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High mobility group 1 and 2 proteins bind preferentially to DNA that contains bulky adducts induced by benzo[*a*]pyrene diol epoxide and *N*-acetoxy-acetylaminofluorene

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Abstract

High mobility group (HMG) proteins 1 and 2 are abundant non-histone chromosomal proteins that bind preferentially DNA that is bent or underwound. Previous studies have shown that these proteins preferentially bind to DNA damaged by the crosslinking agents *cis*-diammine-dichloro-platinum(II), chromium(III) and UV-C radiation. Here we have studied the binding of HMG-1/2 proteins to a duplex oligonucleotide damaged by benzo(a)pyrene diol epoxide or *N*-acetoxy-acetylaminofluorene using an electrophoretic mobility shift assay. Both chemicals induce monoadducts that are known to distort DNA structure. The affinities of HMG-1/2 for DNA damaged by benzo[*a*]pyrene diol epoxide or *N*-acetoxy-acetylaminofluorene were similar to that for UV-irradiated DNA, which were an order of magnitude higher than for undamaged DNA. In contrast, DNA modified by dimethyl sulfate was not preferentially recognised by HMG-1/2. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: High mobility group proteins; Damage recognition; DNA adducts; *N*-Acetoxy-acetylaminofluorene; Benzo[*a*]pyrene diol epoxide

1. Introduction

The high-mobility-group (HMG) proteins are a major group of non-histone chromosomal proteins. Members of the HMG-1/2 family are the largest (28–25 kDa) and most abundant (1 molecule per 10–15 nucleosomes). Proteins from this family are highly conserved; human HMG-1 (215 amino acids) and HMG-2 (209 amino acids) share >80% amino acid identity. There is a large and highly diverse family of DNA-binding proteins that contain a HMG-1/2 DNA-binding domain, called the 'HMG-box' family. HMG-1/2 proteins have the ability to preferentially bind to DNA that is intrinsically bent, underwound or organized as cruciform or four-way junction. HMG proteins also have the ability to non-specifically bind to B-form DNA and to induce bending, unwinding, looping or supercoiling (review in Refs. [1,2]). The actual biological role of HMG-1/2 proteins remains elusive. However, numerous lines of evidence suggest that these proteins participate in the regulation of chromatin structure as well as aspects of DNA replication, transcription and repair. Both HMG-1/2 and linker histones bind to internucleosomal linker DNA in chromatin and may compete for the same binding sites. However, whether they have either opposite or synergistic effects on gene regulation remains unclear (review in Ref. [3]).

The ability of HMG-1/2 to preferentially bind to bent or distorted DNA has also been supported by

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the efficient binding of these proteins to DNA modified by *cis*-platin. *cis*-Platin (*cis*-diamminedichloroplatinum(II), abbreviated *cis*-DDP) is one of the most widely used antineoplastic drugs. The cytotoxicity of *cis*-DDP is believed to be due to the formation of DNA adducts. Major adducts are 1,2-intrastrand d(GpG) and d(ApG) crosslinks, which comprise ~90% of the lesions, while 1,3-intrastrand d(GpNpG) and interstrand crosslinks are minor lesions [4,5]. All these lesions produce severe local distortions in the DNA double helix: 1,2-intrastrand crosslinks bend DNA by 33° and unwind by 13°. The geometric isomer *trans*-DDP, which is ineffective as a chemotherapeutic agents, cannot form 1,2-intrastrand cross-links [5,6]. Pil and Lippard [7] showed that HMG-1/2 proteins bound to DNA modified by *cis*-DDP, but not *trans*-DDP, with an affinity at least 100-fold greater than for unmodified DNA (preferential binding was restricted to 1,2-intrastrand crosslinks). It has been shown that the DNA-binding domain of HMG-1 binds to the widened minor groove of kinked DNA molecules containing such a *cis*-DDP adduct [8]. Other proteins from the 'HMG-box' family: human SSRP1 [9], human UBF [10] and yeast Ixr1 [11], also bind preferentially to DNA modified by *cis*-DDP. The majority of *cis*-DDP-induced lesions are repaired by nucleotide excision repair (NER); cell lines cultured from xeroderma pigmentosum (XP) patients are highly sensitive to *cis*-DDP and do not removed intrastrand crosslinks (review in Ref. [12]). The presence of HMG-1 blocks the repair of the *cis*-DDP-induced intrastrand crosslinks in an in vitro repair system [13]. Disruption of the gene encoding HMG-box protein Ixr1 results in a desensitization of a yeast strain to *cis*-DDP [11].

