samples were then mapped to the resolution of single nucleotides by using LMPCR as we did previously in our study with BPDE (24). After cells have been allowed time for repair, the decrease of frequencies of UvrABC excision at a particular nucleotide position should reflect the decrease of 1-NOP-induced adduct level at that site, i.e., the site-specific rate of repair.

Determination of DNA replication during the time allowed for repair. Any semiconservative DNA synthesis occurring within the period allowed for repair would give an apparent decrease in the frequency of adducts. Under the conditions used, cells synchronized by release from density inhibition and serum deprivation begin DNA synthesis after ~15 h (23). However, carcinogen treatment can cause a dose-dependent delay in the onset of DNA synthesis (28). Before undertaking the study, we determined the extent of daughter strand DNA synthesized during the period from 10 to 20 h after treatment by treating cells in early G_1 with 0.8 μM 1-NOP or with the solvent dimethyl sulfoxide alone (control) and measuring the amount of incorporation of [^{3}H]TdR into DNA during that period. The value for the 1-NOP-treated cells was ~2% of the incorporation in the control cells (data not shown). Therefore, the amount of DNA analyzed for 1-NOP-induced adducts remaining from cells harvested after 20 h was increased by 2% to ensure that we were analyzing the same amount of parental DNA to start with.

Determination of the efficiency of UvrABC excision of 1-NOP-induced adducts. For analysis of the fraction of adducts remaining using UvrABC excinuclease digestion, we used 18 pmol of UvrABC to digest 12 μg of parental DNA. A dose of 1-NOP that yields one adduct per 2×10^4 nucleotides introduces approximately 5×10^{11} adducts per 12 μg of genomic DNA. To test whether 18 pmol of UvrABC is capable of excising this level of 1-NOP-induced adducts, we treated a purified DNA fragment containing exon 3 of the *HPRT* gene with 8 μM 1-NOP. This dose produces ~0.6 adduct per strand of the 354-bp fragment (26). An aliquot of DNA containing ~8×10^{11} adducts (0.25 μg of DNA) was digested with 9 pmol of UvrABC under standard conditions; a second 0.25-μg aliquot was left undigested. The DNA was then purified and denatured. An end-labeled primer complementary to the 3′ end of the nontranscribed strand of exon 3 was annealed and extended with Sequenase 2.0.

The results are shown in Fig. 2. With DNA that had not been digested with UvrABC (lane Con), Sequenase extension terminated principally 1 nucleotide prior to each guanine; and in a few positions, terminations occurred directly across from the guanine. These data indicate that the majority of the 1-NOP-induced adducts involve guanine and that the adducts can stop the Sequenase extension 1 nucleotide prior to or directly across from the corresponding guanine. With DNA that had been digested with UvrABC (lane S3), at the majority of guanine positions, Sequenase extension terminated 4 nucleotides prior to the corresponding guanine. Since UvrABC makes dual incisions, one at the 8th phosphodiester bond 5′ of the adduct and the other at the 4th phosphodiester bond 3′ of the adduct, this result indicated that at most sites, UvrABC can excise 1-NOP-induced adducts completely. However, at a few sites,

