Nucleotide Excision Repair from Site-Specifically Platinum-Modified Nucleosomes†

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ABSTRACT: Nucleotide excision repair is a major cellular defense mechanism against the toxic effects of the anticancer drug cisplatin and other platinum-based chemotherapeutic agents. In this study, mononucleosomes were prepared containing either a site-specific *cis*-diammineplatinum(II)−DNA intrastrand d(GpG) or a d(GpTpG) cross-link. The ability of the histone core to modulate the excision of these defined platinum adducts was investigated as a model for exploring the cellular response to platinum−DNA adducts in chromatin. Comparison of the extent of repair by mammalian cell extracts of free and nucleosomal DNA containing the same platinum−DNA adduct reveals that the nucleosome significantly inhibits nucleotide excision repair. With the GTG-Pt DNA substrate, the nucleosome inhibits excision to about 10% of the level observed with free DNA, whereas with the less efficient GG-Pt DNA substrate the nucleosome inhibited excision to about 30% of the level observed with free DNA. The effects of post-translational modification of histones on excision of platinum damage from nucleosomes were investigated by comparing native and recombinant nucleosomes containing the same intrastrand d(GpTpG) cross-link. Excision from native nucleosomal DNA is ∼2-fold higher than the level observed with recombinant material. This result reveals that post-translational modification of histones can modulate nucleotide excision repair from damaged chromatin. The in vitro system established in this study will facilitate the investigation of platinum−DNA damage by DNA repair processes and help elucidate the role of specific post-translational modification in NER of platinum−DNA adducts at the physiologically relevant nucleosome level.

Cisplatin, *cis*-diamminedichloroplatinum(II), and other platinum-based drugs such as carboplatin, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (Figure 1), are used to treat testicular tumors as well as a variety of other cancers (*1*, *2*). DNA is the principal cellular target of these compounds (*3*), the platinum atom forming covalent bonds to the N7 positions of the purine bases. The major adducts are 1,2-intrastrand and 1,3-intrastrand cross-links (*4*−*6*). Although cisplatin and carboplatin both form these identical bifunctional adducts, the relative frequency of individual platinum−DNA cross-links is quite different. For cisplatin, 1,2-intrastrand adducts comprise 50−90%, and 1,3-intrastrand 10−25%, of all DNA lesions in cultured Chinese hamster ovary (CHO)[1] cells treated with the drug. However, the percentage of 1,2- and 1,3-intranstrand cross-links for carboplatin treatment of the same CHO cells is 35−50% and 30−40%, respectively (*6*). Since carboplatin is widely used in cancer chemotherapy, it is important to study both the 1,2- and 1,3-intrastrand *cis*-diammineplatinum(II) cross-links formed by the two compounds.

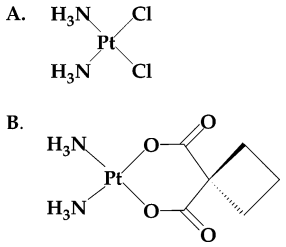

FIGURE 1: Structures of cisplatin and carboplatin. (A) Cisplatin, *cis*-diamminedichloroplatinum(II). (B) Carboplatin, *cis*-diammine-(1,1-cyclobutanedicarboxylato)platinum(II).

The cellular events that are triggered by platinum−DNA damage are the subject of considerable interest, and detailed knowledge of these processes could facilitate the rational design of better platinum-based drugs. For example, understanding how platinum compounds with different ligands block DNA repair and transcription more efficiently may suggest a strategy for designing new drugs. Cells deficient

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[1] Abbreviations: NER, nucleotide excision repair; CHO, Chinese hamster ovary; XPA, xeroderma pigmentosum complementation group A; XPF, xeroderma pigmentosum complementation group F; ERCC, excision repair cross complementing; GTG-Pt, DNA probe containing a site-specific intrastrand cisplatin cross-link at a single d(GpTpG) site; GG-Pt, DNA probe containing a site-specific intrastrand cisplatin cross-link at a single d(GpG) site; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; bp, base pair(s); IE HPLC, ion exchange high-performance liquid chromatography; AAS, atomic absorption spectroscopy; PAGE, polyacrylamide gel electrophoresis; TBE, Tris-borate-EDTA; BSA, bovine serum albumin.