More recently it has been shown that HMG-1/2 bind preferentially to DNA damaged by another carcinogenic metal, chromium(III), which also produces DNA-DNA crosslinks [14]. HMG-1 binds preferentially also to major lesions formed in DNA irradiated by UV-C, such as the cyclobutane pyrimidine dimers and pyrymidine-pyrimidone (6-4) photoproducts, which induce bending and unwinding of DNA. HMG-1 binds to these lesions with an affinity one order of magnitude lower than that for DNA damaged by *cis*-DDP, yet one order of magnitude higher as compared for undamaged DNA [15].

The present study addresses the question of whether monofunctional bulky adducts that do not crosslink DNA, yet which distort its structure, might also be preferentially recognized by HMG-1/2. We have examined the binding of HMG-1/2 to DNA damaged by two polycyclic aromatic hydrocarbons: benzo(a)-pyrene diol epoxide (BPDE) and acetoxy-acetylaminofluorene (AAAF). Both of these compounds are well known genotoxic and carcinogenic agents that induce covalent DNA adducts predominantly at guanine residues [16,17]. We show that the affinity of HMG-1/2 to bind to DNA damaged by these compounds is similar to that of binding to UV-irradiated DNA.

2. Materials and methods

2.1. DNA probes

A synthetic double-stranded 36 bp-long oligonucleotide (5′-AATTCGTAGGCCTAAGAGCAATCGCACCTGTGCGCG-3′, with blunt ends) was used as a model DNA substrate. The oligonucleotide (at 10 μM concentration) was incubated for 4 h at 37°C with 40 μM *N*-acetoxy-*N*-2-acetylaminofluorene (AAAF) or trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) (both obtained from Midwest Research Institute), for 20 h at 37°C with 3 μM *cis*-DDP (Ebewe), or for 15 min at 20°C with 100 μM dimethyl sulfate (DMS, Merck), and then purified by phenol/chloroform extraction and ethanol precipitated. Alternatively, the oligonucleotide was UV-irradiated (5 kJ/m^2) using a 254 nm UV-crosslinker (Stratagene). The treatment with AAAF, BPDE and UV introduced single lesions into about 15% of the DNA molecules (on average), which was verified by a ^{32}P-postlabeling method [18]. The treatment with *cis*-DDP introduces a similar amount of damage, calculated as reported elsewhere [19]. The oligonucleotide was end-labeled by transfer of ^{32}P from ($\gamma^{32}P$)ATP using T4 polynucleotide kinase and purified by polyacrylamide gel electrophoresis [20].

2.2. Preparation of proteins

Nuclei were purified from homogenized liver tissue of adult male WAG rats. To obtain extracts of nuclear proteins, nuclei were incubated for 30 min at 4°C with a buffer consisting of 0.35 M NaCl, 10 mM Hepes–

NaOH (pH 7.9), 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM DTT, 5% glycerol, a protease inhibitor mixture (Complete^{TM}, Boehringer). Insoluble remnants of the extracted nuclei were pelleted by centrifugation for 30 min at 16,000 rev./min at 4°C. HMG proteins were purified from rat liver nuclear extracts according to a published method [21]. Briefly, proteins extracted with 0.35 M NaCl were precipitated with 2% trichloroacetic acid and the acid soluble fraction containing HMG proteins was concentrated by precipitation with acetone. Human recombinant HMG-1 was a gift of Dr M. Bustin. Protein concentration was assayed using a Protein Assay Kit (BioRad).

2.3. Electrophoretic mobility shift assay (EMSA)

Radioactive oligonucleotide (25 ng) was incubated with nuclear extracts (5 μg) or HMG proteins (0.5 μg) for 30 min at 4°C. The binding buffer consisted of 20 mM Tris–HCl (pH 7.6), 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, 5% glycerol and NaCl (150 mM final concentration). Protein-oligonucleotide complexes were formed in a final volume of 20 μl in the presence of non-radioactive DNA competitors added before the proteins. Sonicated *E. coli* DNA was used as a non-specific competitor at an 80-fold excess (2 μg). Either undamaged or damaged oligonucleotide was used as a homologous competitor. Protein-oligonucleotide complexes were resolved by electrophoresis on 6% polyacrylamide gels in $0.5 \times$ Tris/borate/EDTA buffer. Gels were dried, marked with radioactive ink, and autoradiographed. Gel fragments containing radioactive complexes were excised and quantitated by scintillation counting. The dissociation constants (K_d) were determined according to [15].

2.4. Southwestern blot analysis

Nuclear extracts (50 μg of proteins) or HMG proteins (5 μg) were fractionated on 13% polyacrylamide/SDS gels [20] and electrotransferred onto PVDF membranes (Hybond-P; Amersham) in 25 mM Tris, 190 mM glycine and 20% methanol. Filter-bound proteins were renatured by incubation in a hybridization oven for 5 h at 25°C with 25 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$ and 2.5% BSA. After washing with binding buffer (same composition as above but with 0.25% BSA), filters were incubated for 5 h 25°C in binding buffer supplemented with 250 ng of ^{32}P-end-labeled oligonucleotide (that was either non-damaged or *cis*-DDP damaged) and an 80-fold excess of non-radioactive competitor (*E. coli* DNA) in a final volume 10 ml. Filters were then washed with binding buffer and autoradiographed.

