The Inhibitory Effect of HMGB-1 Protein on the Repair of Cisplatin-Damaged DNA Is Accomplished through the Acidic Domain†

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ABSTRACT: The well established inhibitory effect of HMGB-1 on repair of cisplatin-damaged DNA has been studied with two modified forms of the protein, shown to bind platinated DNA with higher affinity than the original protein: in vivo acetylated HMGB-1 and HMGB-1 lacking its C-terminal domain. The native and the modified proteins were assayed for their effects on adduct removal by using cell-free extract capable of repairing cisplatinated DNA in vitro. The inhibition observed with the native HMGB-1 was reduced in the presence of acetylated HMGB-1 and completely abolished when the assay was carried out with the truncated protein. When the repair assay was performed in the presence of a synthetic polypeptide identical to the C-terminal tail, either alone or together with the truncated protein, the inhibitory effect was partially recovered in a concentration-dependent manner. These findings strongly suggest that the HMGB-1-induced inhibition of cisplatin−DNA adduct repair is accomplished through the acidic domain. The results obtained are discussed in terms of the repair events that may occur in the presence of HMGB-1 protein.

The antitumor activity of cisplatin [*cis*-diamminedichloroplatinum(II)] is well established and is now widely used for treatment of many malignancies (*1*). The cytotoxicity of the drug is believed to result from its interaction with DNA via formation of DNA adducts, the major ones being 1,2-d(GpG) and d(ApG) intrastrand cross-links (*2*, *3*). These adducts produce local unwinding and kinking of the helix (*4*, *5*) which affect DNA replication and transcription (*1*, *6*−*8*). The structural distortions in DNA are recognized by a variety of cellular proteins (*9*), including the chromosomal high mobility group box 1 protein (HMGB-1).[1] These proteins, reported to form a stable platinum−DNA−protein ternary complex (*10*), were implicated in modulating the antitumor activity of the drug (*11*). HMGB-1 and other HMG domain proteins were shown to inhibit removal of intrastrand DNA cross-links when added to in vitro repair assay (*12*−*14*). Furthermore, steroid hormone-induced overexpression of HMGB-1 in human cancer cells sensitized the cells to cisplatin and carboplatin (*15*). On the contrary, immunodepletion of HMGB-1 from cell extracts enhanced in vitro repair of damaged DNA (*16*). A similar result was observed in vivo upon deletion of the yeast HMG domain protein Ixr1 (*17*). The potential of HMGB-1 to affect the cytotoxicity of cisplatin was attributed to shielding the lesions in DNA from repair (*11*). If HMGB-1 does protect adducts from repair, any increase of its binding affinity to cisplatin-damaged DNA should enhance the inhibitory effect of the protein on adduct removal and will improve the antitumor activity of the drug. Such a prediction (*10*, *12*) agrees with the observation that the binding affinities of HMGB-1 to DNA modified by various cisplatin derivatives were found to correlate with their antitumor activity (*18*) and was further supported by a recent study with two enantiomeric analogues of cisplatin (*19*). Adducts of these analogues bound HMGB-1 with different affinity (*19*), due to considerable differences in the bending they induce in DNA (*20*). The cross-links formed by both compounds are removed during in vitro nucleotide excision repair with a similar efficiency. The presence of HMGB-1, however, inhibited the repair process, and this effect was much stronger to adducts with higher affinity to the protein.

In this work, the inhibition of cisplatin−DNA adduct removal by HMGB-1 was investigated by using two modified forms of the protein, shown to bind cisplatinated DNA with a higher affinity than the original protein: truncated HMGB-1 (trHMGB-1) lacking its C-terminal tail, and in vivo acetylated HMGB-1 (*21*). The inhibitory effect observed with the native protein was reduced in the presence of its acetylated form and completely abolished when the assay was performed with the truncated protein. The latter finding indicates that HMGB-1-induced inhibition of repair of cisplatinated DNA is accomplished through the acidic domain.

EXPERIMENTAL PROCEDURES

Preparation of Proteins. Nuclei from Guerin ascites tumor cells were used to isolate and purify HMGB-1 and -2 proteins under non-denaturing conditions as described elsewhere (*22*). The acidic C-terminal domain was removed by mild digestion with trypsin (Sigma, TPCK-treated, see ref *23*), and purified (*24*). The purity of the final preparations of HMGB-1 and -2 and their truncated forms lacking the acidic tail was

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[1] Abbreviations: EMSA, electrophoretic mobility shift assay; CFE, cell-free extract; HMGB-1 and -2, high mobility group box proteins 1 and 2; HMGB-1ac, acetylated HMGB-1; trHMGB-1, truncated HMGB-1, lacking the C-terminal tail.