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in DNA repair are hypersensitive to cisplatin, indicating that repair plays an important role in the molecular mechanism of the drug (*7*, *8*).

To date, most studies of platinum−DNA adducts have used platinated DNA substrates, with little work having been done with nucleosomal DNA (*9*−*11*). In eukaryotic cells, however, DNA is packaged into chromatin. The fundamental unit of chromatin structure is the nucleosome, comprising a core particle and linker DNA (*12*). X-ray crystal structure analysis of the nucleosome core particle has revealed how the histone octamer $(H2A, H2B, H3, H4)_2$ is assembled and how the 146 bp DNA duplex is wrapped around the outside of this core to form a shallow superhelix (*13*). Nucleosomes modulate many cellular processes, including DNA recombination, replication, transcription, and repair. (*12*, *14*−*16*). It is therefore of interest to investigate the effects of platinum−DNA adducts at the nucleosome level, which is more physiologically relevant, to achieve a better understanding of the mechanism of action of cisplatin and its analogues.

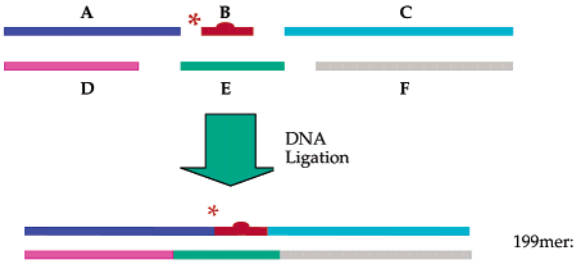
Two major classes of chromatin-modification factors that increase DNA accessibility have recently been identified. The first class, chromatin-remodeling complexes, such as SWI2/SNF2, ISWI, and Mi, alter histone−DNA interactions by utilizing energy released from ATP hydrolysis (*17*). The second class of factors alters DNA−histone interactions through covalent modification of histones, including acetylation, phosphorylation, methylation, and ubiquitination (*18*−*20*).

In the present study, we address how nucleosome structure modulates nucleotide excision repair of platinum−DNA adducts. Since we wished to learn the extent to which nucleosome structure affects the excision of different types of platinum−DNA adducts, it was necessary to prepare substrates containing specific adducts. Accordingly, two site-specifically platinated nucleosomes were prepared, each containing a single 1,2-d(GpG-Pt) or 1,3-d(GpTpG-Pt) intrastrand *cis*-diammineplatinum(II) cross-link. In vitro repair assays using these substrates were carried out to compare the effect of the nucleosome core on the efficiency of platinum removal from DNA. In addition, we studied platinum excision from core particles reconstituted with either native or recombinant histones in order to determine their relative activities as substrates. The results, which are described herein, reveal the first direct evidence linking nucleotide excision repair of platinated mononucleosomes with post-translational modification of histones.

MATERIALS AND METHODS

Materials. Cisplatin was obtained as a gift from Johnson-Matthey. T4 polynucleotide kinase and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA). Phosphoramidites and chemicals for DNA synthesis were obtained from Glen Research. γ-^{32}P-ATP was purchased from ICN Biomedicals.

Synthesis and Characterization of Platinated Oligonucleotides. All oligonucleotides were synthesized on a 1 μmol scale using an Applied Biosystems DNA synthesizer (Model 392) and purified by conventional methods. The platinated oligonucleotide 20-mers, 20GG-Pt or 20GTG-Pt, were prepared as described previously (*21*). In brief, a 179 μL aliquot of cisplatin (0.84 mM) was mixed with a 100 μL portion of the oligonucleotide (1.50 mM) in a 1:1 ratio in



A:83mer; B:20GG-Pt or 20GTG-Pt; C: 96mer; D:72mer; E:40CC or 40CAC; F: 87mer.