3. Results and discussion

Formation of complexes between nuclear proteins and damaged DNA was studied using an EMSA. Fig. 1A shows the results of an experiment in which proteins extracted with 0.35 M NaCl from rat liver nuclei were incubated with radioactive duplex oligonucleotide, either undamaged or damaged by UV-irradiation, *cis*-DDP, DMS, AAAF and BPDE, in the presence of a non-specific competitor (*E. coli* DNA). Significantly, no protein-DNA complexes could be detected when undamaged oligonucleotide was used as the probe (Fig. 1A, lane 1). However, when duplex oligonucleotides damaged by different agents were used, several protein-DNA complexes were detected. Protein-DNA complexes marked as a complex B were detected with all damaged oligonucleotides. Additionally, oligonucleotide damaged by *cis*-DDP or AAAF formed complexes with higher electrophoretic mobility than the former one. These complexes (marked as a complex A) contain a protein band of about 23 kDa, which has a high affinity to DNA damaged by *cis*-DDP or AAAF but does not purify together with HMG proteins (Widlak and Rzeszowska-Wolny, unpublished results). UV-irradiated oligonucleotide formed different major complex with lower electrophoretic mobility that probably contains protein homologous to human UV-DDB protein p125/p48 (marked as a complex C).

Fig. 1B shows the results of an experiment in which purified HMG proteins were incubated with radioactive oligonucleotide probe. DNA-HMG complexes were detected when oligonucleotide damaged by UV, *cis*-DDP, AAAF or BPDE, but not undamaged or DMS-damaged, was used as a probe. Only one complex could be detected for each DNA probe and such complexes had similar elecrophoretic mobility. These complexes co-migrated with the major complex detected with nuclear extracts (complex B on Fig.