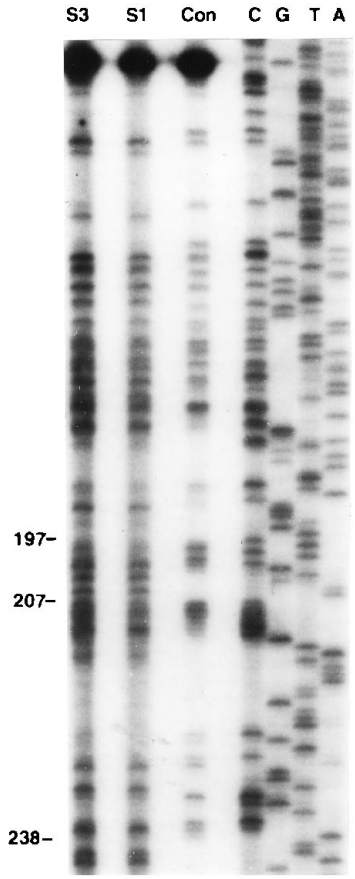

FIG. 2. Autoradiogram showing the termination of Sequenase extension on a 1-NOP-treated DNA fragment. Lane Con shows the termination of Sequenase progression on the adducted DNA fragment that had not been digested with UvrABC. Lanes S3 and S1 show the termination of Sequenase progression on the same adducted DNA fragment after UvrABC digestion. Lanes C, G, T, and A are sequencing controls generated by the standard method of Sanger et al. (18), using the same end-labeled primer on the nonadducted DNA fragment. Therefore, each termination in lane C represents the position of the corresponding guanine on the opposite strand. The nucleotide positions are indicated by the numbers at the left.

such as the region of six consecutive guanines (positions 207 to 212), Sequenase extension terminated at both 1 nucleotide and 4 nucleotides prior to the adducted guanine (see lane S3), indicating that excision by UvrABC had not been complete.

To test whether the incomplete digestion observed at certain nucleotide positions, such as the six consecutive guanines at 207 to 212, resulted from insufficient levels of UvrABC, the same amount of UvrABC (9 pmol) was used to digest one-third of the original template (0.08 μg) under standard conditions. As shown in lane S1 of Fig. 2, the termination pattern of Sequenase extension was essentially the same as that in lane S3, indicating that the incomplete excision at these sites was not a result of too much substrate. Rather, under our standard conditions, the efficiency of UvrABC excision at a particular nucleotide, either complete or incomplete, remained constant. It is of interest to note that it was not possible to digest these undigested 1-NOP-induced adducts by adding more fresh enzyme (data not shown). This suggests that in the first UvrABC reaction, those unexcised adducts had been recognized and in some way protected by UvrABC from later digestion. This hypothesis is further supported by the fact that only after vigorous phenol-chloroform extractions could the remaining

1-NOP-induced adducts be excised by adding fresh UvrABC enzyme (data not shown). Therefore we conclude that 9 pmol of UvrABC is sufficient to recognize 8×10^{11} 1-NOP-induced DNA adducts and to excise most of them. These results strongly suggest that 18 pmol of UvrABC is more than adequate to recognize 5×10^{11} 1-NOP-induced adducts, and what is most important, constant efficiency of excision by UvrABC at each nucleotide position can be achieved under our standard conditions.

Use of LMPCR to quantify rates of repair of 1-NOP-induced adducts. Study of rates of repair using LMPCR involves at least four steps of DNA purification, and at each step of purification some DNA loss is inevitable. Because of technical difficulty, internal controls to allow one to compensate for the DNA loss during handling were not included in previous LMPCR studies, and the variability in the published data reflects this omission. In the present study, we synthesized a [^{3}H]dCTP-labeled DNA fragment that carried a biotin at one of the 5′ ends and included it in our samples as an internal control. Equal counts of the internal control were loaded into each sample before the first DNA purification step (right after UvrABC digestion). Since one end of the internal standard contains a biotin, the internal control fragments remain in the sample even after the step involving Dynabeads purification. Therefore, after the final ethanol precipitation, the radioactivity remaining in the sample was measured and the final products containing equal counts of the internal control were labeled and separated on a sequencing gel to ensure that the relative levels of 1-NOP-induced adducts from the same amount of original genomic DNA were compared.

To quantify adduct levels by using LMPCR, amplification must be in the exponential range. To determine if this was the case under our conditions, we mixed 6 μg of DNA isolated from cells immediately after 0.8 μM 1-NOP treatment with 6 μg of control DNA and analyzed it with UvrABC and LMPCR using our standard conditions. Similar analysis was carried out using 12 μg of DNA from the treated cells. The intensities of the various bands with the former were approximately half that of the corresponding bands in the latter (data not shown), indicating that after 22 cycles of PCR, the amplification was still in the exponential range.