Fragment A:
GCTTGACAACAAAAAGATTGTCTTTTCTGACCAGATGGACGCGGCCACCCT
CAAAGGCATCACCGCGGGCCAGGTGAATATCA

Fragment B:
20GG-PT: AATCCTCCTGGTTTTTCCAC
20GTG-PT: AATCCTCCGTGTTTTTCCAC

Fragment C:
GTATTATGAATTCAGCTGCTCGAGCTCAATTAGTCAGACCCCAACAGCTGG
AGAGAACAGCGACCCGCGGGCCCCGGCAGGCGGCTCAAGCAGGAG

Fragment D:
CTGGCCCGCGGTGATGCCTTTGAGGGTGGCCGCGTCCATCTGGTCAGAAAA
GACAATCTTTTTGTTGTCAAG

Fragment E:
40CC: TTCATAATACGTGGAAAAACCAGGAGGATTTGATATTCAC
40CAC: TTCATAATACGTGGAAAAACACGGAGGATTTGATATTCAC

Fragment F:
GCTCCTGCTTGAGCCGCCTGCCGGGGCCCGCGGGTCGCTGTTCTCTCCAGCT
GTTGGGGTCTGACTAATTGAGCTCGAGCAGCTGAA

FIGURE 2: Strategy for synthesizing site-specifically platinated DNA probes. Six synthetic oligonucleotides, one bearing the platinum intrastrand cross-link and a 5′-^{32}P radiolabel denoted by an asterisk, were annealed and ligated as shown. Sequences of the individual strands are depicted.

0.01 M sodium phosphate buffer (pH 6.75). The mixture was incubated in the dark at 37 °C for 12−14 h. The platinated oligonucleotides were purified by ion exchange HPLC and characterized by mass spectrometry, UV spectroscopy, and atomic absorption spectroscopy (AAS). DNA concentrations were determined by UV absorption at 260 nm using a Hewlett-Packard 8453 UV−vis instrument. The concentrations of bound platinum were measured on a Perkin-Elmer Analyst 300 atomic absorption spectrometer equipped with graphite furnace. The ratio of bound platinum to DNA was calculated by dividing the Pt concentration by the DNA concentration. The molecular masses of platinated oligonucleotides were confirmed by mass spectrometry at the MIT Biopolymers Laboratory.

Preparation of Site-Specifically Modified Platinum−DNA Probes. The synthesis of DNA duplexes containing a unique, site-specific platinum−DNA adduct was performed by enzymatic ligation of three sets of complementary oligonucleotides (Figure 2), as previously described (*21*, *22*). In brief, six fragments were annealed and ligated. The ligation products were then separated by denaturing PAGE, reannealed, and purified by nondenaturing PAGE. The resulting DNA probes contain internal labels with ^{32}P at the 10th phosphodiester bond 5′ to the d(GpG) cross-link and at the ninth phosphodiester bond 5′ to the d(GpTpG) cross-link (Figure 2).

Cell-Free Extract Preparation. Cell-free extracts were prepared from CHO AA8 cells by the method described previously (*23*).

Native Histone Octamer Preparation. Native histones H2A, H2B, H3, and H4 from HeLa S3 cells were isolated and purified as reported (*24*, *25*). HeLa cells were grown in suspension with Dulbecco's minimal essential medium supplemented with 10% fetal calf serum at 37 °C. Thereafter, the cells were harvested and used for whole cell extract preparation according to a published procedure (*23*). The remaining nuclear pellets were lysed with a glass homogenizer and washed with 0.4 and 0.6 M NaCl solutions. The washed nuclear pellets were adsorbed onto a dry hydroxylapatite resin. Subsequently, the core histone proteins were eluted with 2.5 M NaCl.

Expression and Purification of Recombinant Histone Proteins. Expression plasmids encoding each of the four histone proteins were transformed into *E. coli* BL21(DE3) cells and expressed under the control of a T7 promoter. The histones were then purified by following literature procedures (*26*). The histone octamer was refolded and the solution was separated by gel filtration on a 16/60 Superdex 200 (Pharmacia) column. The purified histone octamer was analyzed by 18% SDS−PAGE.