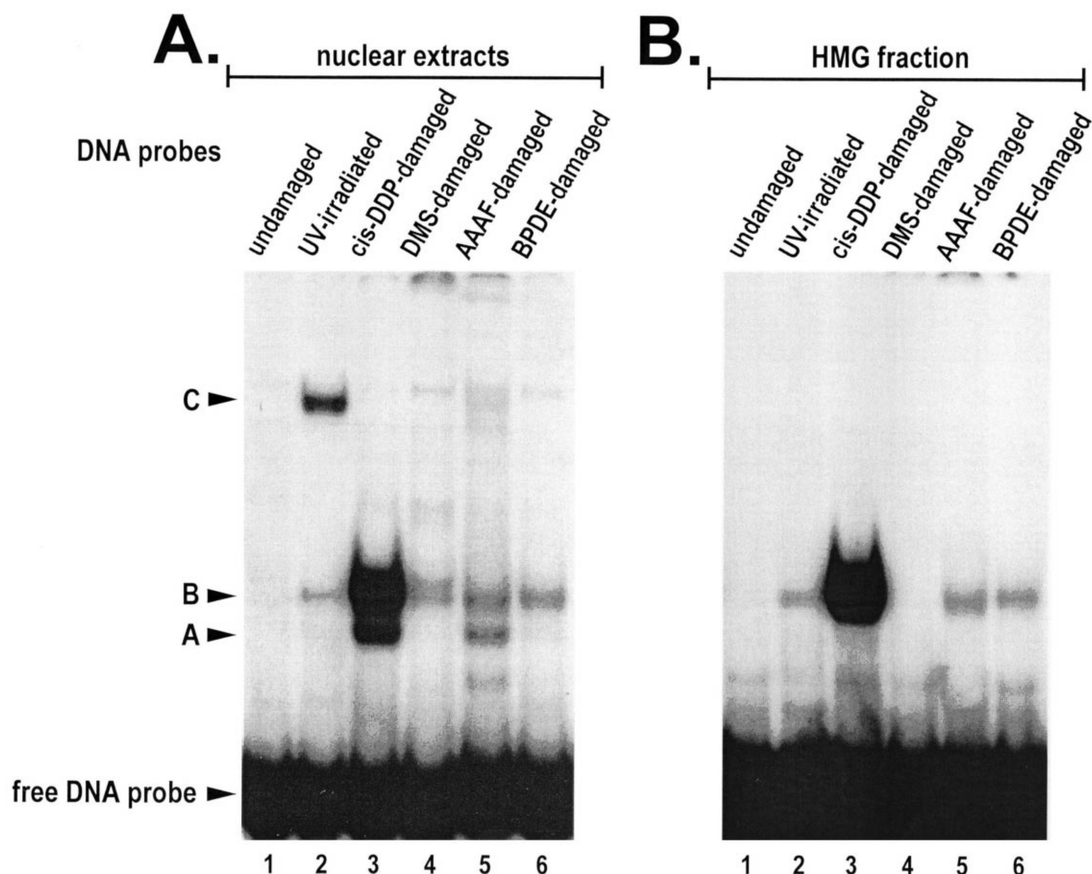

Fig. 1. Analysis of damage recognition proteins by EMSA. (A) In vitro complexes were formed between radioactive DNA probes (either undamaged or damaged by different agents) and nuclear proteins extracted with 0.35 M NaCl. The complexes were formed in the presence of excess of *E. coli* competitor DNA. Protein–DNA complexes are marked with arrowheads. (B) In vitro complexes were formed between radioactive DNA probes and a HMG protein fraction.

1A). Protein–DNA complexes specific for DMS-modified oligonucleotide that could be detected with nuclear extracts (shown on Fig. 1A, lane 4) did not contain HMG proteins, yet showed similar electrophoretic mobility. Oligonucleotide damaged by *cis*-DDP formed a complex with HMG proteins with much higher affinity than those of other damaged oligonucleotides.

This complex could also be demonstrated by a Southwestern blot (Fig. 2), validating that HMG-1/2 are indeed responsible for complex B formation. Fig. 2B shows the result of an experiment in which electrophoretically resolved proteins from nuclear extract were probed with labelled oligonucleotide either undamaged or damaged by *cis*-DDP. The only radioactive complex detected when undamaged oligonucleotide was tested co-migrated with histone H1. When oligonucleotide damaged by *cis*-DDP was tested two strong radioactive complexes were detected in addition to the putative histone H1; one complex of about 28 kDa and the other one of about 26 kDa, which co-migrated with HMG-1 and HMG-2, respectively (Fig. 2A). This was in agreement with previous studies showing that HMG-1/2 are major nuclear proteins binding to DDP-damaged DNA [7,22,23]. Both HMG-1 and HMG-2 proteins were engaged in formation of complexes with *cis*-DDP-modified DNA. Thus the complex detected by an electrophoretic mobility shift assay (complex B on Fig. 1) probably contained both protein species.

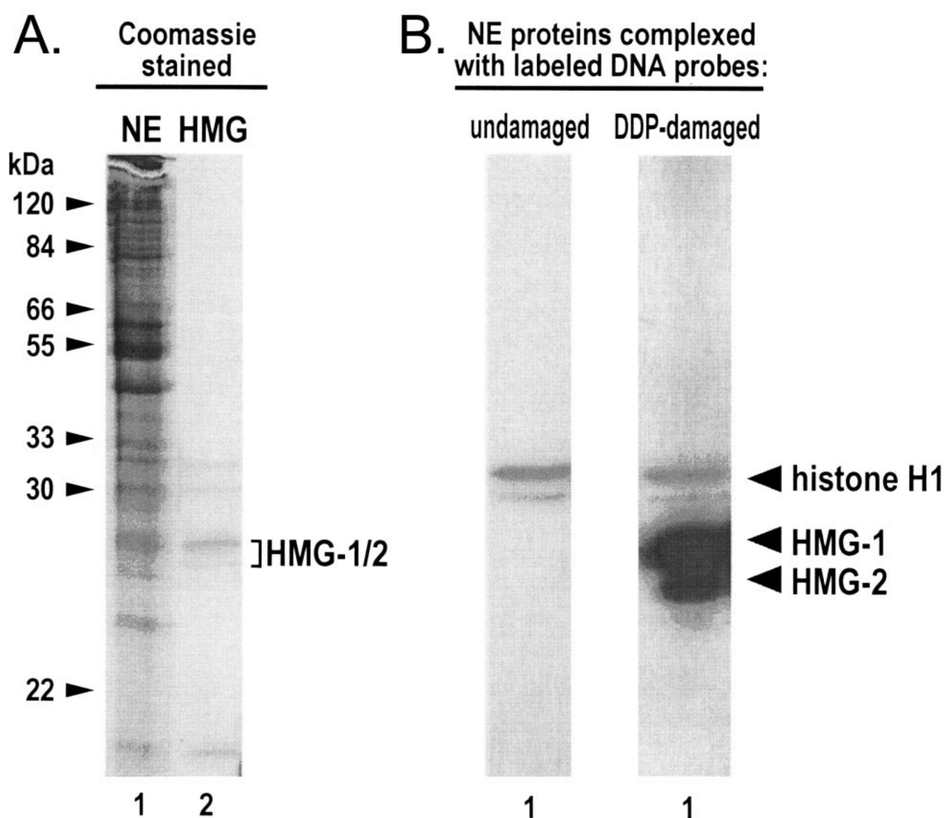

Fig. 2. Analysis of damage recognition proteins by Southwestern blot. (A) SDS-PAGE analysis of proteins in nuclear extracts (NE) and a HMG protein fraction (HMG). Molecular size standards are marked with arrowheads. (B) Southwestern blot analysis of proteins in a rat liver nuclear extract. Radiolabeled oligonucleotide probe was either undamaged or damaged by *cis*-DDP.