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controlled by SDS−polyacrylamide gel electrophoresis. In vivo acetylated HMGB-1 was isolated and purified from Guerin ascites tumor cells grown in the presence of butyrate as described (*21*). The peptide NH_2-DEEDEEDEEEEEEEED-EDEEEDDDDE-OH, representing the last 26 amino acids of the mouse HMGB-1 C-terminal tail, was purchased from Biosynthesis Inc.

DNA Probes. Plasmids pUC19 (2.69kb) and pGEX2T (4.3kb) were propagated in *Escherichia coli* and extensively purified as closed circular DNA by using FlexiPrep Kit (Pharmacia Biotech). Platination of plasmid DNA (200 μg/mL) with cisplatin (drug/nucleotide molar ratio 50:1) was carried out in TE buffer (10 mM Tris, 1 mM EDTA), pH 7.4, in the dark at 37 °C for 16 h. Following ethanol precipitation, DNA was washed twice in 70% ethanol and redissolved in TE buffer, and the superhelical form of the plasmid DNA was purified by ethidium bromide/cesium chloride gradient centrifugation (*25*). Levels of platinum modification were determined by electrothermal atomic absorption spectroscopy carried out on a Perkin-Elmer Zeeman 3030 spectrometer equipped with HGA-600 graphite furnace. The light source used was a hollow cathode lamp for Pt, and the spectral band-pass and the wavelength used were as recommended by Perkin-Elmer. Pyrolitically coated graphite tubes were used as atomizers. Sample solutions (20 μL) were introduced into the graphite furnace using an autosampler. Cisplatin-treated pUC19 contained 20 platinum atoms bound per 1000 nucleotides.

Preparation of Cell-Free Extract. Cell-free extract (CFE) from exponentially growing Guerin ascites tumor cells was prepared using a described protocol (*26*), adapted for in vitro DNA repair studies (*27*) and stored at −80 °C until use. XPA lymphoblastoid cell line was obtained from NIGMS Human Genetic Cell Depository. Cultures were grown in suspension in RPMI1640 medium supplemented with 15% fetal calf serum and antibiotics.

In Vitro DNA Repair Synthesis. Repair of DNA lesions induced by cisplatin was assayed as described (*28*). Briefly, the standard 50 μL reaction mixture contained 400 ng of cisplatin-treated repair substrate pUC19, 400 ng of nondamaged pGEX2T internal control, 45mM HEPES-KOH, pH 7.8, 70 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dTTP, dGTP, dATP, 2μCi [α-^{32}P]dCTP (Amersham, 3000Ci/mmol), 40 mM phosphocreatine, 2.5 μg of creatine phosphokinase, 3% glycerol, 20 μg of bovine serum albumin, and 80−120 μg of cell-free extract at 30 °C for 1 h. Reactions were stopped by addition of EDTA to 20 mM, and mixtures were incubated for 20 min with RNase A (80 μg/mL) followed by another 20 min with proteinase K (200 μg/mL) in the presence of 0.5% SDS. Plasmid DNA was purified with phenol−chloroform (1:1) and precipitated with 2 vol of ethanol in the presence of glycogen (Stratagene, 1 mg/mL) at −70 °C. The plasmids were linearized by digestion with *Eco*RI and resolved by electrophoresis in 0.8% agarose gel containing ethidium bromide (0.5 μg/mL). After electrophoresis, the gel was photographed under UV illumination, dried under vacuum. and exposed to Kodak XAR-5 film for 12 h at −70 °C. The autoradiograph was scanned with Gel-Pro Analyser.

Electrophoretic Mobility Shift Assay (EMSA). DNA-binding assay of HMGB-1 and its truncated form with ^{32}P-labeled cisplatinated DNA was performed as described (*22*).

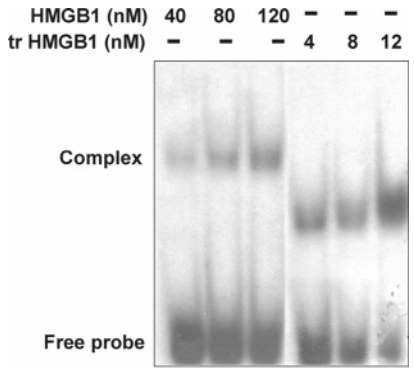

FIGURE 1: Comparative study of the binding affinity of HMGB1 and truncated HMGB1 lacking the C-terminal tail (trHMGB1) to cisplatin-treated DNA by EMSA.