Nucleosome Reconstitution and Purification. The radioactively labeled 199 bp DNA probes were assembled into nucleosomes with the histone octamer as described previously (*27*). A 1 pmol quantity of DNA substrate was incubated with the HeLa core histones in a 1:1 molar ratio, 1 μg of BSA, and 2 M NaCl in a final volume of 10 μL for 15 min at 37 °C. The reaction mixture was serially diluted by adding 3.3, 6.7, 5.0, 3.6, 4.7, 6.7, 10, 30, and 20 μL portions of 50 mM HEPES (pH 7.5), 1 mM EDTA, 5 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) at 15 min intervals over a period of 2.5 h incubating at 30 °C. The resulting solution was reduced to 0.1 M in NaCl by adding 100 μL of 10 mM Tris·HCl (pH 7.5), 1 mM EDTA, 0.1% Nonidet P-40, 5 mM DTT, 0.5 mM PMSF, 20% glycerol, and 100 μg/mL of BSA and incubated for 15 min at 30 °C. After reconstitution, the mononucleosomes were purified from free DNA by centrifugation through an 11 mL, 5−25% sucrose gradient in 10 mM HEPES−KOH (pH 7.9)−1 mM EDTA−0.1% Nonidet P-40 using a SW41 rotor (25 000 rpm, 18 h, 4 °C) according to published methods (*28*). Reconstitution products and fractions separated by sucrose gradient were analyzed by nondenaturing polyacrylamide gel electrophoresis (6% polyacrylamide; 1X Tris-borate-EDTA [TBE]) (*29*). Fractions containing mononucleosomes were used for the excision assay, which measures the release of 24 to 32 nt-long oligomers carrying the damage site (*30*, *31*). The recombinant nucleosomes were prepared in the same manner.

Excision Assay. Cell-free extracts from CHO AA8 cells were used to measure excision with the 199 bp DNA substrates in the form of a mononucleosome or free DNA as described elsewhere (*29*). In brief, in a typical excision assay, 1.5−3 fmol of substrate DNA (either free DNA, native nucleosome or recombinant nucleosome) was incubated with 50 μg of cell-free extracts at 30 °C in 25 μL of excision repair buffer (32 mM HEPES−KOH, pH 7.9, 64 mM KCl, 6.4 mM $MgCl_2$, 0.24 mM EDTA, 0.8 mM DTT, 2 mM ATP, 0.2 mg/mL BSA, 5.5% glycerol, 4.8% sucrose). The reaction mixtures were incubated at 30 °C for 2−4 h. The reaction products were purified by phenol-chloroform extraction and analyzed on a denaturing PAGE gel (8% polyacrylamide; 1X TBE). The extent of excision was determined by measuring the levels of radioactivity in the bands of excised products (20−30 nt range) and unexcised substrate with a phosphorimager and ImageQuant system (Molecular Dynamics).

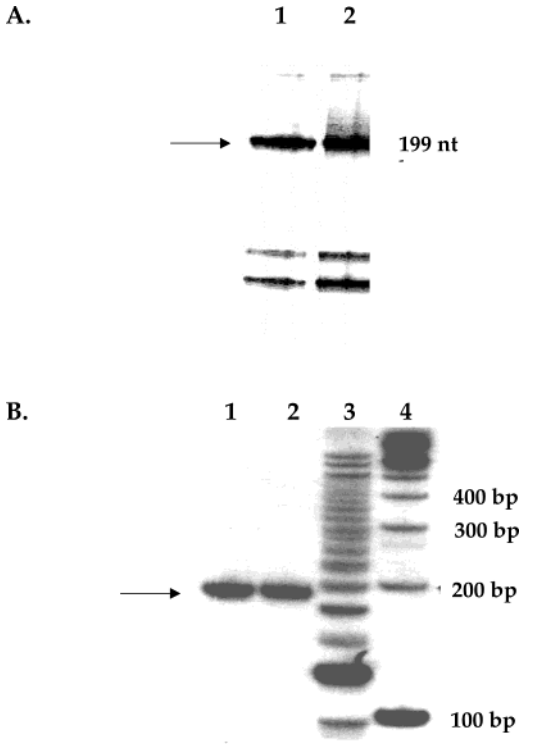


FIGURE 3: (A) Denaturing PAGE gel of ligation products of site-specifically platinated DNA probes. Lane 1, ligation reaction of 199-mers DNA GG-Pt; lane 2, ligation reaction of 199-mer DNA GTG-Pt. The 199-mers are shown by the arrow. (B) Nondenaturing PAGE gel of purified site-specifically platinated ds-DNA probes. Lane 1, ds-199-mer DNA GG-Pt; lane 2, ds-199-mer DNA GTG-Pt; lane 3, 25 bp DNA ladder; lane 4, 100 bp DNA ladder.