To estimate the affinities of HMG-1/2 proteins for oligonucleotides damaged by different agents, complexes between proteins and labelled *cis*-DDP-modified oligonucleotide were formed in the presence of homologous competitors. Fig. 3A shows the results of an experiment in which such complexes were formed in the presence of 13-, 40- and 120-fold excess of non-radioactive homologous oligonucleotides, which were either undamaged or damaged by different agents. The amounts of DNA in complexes that were quantitated by scintillation counting are shown in Fig. 3B. The affinities of HMG-1/2 for oligonucleotides that were damaged by AAAF, BPDE or UV-irradiated were similar, and about 20-fold greater than those for either undamaged or DMS-damaged DNA (the calculation was done according to the assumption that only 15% of the molecules contained a lesion). HMG-1/2 bound to oligonucleotides damaged by AAAF, BPDE or UV with an affinity about 10-fold lower than that for *cis*-DDP-modified oligonucleotide. Calculated dissociation constants of HMG/DNA complexes were about 2×10^{-7}, 3×10^{-6} and 5×10^{-5} M for DDP-damaged, AAAF-damaged and undamaged oligonucleotides, respectively.

The affinity of HMG proteins for DNA damaged by aromatic compounds was confirmed in experiments with human recombinant HMG-1 (Fig. 4). Fig. 4A shows that complexes containing duplex oligonucleotide and HMG proteins purified from rat liver or human HMG-1 co-migrated. To estimate the affinities of HMG-1 for oligonucleotides which were either undamaged or damaged by AAAF and *cis*-DDP, complexes between proteins and radioactive *cis*-DDP-damaged DNA probe were formed in the

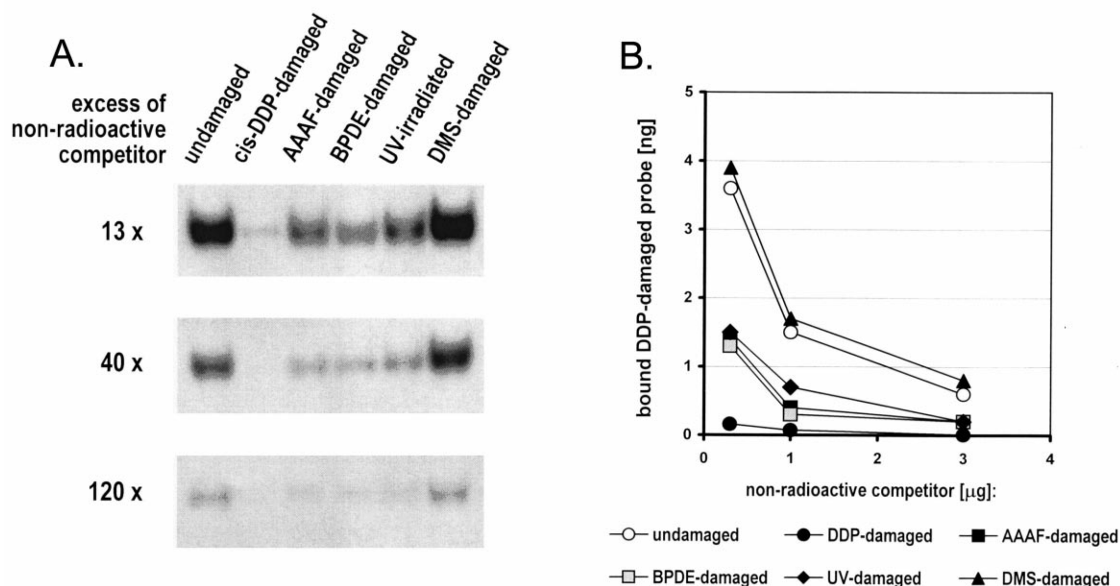

Fig. 3. Analysis of affinity of HMG-1/2 proteins for DNA damaged by different factors. (A) In vitro complexes were formed between radioactive oligonucleotide damaged by *cis*-DDP and a HMG protein fraction in the presence of excess (13-, 40- and 120-fold) of homologous competitor that was either undamaged or damaged by different agents. (B) The effects of excess homologous competitors on amounts of complexes formed between labelled DDP-modified oligonucleotide and HMG-1/2 proteins.

presence of 20-fold excess of an appropriate homologous competitor (Fig. 4B). The affinity of HMG-1 for AAAF-modified oligonucleotide was about 10-fold greater than that for undamaged DNA and about 15-fold lower than that for oligonucleotide damaged by *cis*-DDP. The increased affinity of HMG-1 for DNA damaged by AAAF was also demonstrated by a Southwestern blot analysis (Fig. 4C).