Nonlabeled sonicated salmon sperm DNA was added as a competitor in all experiments except those designed to determine the dissociation constants. On completion of electrophoresis the gel was dried and exposed to Amersham hyperfilm. Quantification of band densities was performed by scanning the autoradiographs with Gel-Pro Analyser. In some assays, the reaction mixture prepared for EMSA was supplemented with CFE preincubated with nonplatinated DNA (500-fold molar excess over the platinated probe) and loaded on the gel.

Determination of the Binding Affinity of HMGB-1 to DNA. Dissociation constants for binding of HMGB-1 and its truncated form lacking the C-terminal tail to cisplatin-treated DNA were determined by evaluating the relative band intensities from EMSA as described (*22*).

RESULTS

The Binding Affinity of HMGB-1 to Cisplatin-Damaged DNA Is Strongly Enhanced upon Removal of the Acidic Tail. Down-regulation of DNA binding affinity of HMGB-1 by the acidic C-terminal domain has been well demonstrated with different DNA substrates but not with cisplatin-damaged DNA. Binding of truncated and full-length protein to cisplatin−DNA adducts was compared by gel retardation assay. As Figure 1 shows, 10 times lower concentrations of trHMGB-1 shifted more DNA than the full-length protein. By quantifying the gel shift assay, binding curves were built (not shown) and used to determine the dissociation constants. The values obtained, 1.17 ± 0.1 μM and 0.09 ± 0.01 μM, respectively, showed that trHMGB-1 lacking the acidic tail binds cisplatin-modified DNA with more than an order of magnitude higher affinity than the full-length protein. Similar data were obtained when the original incubation mixture prepared for EMSA was supplemented with CFE and a 500-fold excess of nonplatinated DNA over the platinated probe. This experiment shows that trHMGB-1 protein still binds with a higher affinity to cisplatinated DNA than does the full-length protein in CFE employed in repair experiments (not shown).

The Inhibitory Effect of HMGB1 on Repair of Cisplatin-Damaged DNA by Cell Free Extracts Is Reduced upon Acetylation of the Protein and Completely Alleviated upon Tail Removal. Conditions for repair synthesis of cisplatin-damaged DNA by CFE were tested in a separate experiment. Cisplatin-treated pUC19 and nondamaged pGEX2T serving as an internal control were incubated together in a standard

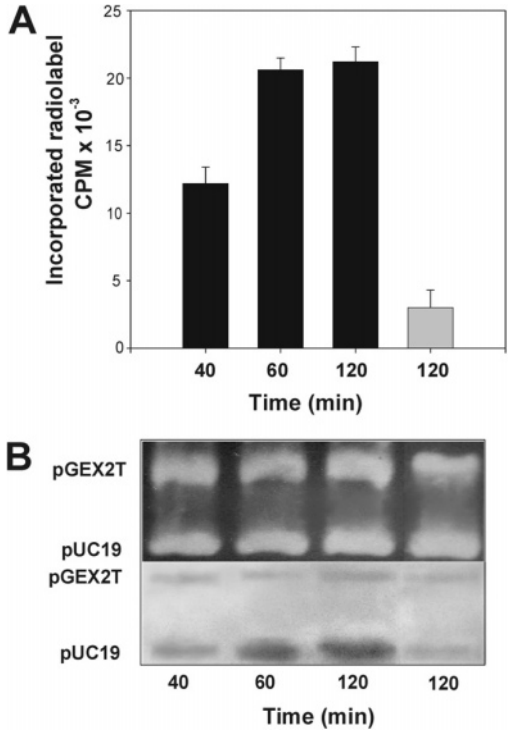

FIGURE 2: Repair synthesis assay for cisplatin-treated DNA by CFE. Four hundred nanograms each of pGEX2T (nondamaged DNA) and pUC19 (cisplatin-treated) were incubated together under standard reaction conditions for 40, 60, and 120 min. Following purification, the plasmid DNA was counted (panel A, black columns), linearized with *Eco*RI, separated on an agarose gel, and visualized by ethidium bromide staining (panel B, upper part) and by autoradiography (panel B, bottom part). Label incorporation in the presence of CFE from repair-deficient XPA lymphoblastoid cell line is shown in panel A (gray column) and panel B (the last lane).