RESULTS

Synthesis and Purification of DNA 199GG-Pt and 199GTG-Pt Probes. The oligonucleotides were prepared and purified by PAGE and the purity was confirmed by HPLC and mass spectrometry. The platinated oligonucleotides (GG-Pt and GTG-Pt) were characterized by UV−vis spectroscopy, AAS (*32*), and mass spectrometry. The 199-mer was synthesized by modification of a previously described strategy (Figure 2) (*22*). The ligation products were then purified by PAGE (Figure 3), and the bands corresponding to the 199-mer (Figure 3A) were excised and eluted. The double-stranded 199-mer (ds-199-mer) was purified by nondenaturing PAGE (Figure 3B). The total yield of ds-199-mer ranged from 1% to 5% based on starting material.

Reconstitution and Purification of Nucleosomes. The recombinant histone octamer was refolded and purified. The SDS−PAGE gel analysis revealed that the individual recombinant histones and recombinant histone octamer are >95% pure (Figure 4). The internally ^{32}P-labeled DNA probes and octamer were reconstituted into nucleosomes with native or recombinant histones. The reconstituted nucleosomes were separated from free DNA by sucrose gradient centrifugation. Analysis on a nondenaturing gel (Figure 5) revealed that the nucleosome band migrates more slowly than

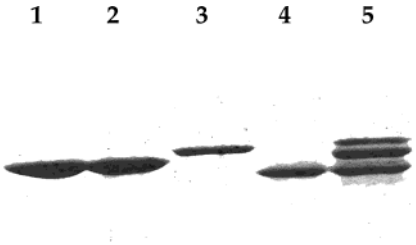


FIGURE 4: SDS−PAGE gel of recombinant histones and histone octamer. Lane 1, H2A; lane 2, H2B; lane 3, H3; lane 4, H4; lane 5, octamer.

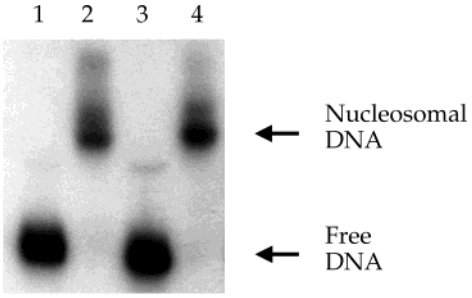


FIGURE 5: Nondenaturing PAGE gel of nucleosome and free DNA. Lane 1, free 199GG-Pt DNA; lane 2, nucleosomal 199GG-Pt DNA; lane 3, free 199GTG-Pt DNA; lane 4, nucleosomal 199GTG-Pt DNA.

its DNA component, in agreement with previous work (*29*). The GTG-Pt and GG-Pt modifications did not significantly alter the migration of either free or nucleosomal DNA in the gel. The presence of a sharp band in lanes 2 and 4 (Figure 5) indicates good substrate positioning of the nucleosomal DNA wrapped around the histone octamer.

Excision Assay on Nucleosomal DNA. The nucleosomal DNAs were used as substrates for in vitro nucleotide excision repair under the same conditions as for free DNA. The reaction products were analyzed by 8% denaturing PAGE (Figure 6A). Comparison of repair signals for free and native nucleosomal DNA containing the same cisplatin adduct clearly indicates that the nucleosome inhibits excision under our assay conditions. For the free GTG-Pt DNA substrates, the excision is about 10% ± 2% (averaged results from 4 experiments) of total DNA, whereas for the native nucleosomal GTG-Pt DNA, the excision is only 1.2% ± 0.4% (averaged results from 3 experiments). Upon changing from free GG-Pt DNA to native nucleosomal GG-Pt DNA, the excision signal decreases from 1.1% ± 0.3% (averaged results from 4 experiments) to 0.35% ± 0.14% (averaged results from 3 experiments) of total DNA. Thus, the efficiency of excision repair of nucleosomal DNA GG-Pt is about 33% of free DNA GG-Pt, whereas the efficiency of excision of nucleosomal DNA GTG-Pt is about 12% that of free DNA GTG-Pt (Figure 6B).