All DNA lesions that we have found to preferentially bind HMG-1/2 proteins deform the DNA helix at the damaged site. Angles of DNA unwinding and bending induced by each of these lesions have been determined experimentally (see Table 1). The most preferable substrate for HMG-1/2 was oligonucleotide damaged by *cis*-DDP while the other three damaged DNA species bound these proteins with lower affinities (yet an order of magnitude stronger than that of undamaged DNA). Although there are some disagreements in the literature, degrees of bending and unwinding of DNA are distinct in the sites of damage induced by UV, BPDE or AAAF (Table 1). Because of this discrepancies, one cannot make simple conclusion which type of the DNA helix distortion affects the binding of HMG-1/2 proteins in the most efficient way.

Several models have been proposed to explain the effects of 'HMG-box' proteins upon cellular resistance/sensitivity to *cis*-DDP. According to the 'repair shielding' model, the binding of HMG proteins to drug-modified DNA might prevent recognition of adducts by the repair machinery, blocking their repair and leading to cell death [7]. According to alternative model of the 'repair recognition', 'HMG-box' proteins may act as damage sensors and recruit the repair complex to the sites of lesions [12]. Adducts induced by *cis*-DDP may also function by non-specifically trapping essential 'HMG-box' proteins (e.g. hUBF [10]) or may relocate HMG to genomic regions where they are not normally present, altering the transcription of critical genes and thereby leading to cellular toxicity. Binding of HMG proteins to damaged DNA might also facilitate local repair due to promoting chromatin remodeling (e.g. nucleosome unfolding) at sites of lesions. Other abundant chromatin