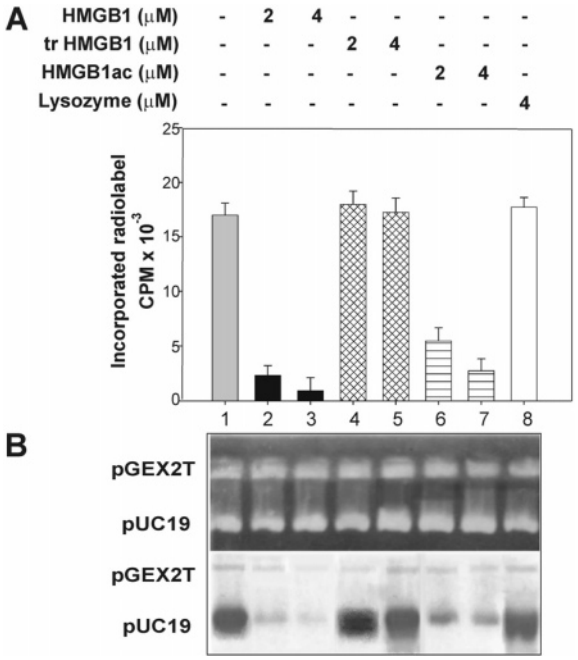

FIGURE 3: Effect of HMGB-1, truncated HMGB-1 lacking its acidic tail, and in vivo acetylated HMGB-1 on repair synthesis of cisplatin-treated DNA by CFE. The plasmids pGEX2T (nondamaged) and pUC19 (cisplatin-treated) were incubated together for 60 min in a standard assay as well as upon addition of one of the following proteins: HMGB-1, truncated HMGB-1 (trHMGB-1), acetylated HMGB-1 (HMGB-1ac), and lysozyme. Upon completion of the reaction, plasmid DNA was purified and counted (panel A). DNA was then linearized with *Eco*RI, separated by agarose gel electrophoresis, and visualized by ethidium bromide staining (panel B, upper part) and by autoradiography (panel B, bottom part).

reaction assay containing repair competent CFE for various times. Following extraction with phenol−chloroform, DNA was precipitated with ethanol and counted. After linearization with *Eco*RI, DNA was run on an agarose gel and visualized by staining with ethidium bromide and by autoradiography (Figure 2). As seen, the maximum of label incorporation into plasmid DNA is achieved after the first hour of incubation (Figure 2, panel A). The autoradiography of the two linearized plasmids reveals that the label is incorporated entirely into the cisplatin-treated pUC19 (Figure 2, panel B, bottom part, pUC19). A control staining of the gel with ethidium bromide demonstrates that the loaded DNA contained the two plasmids in comparable amounts (Figure 2, panel B, upper part, compare pGEX2T and pUC19). The same standard reaction assay was performed by using CFE from repair-deficient XPA lymphoblast cells. The autoradiography showed no label incorporation in both linearized plasmids (Figure 2, panel A, the gray column, and panel B, the last lane).

The effect of HMGB-1 protein on repair synthesis of cisplatin-treated pUC19 was investigated with three protein preparations: full-length HMGB-1, truncated HMGB-1 lacking its acidic tail, and in vivo acetylated HMGB-1, isolated from cells grown in the presence of butyrate. Repair synthesis obtained with these proteins (each one in two concentrations) was compared to that registered with two control experiments: in the absence of HMGB-1 and in the presence of lysozyme (Figure 3). We find that (i) HMGB-1 inhibits the repair synthesis of cisplatin-treated DNA (Figure 3, panel A, compare column 1 with columns 2 and 3) thus confirming previously reported data (*12*−*14*); in our experiments, label incorporation into the damaged DNA in the presence of 4 μM HMGB-1 was inhibited by 95% relative to the control assays. This result is further proved by the autoradiography of the two linearized plasmids after electrophoresis (Figure 3, panel B, bottom part, pUC19, compare the first three lanes); (ii) HMGB-1 lacking its acidic tail has no effect on repair of cisplatinated DNA (Figure 3, panel A, compare column 1 with columns 4 and 5): the level of label incorporation did not differ from that registered in the controls (columns 1 and 8). The autoradiography of the two linearized plasmids confirms these observations (Figure 3, panel B, bottom part, pUC19, compare lane 1 with lanes 4 and 5); (iii) the inhibitory effect of acetylated HMGB-1 was 2−3 times weaker than that observed with the parental molecule, depending on the concentrations used. This is well demonstrated both by label incorporation counting (Figure 3, panel A, compare column 1 with columns 6 and 7) and by the autoradiography of the linearized plasmids (Figure 3, panel B, bottom part, pUC19, compare lane 1 with lanes 6 and 7). In all experiments the amount of DNA loaded for electrophoresis was controlled by staining with ethidium bromide (Figure 3, panel B, upper part).

