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Analysis of HMG protein binding to DNA modified with the anticancer drug cisplatin

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Abstract Cisplatin (CDDP) is an effective and widely used cancer chemotherapy drug. High mobility group (HMG) proteins 1 and 2 have been shown to bind with high affinity to CDDP-DNA. In this study we analyzed the interaction of HMG proteins with CDDP-DNA. We demonstrate that after binding, HMG proteins can be removed from CDDP-DNA leaving the Pt adducts intact and capable of rebinding HMG proteins. Furthermore, the very HMG proteins that have been removed remain functionally viable and capable of rebinding CDDP-DNA. We also investigated the role that Cys residues play in protein binding. Replacement of Cys 45 or Cys 106 with a Ser residue reduced HMG2 protein binding to CDDP-DNA. These results indicate that Cys residues play a critical role in the high affinity binding of this protein to CDDP-DNA. From these findings, we speculate that the intracellular oxidative environment could affect the redox state of protein thiols in HMG1 and HMG2 and in addition, regulate the ability of these proteins to recognize *cis*-Pt-DNA adduct formation in tumor cells.

Key words HMG proteins · CDDP-DNA · Cisplatin

Abbreviations *CDDP* cis-diamminedichloroplatinum (II) *CDDP-DNA* DNA modified by CDDP, *DTT* dithiothreitol, *EDTA* ethylenediaminetetraacetic acid, *IPTG* isopropyl β-D-thiogalactoside, β*ME* β-mercaptoethanol, M_r molecular mass, R_f molar ratio of free platinum to nucleotide-phosphate at onset of incubation with DNA, *SDS* sodium dodecyl sulfate, *TDDP* trans-diamminedichloroplatinum (II)

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Introduction

cis-Diamminedichloroplatinum (II) (cisplatin; CDDP) is a frequently used chemotherapeutic agent employed in the treatment regimens of several human malignancies including bladder [26], cervical [1], esophageal [22], head and neck [10], ovarian [23, 24], testicular [12] and small-cell lung cancers [29]. It is generally accepted that the antitumor activity of CDDP results from its interaction with cellular DNA. The primary adducts formed by CDDP are intrastrand crosslinks between adjacent purines in genomic DNA [9]. A major clinical problem associated with the use of CDDP is that of acquired resistance [28]. While resistance to CDDP is multifactorial [2, 28], we believe that one mechanism influencing the response of tumor cells to CDDP may be the levels of specific proteins which recognize CDDP-DNA [6].

High mobility group (HMG) proteins 1 and 2 are highly conserved basic chromatin proteins which are found in mammalian nuclei [7]. These proteins are relatively abundant and are believed to play essential roles in chromatin structure and function. HMG1 and HMG2 contain two similar DNA binding segments known as HMG boxes or HMG domains [4]. HMG boxes consist of about 80 amino acid regions which are rich in basic amino acids and contain several highly conserved aromatic residues [4, 13, 15, 20]. HMG boxes have been identified in many proteins involved in the control of gene expression. Two classes of HMG box-containing proteins have been described: (1) proteins with two or more HMG boxes such as chromatin proteins HMG1 and HMG2 [4, 13] and upstream

binding factor, a general RNA polymerase 1 transcription factor [18], and (2) proteins containing a single HMG box such as the sex-determining region Y testis determining factor (reviewed in references 15 and 20; [27]). Proteins in the first group bind DNA in a sequence-independent manner, while those in the second group exhibit sequence-specific binding [15, 20]. All HMG box-containing proteins have the ability to recognize kinked or bent DNA conformations [15] and induce sharp bends in DNA after binding [8].

HMG1 and HMG2 bind with high affinity to CDDP-DNA [5, 17, 25]. Several lines of evidence suggest that these proteins recognize intrastrand Pt-DNA adducts and may also play a role in the response of tumor cells to CDDP toxicity: (1) these proteins bind with high affinity to DNA modified with CDDP and carboplatin but do not recognize DNA modified with the clinically ineffective isomer, TDDP [17, 25]; (2) increased levels of HMG1 and HMG2 binding to CDDP-DNA have been observed in HeLa cell lines resistant to CDDP [6], and (3) HMG1 blocks excision of CDDP adducts in an in vitro repair system [16].

In the study reported here, we analyzed the binding of HMG1 and HMG2 to CDDP-DNA. Our results indicate that the Pt-DNA adducts remain intact following binding of HMG1 and HMG2 to CDDP-DNA and that HMG protein binding is reversible. We also demonstrated that Cys residues at positions 45 and 106 play a critical role in the binding of HMG2 to CDDP-DNA.