We then determined the rates of repair at specific sites in cells treated with 0.8 μM 1-NOP in early G_1 phase and allowed 0, 5, 10, or 20 h for repair (Fig. 3). Three independent experiments were performed to obtain an average value and the standard deviation. As shown in Fig. 3, the UvrABC excision pattern from 1-NOP-treated DNA samples was very similar to the guanine ladder in the sequencing control, except that each fragment was 4 nucleotides shorter, indicating that the majority of DNA adducts induced by 1-NOP in vivo involved guanine, as shown by previous studies (1, 15). The progressive decrease of the intensity of the corresponding bands is indicative of the repair of adducts at specific sites. The intensities of the bands were measured with a PhosphorImager, and the percentage of intensities remaining at a particular nucleotide position after repair for various lengths of time was calculated and taken as indicative of the rate of repair at that nucleotide position.

Our results indicate that 1-NOP-induced adducts at the region of interest were repaired in a site-specific manner. As shown in Fig. 3, the adducts at nucleotide 226 had been almost completely removed (95%) after only 5 h, while a significant amount of adducts still remained at other sites. At 0 h, the initial adduct levels at nucleotides 207 to 212 showed a progressive decrease from position 207 to 212, whereas after 10 h of time for repair, the adduct levels at nucleotides 207 to 212 were essentially even. After 20 h of time for repair, 74% of the original adducts at nucleotide 229 remained unexcised, while only 24% of the adducts formed at nucleotides 237 to 238 and only 5% of the adducts at nucleotides 197, 226, and 234 remained unexcised by the cells at the time of harvest (Table 1).

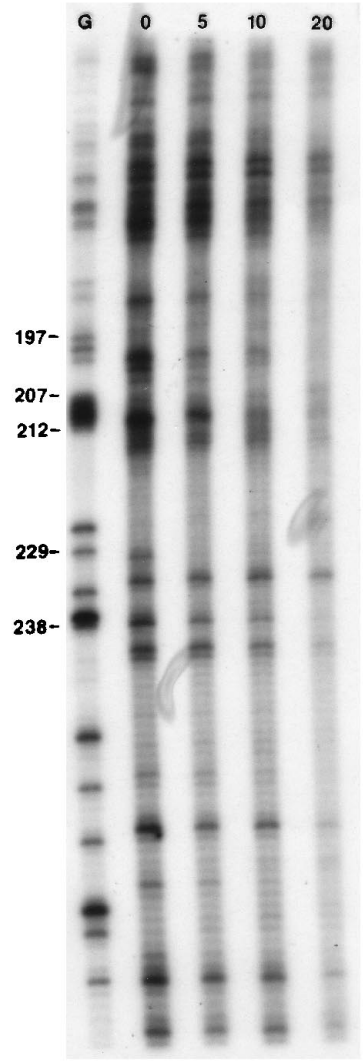

FIG. 3. Autoradiogram illustrating extent of repair in the nontranscribed strand of exon 3 of the *HPRT* gene of cells treated with 0.8 μM 1-NOP. Lane G contains the products of the G reaction of the Maxam-Gilbert sequencing method as described previously (10, 24); lanes 0, 5, 10, and 20 show analysis of 1-NOP adducts in DNA isolated from cells treated in early G_1 phase and harvested after incubation for the indicated number of hours. The nucleotide positions of several guanines in the region of interest are indicated by the numbers at the left. Note that the DNA fragments after UvrABC digestion are four nucleotides shorter than the fragments in the G sequencing ladder.

DISCUSSION

CPDs and BPDE adducts are known to be repaired site specifically by NER in human cells (6, 19, 24). To test whether site-specific excision repair is a common phenomenon for bulky DNA adducts, we investigated the rates of excision repair of 1-NOP adducts at the nucleotide level in the nontranscribed strand of exon 3 of the *HPRT* gene of normal human fibroblasts, using the same LMPCR assay that we used for BPDE adducts (24). Our results showed that the majority of

TABLE 1. Percentages of the original adducts remaining at particular nucleotide positions after 20 h of repair[a]

Nucleotide position	% BPDE adducts	% 1-NOP-induced adducts
197	19.8 ± 3.4	5.4 ± 6
199	6.15 ± 3.9	15.4 ± 4.4
201	25.5 ± 8.7	9.7 ± 3.4
207	62.3 ± 15.8	10.5 ± 4.7
226	46.2 ± 17.7	5.9 ± 1.6
229	91.6 ± 10.8	74.0 ± 6.8
234	63.9 ± 8.2	7.1 ± 7.2
237–238	90.5 ± 8	23.9 ± 14.3

[a] The adduct level at a particular nucleotide position at time zero is taken as 100%. Data are averages ± standard deviations calculated from the results of three independent experiments.