The same results were obtained with full-length and truncated HMGB-2 (not shown).

Repair Synthesis of Cisplatin-Damaged DNA by CFE Is Inhibited in the Presence of a Synthetic Peptide, Corresponding to the Acidic Tail. The effect of the acidic

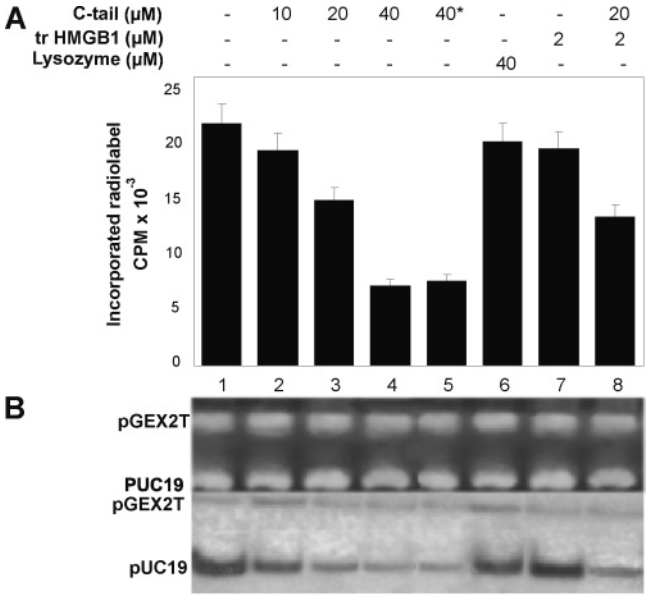

FIGURE 4: Repair of cisplatin-treated DNA by CFE in the presence of a synthetic peptide corresponding to the C-terminal tail of HMGB-1. The plasmids pGEX2T (nondamaged) and pUC19 (cisplatin-treated) were incubated together for 60 min in a standard assay in the presence of the synthetic peptide, either alone or mixed with trHMGB-1. (A) Incorporation of the radiolabel into the plasmids. The asterisk marks an experiment in which a native tail of HMGB-2 substitutes for the synthetic peptide (40*). (B) Agarose gel electrophoresis of linearized plasmid DNA and subsequent visualization by ethidium bromide staining (upper part) and by autoradiography (bottom part).

C-terminal domain of HMGB-1 on repair of cisplatin-damaged DNA was further investigated by supplementing the standard repair assay with a synthetic peptide corresponding to the acidic tail of HMGB-1 either alone or together with the truncated protein (Figure 4). In the first case, a concentration-dependent reduction of label incorporation into the cisplatin-treated DNA was observed (Figure 4, compare columns 2−4 with the control columns 1 and 6; panel B, bottom part, compare lanes 2−4 with lanes 1 and 6). In the second case, addition of trHMGB-1 did not change the effect of the tail at the selected concentration (Figure 4, panel A, compare columns 3 and 8; panel B, bottom part, compare lanes 3 and 8; the effect of trHMGB-1 alone is also shown, see column 7). Similar behavior demonstrated the native tail of HMGB-2 (Figure 4, compare lanes 4 and 5 of both panel A and panel B, bottom part). Again, the DNA samples loaded on the gel are in comparable amounts as judged by the ethidium bromide staining (Figure 4, panel B, upper part).

DISCUSSION

After the demonstration that HMGB-1 binds specifically to cisplatin-damaged DNA (*29*, *30*), the protein was implicated in the antitumor effect of the drug. Regarded initially as a signal for damage recognition that promotes repair, it was soon reported that HMGB-1 protein inhibits removal of DNA adducts probably by shielding them from repair (*11*). Moreover, it was reasonably predicted that "platinum complexes forming DNA adducts with higher affinity for HMG domain proteins would be better shielded from excision repair and thus better antitumor agents" (*12*). This was confirmed by a recent study with two bifunctional enantiomeric cisplatin analogues forming 1,2 intrastrand DNA cross-links with different affinity to HMGB-1 (*19*). While the cross-links of both compounds were removed by nucleotide excision repair with a similar efficiency, the presence of HMGB-1 in repair reaction inhibited removal of the adduct with a high affinity to HMGB-1 and had no effect on the lesion with low affinity to the protein.