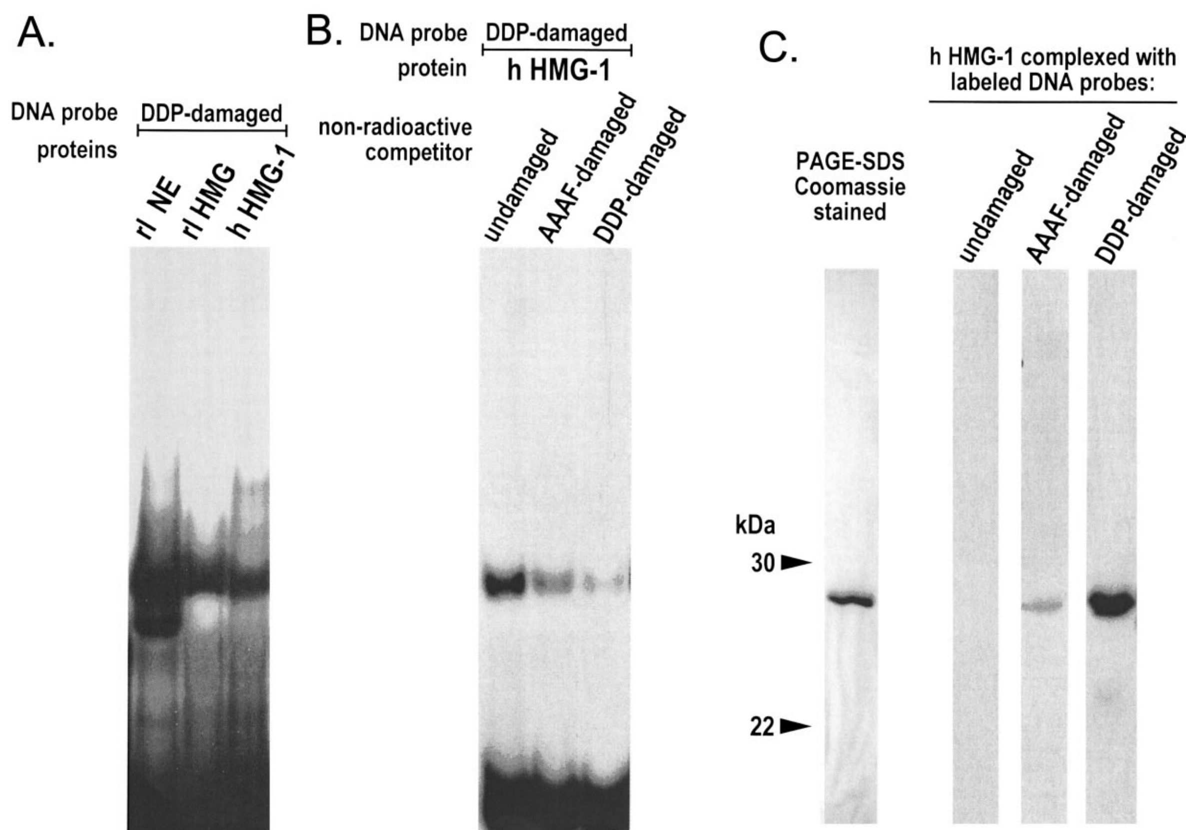

Fig. 4. Binding of human HMG-1 to damaged DNA. (A) Complexes were formed between *cis*-DDP-damaged oligonucleotide and rat liver nuclear extract (rl NE), rat liver HMG protein fraction (rl HMG) or human HMG-1. (B) Complexes were formed between HMG-1 and radioactive *cis*-DDP-damaged probe in the presence of 20-fold excess of homologous competitor (either undamaged or damaged by AAAF and *cis*-DDP). (C) Southwestern blot analysis of HMG-1. Proteins were stained with Coomassie or complexed with radiolabeled oligonucleotide probe that was either undamaged or damaged by AAAF and *cis*-DDP.

proteins, like histone H1 that was also found to preferentially bind *cis*-DDP-modified DNA [24], may similarly affect repair processes. Our findings that HMG-1/2 proteins preferentially bind to DNA damaged by

Table 1
Conformational changes induced in DNA by modified nucleotide residues

Damaging agent	DNA lesion	Induced bend (°)	Induced unwinding (°)
cis-DDP	1,2-intrastrand crosslink	32–34 [6]	~13 [5]
	1,3-intrastrand crosslink	~35 [6]	~23 [5]
UV-C	Cyclobutane pyrimidine dimers	~29 [25]	~14 [27]
		~7 [26]	
	(6-4) photoproduct	~44 [28]	
AAAF	AAF-C8-dG	Hinge point [29]	~22 [30,31]
			~29 [32]
BPDE	*anti*-BPDE-N^2-dG	21–26 [33]	~12 [34]
			~22 [30]

polycyclic aromatic hydrocarbons extend these models to additional DNA lesions. However, as binding of chromatin proteins to distorted DNA structures in a place of bulky adduct could potentially lead to either repair inhibition or facilitation, the final effect may depend on type of DNA lesion and protein that binds to it.

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References

[1] D. Landsman, M. Bustin, A signature for the HMG-1 box DNA-binding proteins, Bioessays 15 (1993) 539–546.

[2] M. Bustin, R. Reeves, High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function, Progress in Nucleic Acid Res. Mol. Biol. 54 (1996) 35–100.

[3] J. Zlatanova, K. van Holde, Linker histones versus HMG1/2: a struggle for dominance? Bioessays 20 (1998) 584–588.

[4] A. Eastman, Reevaluation of interaction of *cis*-(ethylenediammine)platinum (II) with DNA, Biochemistry 25 (1986) 3912–3915.

[5] S.F. Bellon, J.H. Coleman, S.J. Lippard, unwinding produced by site-specific intrastrand cross-links of the antitumor drug *cis*-diamminedichloroplatinum (II), Biochemistry 30 (1991) 8026–8035.

[6] S.F. Bellon, S.J. Lippard, Bending studies of DNA site-specifically modified by cisplatin, *trans*-diamminedichloroplatinum(II), and *cis*-$[Pt(NH_3)_2(N3\text{-cytosine})Cl]^+$, Biophys. Chem. 35 (1990) 179–188.

[7] P.M. Pil, S.J. Lippard, Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin, Science 256 (1992) 234–237.

[8] U.-M. Ohndorf, M.A. Rould, Q. He, C.O. Pabo, S.J. Lippard, Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins, Nature 399 (1999) 708–712.

[9] S.L. Bruhn, P.M. Pil, J.M. Essigmann, D.E. Housman, S.J. Lippard, Isolation and characterization of human cDNA clones encoding a high mobility group box protein that recognizes structural distortions to DNA caused by binding of the anticancer agent cisplatin, Proc. Natl. Acad. Sci. USA 89 (1992) 2307–2311.

[10] D.K. Treiber, X. Zhai, H.-M. Jantzen, J.M. Essigmann, Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor), Proc. Natl. Acad. Sci. USA 91 (1994) 5672–5676.

[11] S.J. Brown, P.J. Kellett, S.J. Lippard, Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin, Science 261 (1993) 603–605.

[12] D.B. Zamble, S.J. Lippard, Cisplatin and DNA repair in cancer chemotherapy, Trends Biochem. Sci. 20 (1995) 435–439.

[13] J.-C. Huang, D.B. Zamble, J.T. Reardon, S.J. Lippard, A. Sancar, HMG-domain proteins specifically inhibit the repair of the major DNA adducts of the anticancer drug cisplatin by human excision nuclease, Proc. Natl. Acad. Sci. USA 91 (1994) 10394–10398.

[14] J.F. Wang, M. Bashir, B.N. Engelsberg, C. Witmer, H. Rozmiarek, P.C. Billings, High mobility group proteins 1 and 2 recognize chromium-damaged DNA, Carcinogenesis 18 (1997) 371–375.