To investigate the role of histone post-translational modification in modulating NER of platinum−DNA adducts at the mononucleosome level, native histone octamers composed of post-translationally modified histones and recombinant histone octamers composed of nonmodified histones were prepared and purified. In vitro nucleotide excision repair of the native and recombinant nucleosomal DNA probes were carried out under the same conditions. The reaction products were analyzed by 8% denaturing PAGE (Figure 7). Comparison of excision signals for native and recombinant nucleosomal DNA containing the same cisplatin adduct clearly indicates that the efficiency of NER of native nucleosomal DNA GTG-Pt is 2.5 ± 0.4-fold higher than that of recombinant nucleosomal DNA GTG-Pt. Similar stimulation of NER (2.2 ± 0.4-fold) was also observed from native nucleosomal GG-Pt, compared to that of recombinant nucleosome GG-Pt (data not shown).

DISCUSSION

In this work we prepared site-specifically platinated mononucleosomes and compared the relative excision of platinum damage from free versus nucleosomal DNA, and from nucleosomes reconstituted with native versus recombinant histone core proteins.

Comparison of NER from Free versus Nucleosomal DNA. This study reports the first synthesis and characterization of

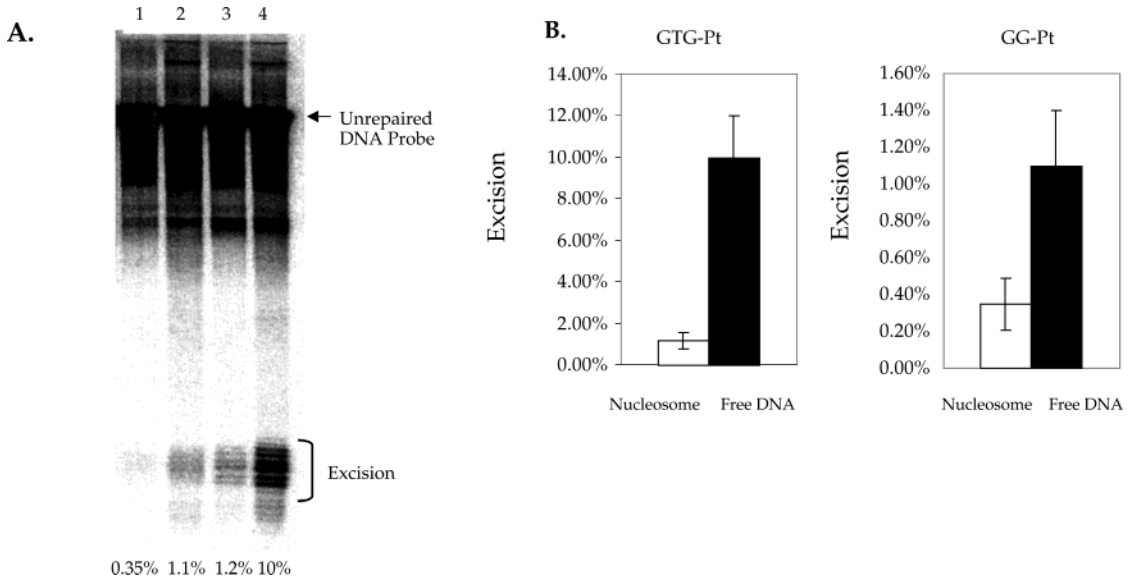


FIGURE 6: (A) Excision assay with nucleosomal and free DNA. Lane 1, nucleosomal 199GG-Pt DNA; lane 2, free 199GG-Pt DNA; lane 3, nucleosomal 199GTG-Pt DNA; lane 4, free 199GTG-Pt DNA. The numbers the bottom of each lane are the excision percentages of the repair reactions. (B) Efficiency of excision of cisplatin lesions from nucleosomal DNA. The efficiency of excision of nucleosomal DNA GTG-Pt is about 12% of free DNA GTG-Pt, whereas the efficiency of nucleosomal GG-Pt is about 33% of free DNA GG-Pt.