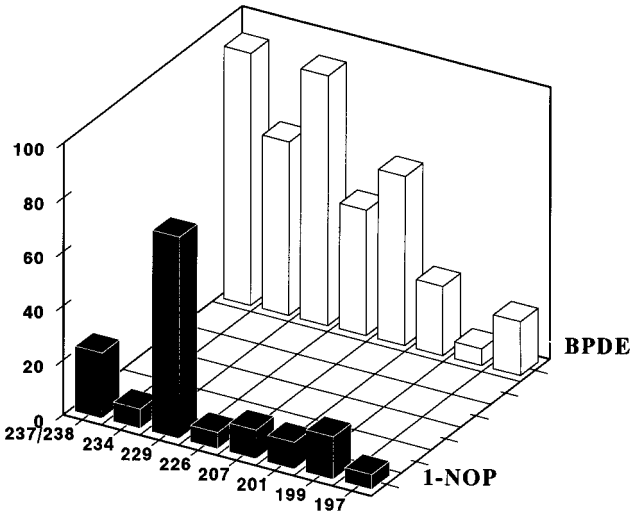

FIG. 4. A three-dimensional presentation of relative percentages of 1-NOP-induced adducts (filled bars) and BPDE adducts (blank bars) remaining in the region of interest after 20 h of repair. The nucleotide positions are shown on the *x* axis and the percentages are shown on the *y* axis. It is obvious that the pattern of site-specific repair of 1-NOP-induced adducts differs significantly from that of BPDE adducts.

the 1-NOP-induced adducts involve guanine and that the rates of repair at different sites vary significantly. No consensus DNA sequence can be found to account for rapidly or slowly repaired sites. 1-NOP adducts at nucleotide 226 were repaired very fast (5% remaining after 5 h), while only 3 nucleotides away, 1-NOP adducts at nucleotide 229 were repaired very slowly (74% remaining after 20 h). In the region of six consecutive guanines, a gradient of initial adduct level formation, ranging from position 207 (the highest) to 212 (the lowest), was observed in DNA from cells allowed no time for repair. In cells allowed 10 h of repair, the adducts remaining at nucleotides 207 to 212 were essentially evenly distributed, indicating that rates of repair at each individual guanine in this region, even though they are only 1 nucleotide apart from each other, are also graduated, decreasing from 207 to 212. It is unlikely that the gradient of site-specific repair in this region is the result of steric hindrance caused by DNA-protein interactions because in a separate study using micrococcal nuclease to carry out in vivo footprinting at exon 3 of the *HPRT* gene, we found no evidence of phased nucleosomal structure or other DNA-protein interaction at this region of interest (24a). Rather, the gradient for rates of repair in the region of six consecutive guanines must be a result of the specific local DNA conformation at each particular nucleotide position.

Yang et al. (26) showed that in a human embryonic cell line, tritiated 1-NOP adducts were removed from the overall genome three to four times faster than tritiated BPDE adducts. Similarly, McGregor et al. (11) showed that the rate of excision repair of 1-NOP-induced adducts in the individual strands of the *HPRT* gene in normal human fibroblasts is twice as fast as that of BPDE adducts (4). In the present study, the average rate of repair of 1-NOP adducts in exon 3 of the *HPRT* gene, i.e., ~60% excised in 10 h and 85% excised in 20 h, was two to three times faster than that of BPDE adducts, i.e., 22% in 10 h and 34% in 20 h (24). The reasons underlying this difference are not clear. BPDE binds principally to the N^2 position of guanine, forming a pentacyclic adduct that lies in the minor groove of the DNA molecule with the pyrenyl ring oriented toward the 5′ end of the modified strand, where it causes little helical distortion (5). 1-NOP undergoes a further intracellular reduction step in human fibroblasts before it forms an unstable reactive intermediate that binds principally to the C8 position of guanine to form the stable tetracyclic adduct *N*-(deoxyguanosine-8-yl)-1-aminopyrene. This adduct lies in the major groove of the DNA and causes little helical distortion (14). It is possible that adducts located in the major groove are more accessible to factors involved in NER than adducts located in the minor groove and thus are repaired faster. However, a structurally related adduct, *N*-(deoxyguanosine-8-yl)-1-aminofluorene, which also is located in the major groove and causes little helical distortion, is repaired even more slowly than BPDE-induced adducts (11).