In this work, we used an alternative approach: the stronger binding of HMGB-1 to cisplatinated DNA was achieved by manipulating the protein molecule instead of the target DNA adduct. Previous studies have demonstrated that removal of the acidic tail enhances the affinity of HMG box in vitro to most DNA substrates such as linear DNA, four-way junctions, and UV-irradiated DNA (*22*, *23*, *31*, *32*). Here, we show that the affinity of the truncated HMGB-1 to cisplatinated DNA is at least an order of magnitude higher than the affinity of the full-length protein. Furthermore, we recently demonstrated that the affinity of HMGB-1 to the same DNA was enhanced about 6 times upon in vivo acetylation (*21*). Surprisingly, the expected increase of the inhibitory effect of both modified HMGB-1 proteins as a result from eventual more efficient shielding was not observed. On the contrary, acetylated HMGB-1 was a weaker inhibitor of adduct repair than the parental protein, while the truncated HMGB-1 was without any effect, i.e., the inhibitory effect was completely alleviated. It is worth mentioning that the difference in the binding affinities of various HMGB-1 preparations was preserved in the presence of CFE employed in the repair experiments. When the repair assay was performed in the presence of a synthetic polypeptide identical to the C-terminal tail, either alone or together with the trHMGB-1, the inhibition of repair was partially recovered in a concentration-dependent manner: 40 μM peptide inhibited repair by ~60%. In other words, full-length HMGB-1 inhibits repair synthesis more efficiently than the acidic tail when used in comparable molar concentrations (compare Figure 3, panel A, the first three columns with Figure 4, panel A, the first four columns). We attribute this finding to the difference in the local concentrations of HMGB-1-associated acidic tail and the "free" synthetic tail at the site of the lesion. Recognition of the cisplatin−DNA adducts by the HMG box of full-length protein recruits the tail to the lesion and creates a local tail concentration which is higher than that of the tail when free in solution. When the acidic tail was added to the assay together with trHMGB-1, the inhibitory effect was similar to that registered with the tail alone, thus suggesting that the tail is the major player in inhibiting the repair of cisplatin−DNA adducts. The same results were obtained when the native acidic tail of HMGB-2, prepared by a tryptic cleavage of the protein, was used in the repair assay to substitute for the synthetic peptide, corresponding to the tail of HMGB-1. These data, together with the finding that HMGB-2 had the same inhibiting capacity as HMGB-1, favor a nonspecific mechanism of repair inhibition most probably due to the highly negative charge of the peptide. Such a conclusion agrees with the general observation that HMGB proteins may interact with many diverse protein sites (*33*).

The main findings in this report concern the well-known inhibitory effect of HMGB-1 on repair of cisplatinated DNA. The observation that this effect is alleviated upon removal of the C-terminal domain of the protein demonstrates that

inhibition is accomplished via the acidic tail. Repair experiments employing a synthetic peptide corresponding to the acidic tail support such a conclusion. For the present, we cannot propose a definite explanation of these findings. One possibility suggests the inhibition of adduct removal by HMGB-1 to result from an interaction of its acidic domain with a protein(s) of the repair machinery, reported to consist of at least 30 different components (*34*, *35*). An example of acidic tail-driven interactions of HMGB-1 with other nuclear proteins is the binding of HMGB-1 to the TATA-binding protein (TBP), a universal transcription factor that is essential for eukaryotic transcription by all three RNA polymerases (*36*). The formation of HMGB-1/TBP/TATA complex is proposed to inhibit the assembly of the preinitiation complex and, respectively, transcription from RNA polymerase (*37*). The operation of protein−protein interactions during excision repair of cisplatin-damaged DNA in the presence of HMGB-1 cannot be ruled out. Moreover, the evidence that TATA-binding protein interacts with cisplatin-modified DNA and specifically shields 1,2-d(GpG) intrastrand cross-links from repair supports the possibility that, besides a role in transcription, the HMGB-1/TBP complex may participate in cisplatin adduct repair (*38*). Furthermore, a recent report demonstrates that the domains that are essential for the formation of a stable HMGB-1/TBP complex are the acidic tail of HMGB1 and the N-terminus of TBP (*39*).

As for the finding that acetylation of HMGB-1 at Lys2 reduces the inhibitory effect of the parental molecule, the mechanism is not clear. Considering the consequences for the properties of HMGB-1, acetylation mimics tail removal in two aspects: enhancement of the binding affinity to cisplatin-damaged DNA and reduction (yet without alleviation) of the inhibitory effect of HMGB-1 on repair of damaged DNA. One may assume, therefore, that both effects of acetylation can be mediated by the acidic tail. Indeed, interactions of this domain with one or both HMG boxes have been reported (*40*, *41*), and these tail−HMG box contacts may be affected by the acetylation of Lys2. An alternative explanation, however, cannot be ruled out: the way acetylation influences the ability of HMGB-1 to inhibit repair of cisplatinated DNA may have nothing to do with the acidic domain. Further investigation is certainly needed to clarify this point.