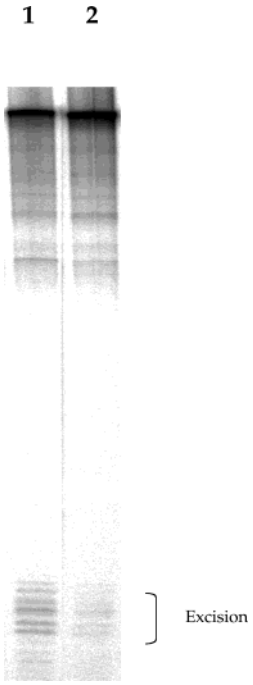


FIGURE 7: Analysis of repair of native and recombinant nucleosomal platinated DNA by the excision assay. Lane 1, native nucleosomal 199GTG-Pt DNA; lane 2, recombinant nucleosomal 199GTG-Pt DNA. The efficiency of excision from native nucleosomal DNA was 5.3% (lane 1) and from nucleosomes reconstituted from recombinant histones was 2.2% (lane 2). The absolute percent efficiency in lane 1 cannot be compared with that in lane 3 of Figure 6 because of different incubation times, buffer conditions, and batch of cell extract.

mononucleosomes containing site-specific cis-$\{Pt(NH_3)_2\}^{2+}$ d(GpG) or d(GpTpG) intrastrand cross-links. With the use of these substrates, we found the excision of damage from nucleosomal DNA to be significantly less than that from free DNA. This observation reveals that nucleosomes protect platinum−DNA adducts from being repaired. There are several possible reasons for this result. First, core histone−DNA interactions reduce the association constants for damaged DNA-binding proteins of the repair complex by 10-fold (*25*, *29*). Second, the site of damage might be shielded by the histone octamer surface or histone protein tails, thus limiting access of the repair machinery. Third, the nucleosome significantly alters DNA structure. Nucleosomal DNA is overwound by about 0.3 bp per turn compared to free DNA (*13*). Finally, the distortion caused by the cisplatin adducts on nucleosomal DNA might differ from that produced by the same adduct on free DNA, thus lowering the overall recognition efficiency of damage sensor proteins. Taken together, these effects can limit the ability of NER proteins to access or recognize the platinum cross-link owing to a modulation of nucleosome structure. This result is consistent with the previous studies of UV- or AAF-damaged nucleosomes (*25*, *29*, *33*, *34*).

Our results are also in accord with the observation that repair in actively transcribed genes is more rapid and efficient than in nontranscribed regions of the genome (*35*). We suggest that one possible factor contributing to this phenomenon of transcription-coupled repair is that, in the transcribed gene, when damage is in the template strand, DNA is more exposed compared to its tight packaging in nucleosomes comprising chromatin of inactive genes (*36*).

The degree of nucleosome protection differs for the GG-Pt and GTG-Pt cross-links. This difference probably reflects the degree of distortion of GG-Pt and GTG-Pt adducts. Previous structural studies reveal that DNAs containing GG-Pt and GTG-Pt lesions are strikingly different (*2*, *37*). Gel electrophoresis experiments indicate that the 1,2-d(GpG) cisplatin adduct bends the DNA helix by 32−34° toward the major groove of DNA and unwinds the DNA helix by 13°, whereas the 1,3-d(GpTpG) adduct bends by 35° and unwinds by 23° the DNA helix (*38*, *39*). Further confirmation is provided by X-ray (*40*, *41*) and NMR structural studies. The NMR structure of a single 1,3-d(GpTpG) cisplatin adduct shows it to be more distorted than the structure of DNA containing a single 1,2-d(GpG) cross-link (*42*−*45*). In the 1,3-adduct, the central T is extruded into the minor groove, and base pairing is lost at the 5′ G and central T/A base pair (*45*). These significantly different structural distortions between 1,2- and 1,3-intrastrand cross-links are already known to produce differential recognition and processing by cellular proteins (*46*). The nucleosome modulation adds another level of complexity, the detailed nature of which must await the availability of structural information on site-specifically platinated nucleosomes.