As shown in table 1 and Fig. 4, the pattern of site-specific repair of 1-NOP-induced adducts differed significantly from that of BPDE adducts. For the majority of positions in exon 3, the former were repaired faster than the latter. Even at positions at which BPDE-induced adducts were repaired very quickly, e.g., nucleotides 197, 201, and 226 (20, 26, and 46% remaining after 20 h, respectively), the repair of 1-NOP-induced adducts was significantly faster (only 7, 8, and 6% left on after 20 h, respectively). At positions where BPDE adducts are excised much less quickly, e.g., positions 207 and 237 to 238 (63 and 92% remaining after 20 h, respectively), 1-NOP adducts were repaired significantly faster (only 11 and 29% left on after 20 h, respectively). These data strongly suggest that the faster rate of repair of a particular type of adduct results from the specific adduct conformation being recognized by the NER system more efficiently. However, 1-NOP adducts were not repaired faster than BPDE adducts at every position. At position 229, where BPDE adducts are repaired very slowly (91% remaining after 20 h), the repair of 1-NOP adducts was comparably slow (74% left on after 20 h), and at position 199, 1-NOP adducts were repaired more slowly than BPDE adducts, i.e., 16% remained after 20 h, compared with only ~6% of BPDE adducts. These data suggest that even though the conformation of the adduct plays a major role, other factors having to do with the local conformation of the DNA are critical for determining the rate of NER. What is more, the data suggest that the contribution of the local conformation of DNA to the rates of excision repair at specific sites varies with the type of adduct, resulting in different patterns of site-specific repair at the same nucleotide positions in the same region. Thus, it is unlikely that the pattern of site-specific repair is merely a reflection of the local DNA conformation. What matters is the final local conformation adopted by the DNA after formation of the adduct. In other words, site-specific rates of repair reflect the interactive effect of the adduct con-

formation and the specific local conformation of the DNA where the adduct has formed.

The model recently proposed by Huang and colleagues (8) for the human excinuclease system hypothesizes that excision repair in human cells starts with nonspecific binding of the recognition subunits to DNA, and this transient, nonspecific binding results in changes in the local DNA conformation, such as DNA kinking and unwinding. It proposes that if the presence of an adduct facilitates this conformational change, a higher affinity interaction between the recognition subunits and DNA occurs, and this long-lived complex allows binding of the nuclease subunits that induce the double incision. This model implies that the final conformation of the adducted site determines the efficiency of NER. Our data in Fig. 4, showing that the rate of excision repair is determined by the interactive effect of the adduct conformation and the specific local conformation of DNA where the adduct is located, provide experimental support for this model and also explain why no consensus DNA sequence was found for either rapidly or slowly repaired sites in DNA (6, 19, 24).

An unanswered question has been how the NER system can recognize and repair such a wide range of DNA damage. Our results, indicating that the signal for NER is the special DNA conformation adopted at a specific site as a result of adduct formation, shed light on this question. The NER system may detect and repair not just a specific kind of DNA damage, but rather any section of DNA that has adopted a dynamic conformation that differs from that of the standard conformation of DNA.

ACKNOWLEDGMENTS

We thank Frederick Beland of the National Center for Toxicological Research (Jefferson, Ark.) for kindly providing 1-NOP and Pieter van de Putte at Leiden University (Leiden, The Netherlands) for kindly providing UvrABC excinuclease. The helpful discussions with W. Glenn McGregor and Beatrice Tung are gratefully acknowledged.

This work was supported by DHHS grants CA56796 and CA21253 from NCI.

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