REFERENCES

1. Jamieson, E. R., and Lippard, S. J. (1999) Structure, recognition, and processing of cisplatin-DNA adducts, *Chem. Rev. 99*, 2467−2498.
2. Fichtinger-Schepman, A. M. J., van der Veer, J. L., den Hartog, J. H. J., Lohman, P. H. M., and Reedijk, J. (1985) Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation, *Biochemistry 24*, 707713.
3. Eastman, A. (1986) Reevaluation of interactions of *cis*-dichloro-(ethylendiaminne)platinum(II) with DNA, *Biochemistry 25*, 3912−3915.
4. Yang, D., and Wang, A. J. (1996) Structural studies of interactions between anticancer platinum drugs and DNA, *Prog. Biophys. Mol. Biol. 66*, 81−111.
5. Ohndorf, U.-M., Rould, M. A., He, Q., Pabo, C. O., and Lippard, S. J. (1999) Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins, *Nature 399*, 708−712.
6. Ciccareli, R. B., Solomon, M. J., Varshavsky, A., and Lippard, S. J. (1985) In vivo effects of *cis*- and *trans*-diamminedichloroplatinum(II) on SV40 chromosomes: differential repair, DNA-protein crosslinling, and inhibition of replication, *Biochemistry 24*, 7533−7540.
7. Comess, K. M., Burstyn, J. N., Essigmann, J. M., and Lippard, S. J. (1992) Replication inhibition and translesion synthesis on templates containing site-specifically placed cis-diamminedichloroplatinum(II) DNA adducts, *Biochemistry 31*, 3975−3990.
8. Mello, J. A., Lippard, S. J., and Essigmann, J. M. (1995) DNA adducts of cis-diamminedichloroplatinum(II) and its trans isomer inhibit RNA polymerase differentially in vivo, *Biochemistry 34*, 14783−14791.
9. Zlatanova, J., Yaneva. J., and Leuba, S. H. (1998) Proteins that specifically recognize cisplatin-damaged DNA: a clue to anticancer activity of cisplatin, *FASEB J. 12*, 791−799.
10. McA'Nulty, M. M., and Lippard, S. J. (1995) Consequences of HMG domain protein binding to cisplatin-modified DNA, *Nucleic Acids Mol. Biol. 9*, 264−284.
11. Zamble, D. B. and Lippard, S. J. (1995) Cisplatin and DNA repair in cancer chemotherapy, *Trends Biochem. Sci. 20*, 435−439.
12. Huang, J.-C., Zamble, D. B., Reardon, J. T., Lippard, S. J., and Sancar, A. (1994) HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease, *Proc. Natl. Acad. Sci. U.S.A. 91*, 10394−10398.
13. Zamble, D. B., Mu, D., Reardon, J. T., Sancar, A., and Lippard, S. J. (1996) Repair of cisplatin-DNA adducts by the mammalian excision nuclease, *Biochemistry 35*, 10004−10013.
14. Trimmer, E. E., Zamble, D. B., Lippard, S. J. and Essigmann, J. M. (1997) Human testis-determining factor SRY binds to the major DNA adduct of cisplatin and a putative target sequence with comparable affinities, *Biochemistry 37*, 352−362.
15. He, Q., Liang, C. H., and Lippard, S. J. (2000) Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin, *Proc. Natl. Acad. Sci. U.S.A. 97*, 5768−5772.
16. Li, L., Liu, X., Glassmann, A. B., Keating, M. J., Stros, M., Plunkett, W., and Yang, L. (1997) Fludarabine triphosphate inhibits nucleotide excision repair of cisplatin-induced DNA adducts in Vitro, *Cancer Res. 57*, 1487−1494.
17. Brown, S. J., Kellet, P. J., and Lippard, S. J. (1993) Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin, *Science 261*, 603−605.
18. Pasheva, E. A., Ugrinova, I., Spassovska, N. C., and Pashev, I. G. (2002) The binding affinity of HMG protein to DNA modified by cisplatin and its analogs correlated with their antitumor activity, *Int. J. Biochem. Cell Biol. 34*, 87−92.
19. Malina, J., Kasparkova, J., Natile, G., and Brabec, V. (2002) Recognition of major DNA adducts of enantiomeric cisplatin analogs by HMG box proteins and nucleotide excision repair of these adducts, *Chem. Biol. 9*, 629−638.
20. Malina, J., Hofr, C., Maresca, L., and Brabec, V. (2000) DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands, *Biophys. J. 78*, 2006−2021.
21. Ugrinova, I., Pasheva, E. A., Armengaud, J., and Pashev, I. G. (2001) In vivo acetylation of HMG1 protein enhances its binding affinity to distorted DNA structures, *Biochemistry 40*, 14655−14660.
22. Pasheva, E. A., Pashev, I. G., and Favre, A. (1998) Preferential binding of high mobility group 1 protein to UV-damaged DNA, *J. Biol. Chem. 273*, 24730−24736.
23. Stros, M., Storkova, J., and Thomas, J. O. (1994) DNA looping by the HMG-box domains of HMG1 and modulation of DNA binding by the acidic C-terminal domain, *Nucleic Acids Res. 22*, 1044−1051.
24. Yoshida, M. (1987) High glutamic and aspartic region in non-histone protein HMG (1+2) unwinds DNA double helical structure, *J. Biochem. (Tokyo) 101*, 175−180.
25. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) *Molecular cloning: A laboratory manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
26. Tanaka, M., Lai, L. S., and Herr, W. (1992) Promoter-selective activation domains in Oct-1 and Oct-2 direct differential activation of a snRNA and mRNA promoter, *Cell 68*, 755−767.
27. Biade, S., Sobol, R. W., Wilson, S. H., and Matsumoto, Y. (1998) Impairment of proliferating cell nuclear antigen-dependent apurinic/apyrimidinic site repair on linear DNA, *J. Biol. Chem. 273*, 898−902.