Comparison of NER from Nucleosomes with Native versus Recombinant Histones. Repair synthesis of UV-damaged DNA is enhanced within nucleosome cores of hyperacetylated chromatin in butyrate-treated human cells (*47*). The origin of enhanced repair synthesis was not identified, however, and could have arisen from a greater population of minor UV photoproducts, such as pyrimidine−pyrimidone (6−4) dimers, in the highly acetylated nucleosomes. The substrates employed in this study were globally modified and then digested to mononucleosomes. Since the DNA sequence differs for each mononucleosome, the types, the amount, and the locations of UV damage are also variable. Thus, it is difficult to evaluate the effects of post-translational modification on a specific type of DNA damage or location within the nucleosome.

The present study, which reports the first reconstituted, site-specifically platinated mononucleosome containing either recombinant or native, and therefore post-translationally modified, histones, provides a powerful tool to investigate these above questions. In our biochemically well-defined system, the excision efficiency of the native nucleosomes is significantly greater than that of recombinant nucleosomes, revealing that overall post-translational modification of histones stimulates NER in this context.

There are at least three possible reasons for this effect. First, histone modification changes the nucleosome structure and surface environment, and thus can improve the accessibility of nucleosomal DNA to the repair apparatus. Acetylation occurs on lysine residues at the basic, N-terminal tail domains of the core histones. One consequence of acetylated N-terminal tails is a reduced affinity for DNA owing to charge neutralization, thus destabilizing chromatin structure (for reviews, see refs *48* and *49*).

Second, the histone modification can recruit remodeling factors. Transient histone hyperacetylation acts as a signal for ATP-dependent remodeling complexes at the PHO8 promoter in vivo (*50*). Moreover, acetylation of histone H4 K8 mediates recruitment of the SWI/SNF complex to DNA

(*51*), and it was recently reported that SWI/SNF can stimulate excision repair of human excision nuclease in the mononucleosome core particle, made with post-translationally modified histones, by increasing DNA accessibility (*25*). Given these findings, we conclude that histone covalent modifications, nucleosome remodeling and nucleotide excision repair are linked processes.

Third, histone modification also enlists proteins involved in NER. Access of DNA repair machinery to UV lesions within chromatin is facilitated by the TBP-free-TAF_{II} complex (TFTC) via covalent modification of chromatin. A subunit of TFTC (SAP130) shares homology with the large subunits of UV-damaged DNA binding factor (DDB). TFTC is recruited to UV damaged DNA in parallel with the nucleotide excision repair protein XPA. The fact that TFTC can recognize UV-damaged DNA and preferentially acetylate nucleosomes assembled on UV-irradiated DNA suggests a possible role for TFTC in making the DNA damage accessible for repair in the context of chromatin (*52*). In addition, CREB binding protein (CBP) and p300 histone acetyl-transferases can interact with the small subunit of XPE damage-specific DNA binding protein (DDB) (*53*). Taken together, these findings may indicate that selective acetylation of histones on damaged nucleosomes may provide a general strategy for recruiting NER factors more efficiently to overcome the nucleosome barrier to excision repair. These three possibilities are not mutually exclusive. Our results are consistent with the previous studies of the function of histone post-translational modification and ability to stimulate transcription (*54*−*56*). It is likely that cells utilize post-translational modifications as a signal or factor, through some common mechanism, to regulate NER and transcription on chromatin.

The present experiments were carried out at the mononucleosome level in vitro. Histone modifications can significantly alter higher order nucleosome packing, however, and thus change the accessibility of nucleosome to NER (for reviews, see refs *18* and *19*). We suggest that post-translational modification of histones will have an important effect on NER in vivo, as has already been demonstrated for transcription regulation (*54*−*56*).

Conclusion. With the use of site-specifically platinated DNA in mononucleosomes, we determined that nucleotide excision repair in mammalian cell extracts is substantially diminished compared with free DNA containing the same adducts. A comparison of the extent of repair of native and recombinant nucleosome substrates revealed excision from native nucleosomal DNA to be about 2-fold higher than the level observed with recombinant, unmodified nucleosomal DNA. This result indicates that post-translational modification of histones can play a key role in modulating nucleotide excision repair of platinum−DNA adducts in chromatin. The relative effect of the nucleosome does not depend on the nature of the adduct. The in vitro system we established in this study will facilitate investigation of other cellular processing of platinum−DNA damage and help to elucidate the role of specific post-translational modifications in NER of platinum−DNA adducts at the nucleosome level.

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