[15] E.A. Pasheva, I.G. Pashev, A. Favre, Preferential binding of high mobility group 1 protein to UV-damaged DNA. Role of the COOH-terminal domain, J. Biol. Chem. 273 (1998) 24730–24736.

[16] E. Kriek, Persistent binding of a new reaction product of the carcinogen *N*-hydroxy-*N*-2-acetylaminofluorene with guanine in rat liver DNA in vivo, Cancer Res. 32 (1972) 2042–2048.

[17] T. Meehan, K. Straub, M. Calvin, Benzo[a]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA, Nature 269 (1977) 725–727.

[18] J. Rzeszowska-Wolny, P. Widłak, Damaged DNA-binding proteins: recognition of *N*-acetoxyacetylaminofluorene-induced DNA adducts, Acta Biochim. Polon. 46 (1999) 173–180.

[19] H.M. Ushay, T.D. Tullius, S.J. Lippard, Inhibition of the BamHI cleavage and unwinding of pBR322 deoxyribonucleic acid by the antitumor drug *cis*-dichlorodiammineplatinum(II), Biochemistry 20 (1981) 3744–3748.

[20] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.

[21] S. Spiker, High-mobility group proteins of wheat, J. Biol. Chem. 259 (1984) 12007–12013.

[22] E.N. Hughes, B.N. Engelsberg, P.C. Billings, Purification of nuclear proteins that bind to cisplatin-damaged DNA: Identity with high mobility group proteins 1 and 2, J. Biol. Chem. 267 (1992) 13520–13527.

[23] J.J. Turchi, M. Li, K.M. Henkels, Cisplatin-DNA binding specificity of calf high-mobility group 1 protein, Biochemistry 35 (1996) 2992–3000.

[24] J. Yaneva, S.H. Leuba, K. van Holde, J. Zlatanova, The major chromatin protein histone H1 binds preferentially to *cis*-platinum-damaged DNA, Proc. Natl. Acad. Sci. USA 94 (1997) 13448–13451.

[25] I. Husain, J. Griffith, A. Sancar, Thymine dimers bent DNA, Proc. Natl. Acad. Sci. USA 85 (1988) 2558–2562.

[26] C.-I. Wang, J.-S. Taylor, Site-specific effects of thymine dimer formation on dA_n*dT_n tract bending and its biological implications, Proc. Natl. Acad. Sci. USA 88 (1991) 9072–9076.

[27] G. Ciarrocchi, A.M. Pedrini, Determination of pyrimidine

dimer unwinding angle by measurement of DNA electrophoretic mobility, J. Mol. Biol. 155 (1982) 177–183.

[28] J.-K. Kim, B.-S. Choi, The solution structure of DNA duplex-decamer containing the (6-4) photoproduct of thymidylyl(3′-5′)thymine by NMR and relaxation matrix refinement, Eur. J. Biochem. 228 (1995) 849–854.

[29] A. Schwartz, L. Marrot, M. Leng, The DNA bending by acetylaminofluorene residues and by apurinic sites, J. Mol. Biol. 207 (1989) 445–450.

[30] N.R. Drinkwater, J.A. Miller, E.C. Miller, N.-C. Yang, Covalent intercalative binding to DNA in relation to the mutagenicity of hydrocarbon epoxides and *N*-acetoxy-2-acetylaminofluorene, Cancer Res. 38 (1978) 3247–3255.

[31] M.C.E. Lang, A.M. Freund, G. de Murcia, R.P.P. Fuchs, M.P. Daune, Unwinding of supercoiled $ColE_1$-DNA after covalent binding of the ultimate carcinogen *N*-acetoxy-*N*-2-acetylaminofluorene and its 7-iodo derivative, Chem. Biol. Interact. 28 (1979) 171–180.

[32] L. Marrot, E. Hebert, G. Saint-Ruf, M. Leng, Comparison of the reactivity of B-DNA and Z-DNA with two isosteric chemical carcinogens: 2-*N*,*N*-acetoxyacetylaminofluorene and 3-*N*,*N*-acetoxyacetylamino-4,6-dimethyldipyrido-(1,2-a:3′,2′-d) imidazole, Nucleic Acids Res. 15 (1987) 5629–5642.

[33] R. Xu, B. Mao, S. Amin, N.E. Geacintov, Bending and circularization of site-specific and stereoisomeric carcinogen-DNA adducts, Biochemistry 37 (1998) 769–778.

[34] R. Xu, S. Birke, S.E. Carberry, N.E. Geacintov, C.E. Swenberg, R.G. Harvey, Differences in unwinding of supercoiled DNA induced by the two enantiomers of *anti*-benzo[a]pyrene diol epoxide, Nucleic Acids Res. 20 (1992) 6167–6176.