28. Satoh, M. S., and Lindahl, J. (1992) Role of poly(ADP-ribose) formation in DNA repair, *Nature 356*, 356−358.
29. Pil, P. M., and Lippard, S. J. (1992) Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin, *Science 256*, 234−237.
30. Hughes, E. N., Engelsberg, B. N., and Billings, P. C. (1992) Purification of nuclear proteins that bind to cisplatin damaged DNA: identity with high mobility group proteins 1 and 2, *J. Biol. Chem. 267*, 13520−13527.
31. Sheflin, L. G., Fucile, N. W., and Spaulding, S. W. (1993) The specific interactions of HMG1 and 2 with negatively supercoiled DNA are modulated by their acidic C-terminal domains and involve cysteine residues in their HMG1/2 boxes, *Biochemistry 32*, 3238−3248.
32. Lee, K.-B., and Thomas, J. O. (2000) The effect of the acidic tail on the DNA-binding properties of the HMG1, 2 class of proteins: insights from tail switching and tail removal, *J. Mol. Biol. 304*, 135−149.
33. Travers, A., and Thomas, J. O. (2004) Chromosomal HMG-box proteins, in *Chromatin Structure and Dynamics: State-of-the-Art* (Zlatanova, J., and Leuba, S. H., Eds.) pp 103−134, Elsevier B.V., Amsterdam.
34. Aboussekhra, A., Biggerstaff, M., Shivji, M. K. K., Vilpo, J. A, Moncollin, Podust, V. A., Protic, Hubscher, U., Egly, J.-M., and Wood, R. D. (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components, *Cell 80*, 859−868.
35. Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T., and Sancar, A. (1995) Reconstitutions of human DNA repair excision nuclease in a highly defined system, *J. Biol. Chem. 270*, 2415−2418.
36. Burley, J. K. and Roeder, R. G. (1996) Biochemistry and structural biology of transcription factor DII (TFIID), *Annu. Rev. Biochem. 65*, 769−799.
37. Ge, H., and Roeder, R. G. (1994) The high mobility group protein HMG1 can reversibly inhibit class II gene transcription by interaction with the TATA binding protein, *J. Biol. Chem. 269*, 17136−17140.
38. Jung, Y., Mikata, Y., and Lippard, S. J. (2001) Kinetic studies of the TATA-binding protein interaction with cisplatin-modified DNA, *J. Biol. Chem. 276*, 43589−43596
39. Das, D., and Scovell, W. M. (2001) The binding interaction of HMG-1 with the TATA-binding protein/TATA complex, *J. Biol. Chem. 276*, 32597−32605.
40. Jung, Y., and Lippard, S. J. (2003) Nature of full-length HMGB-1 binding to cisplatin-modified DNA, *Biochemistry 42*, 2664−2671.
41. Knapp, S., Müller, S., Digilio, Bonaldi, T., Bianchi, M. E., and Musco, G. (2004) The long acidic tail of high mobility group box 1 (HMGB1) proteins forms an extended and flexible structure that interacts with specific residues within and between the HMG boxes, *Biochemistry 43*, 11992−11997.

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