Materials and Methods

Chemicals

Calf thymus double-stranded-DNA cellulose was obtained from Sigma (St. Louis, Mo.). CDDP was obtained from Aldrich (Milwaukee, Wis). Precast sodium dodecyl sulphate (SDS)-polyacrylamide gels were purchased from Novex (San Diego, Calif.). Oligonucleotides were supplied by the Nucleic Acid Facility, University of Pennsylvania Cancer Center (Philadelphia, Pa.). The prokaryotic expression vector pTrcHis was obtained from InVitrogen (San Diego, Calif.).

Purification of HMG proteins

HMG proteins were purified from calf thymus by perchloric acid extraction and acetone precipitation, followed by ion exchange chromatography on MonoQ and MonoS [5].

Preparation of damaged DNA cellulose

DNA was modified by CDDP as previously described [5]. Briefly, stock solutions of CDDP (1 mg/ml, 3 m*M*) were made up in 1 m*M* phosphate buffer (pH 7.4), 3 m*M* NaCl. ds-DNA cellulose (4 mg DNA/g cellulose) was suspended in 1 m*M* sodium phosphate buffer (pH 7.4), 3 m*M* NaCl and mixed with CDDP ($R_f = 0.03$) for 18 h. Following incubation, the DNA cellulose suspension was washed extensively in 20 m*M* Tris-HCl (pH 7.5), 1 m*M* EDTA, 500 m*M* NaCl, 10% glycerol, 0.5 m*M* DTT, 0.1% nonidet P-40; damaged DNA preparations were stored in 10 m*M* Tris-HCl (pH 7.5) at 4°C. The amount of platinum bound to DNA was measured with a Varian SpectrAA-300 atomic absorption spectrometer equipped with a GTA96Z graphite tube atomizer.

Damaged DNA-affinity precipitation assay

Purified protein samples (5–10 μg) were mixed with 30 μl (10.5 μg DNA) undamaged or CDDP-DNA cellulose and 26.5 μg sheared salmon sperm DNA to serve as competitor. The samples were incubated on a rotating platform (4°C, 60 min) and subsequently centrifuged (3000 *g*, 2 min). The pellet is washed three times with 10 m*M* Tris (pH 7.5), 0.5 *M* NaCl, 1 m*M* EDTA, 0.5 m*M* DTT, 10% glycerol and 0.1% NP-40, followed by one wash with 10 m*M* Tris (pH 7.5). Bound proteins were extracted from the DNA-cellulose pellet by incubation (100°C, 3 min) in 125 m*M* Tris (pH 6.8), 10 m*M* βME, 4% SDS, and 20% glycerol, and analyzed on 8–16% SDS-polyacrylamide gradient gels [19].

Removal of HMG protein from CDDP-DNA

HMG1 and HMG2 bound to CDDP-DNA were removed by incubation of the protein–CDDP-DNA complexes in 1 *M* KI (50°C, 30 min). The eluted protein was concentrated and dialyzed in a Microcon 10 microconcentrator (Amicon, Beverly, Mass.).

Subcloning of HMG2

Full length human HMG2 cDNA containing the Met initiation codon and a 3′ termination signal was cloned into the BamH1/HindIII multiple cloning sites of pTrcHisA expression vector. The bacterial expressed protein consisted of an N-terminal fusion protein containing 34 residues on the 5′ (N-terminus) of HMG2. The vector-derived upstream sequence contained a tract of six His residues which function as a metal binding domain and facilitate the purification of recombinant proteins (see below). The recombinant protein bound to CDDP-DNA with the same affinity as wild-type HMG2, as observed previously [21].

Site-specific mutagenesis

Site-specific mutagenesis was performed using the procedures originally described by Deng and Nikoloff [11]. In this system, selection and mutagenic oligonucleotides were used to alter the unique Sca 1 site (position 1378–1384) in the vector and a specific codon in the cloned sequence, respectively. The mutagenic oligonucleotides used in these studies are listed in Table 1.

Phosphorylation of oligonucleotides

The mutagenic and the selection oligonucleotides were phosphorylated at their 5′ ends to facilitate future ligation reactions. Kinase reaction mixtures contained 2 μg oligonucleotide, 2 μl 10 × T4 kinase buffer (500 m*M* Tris-HCl, pH 7.5, 100 m*M* $MgCl_2$, 50 m*M* DTT, 10 m*M* ATP), 10 U T4 polynucleotide kinase and H_2O to 20 μl. The reaction mixtures were incubated for 1 h at 37°C and then at 65°C for 10 min to inactivate the kinase.

For the annealing step, 0.1 μg of selection oligo, 0.2 μg mutagenic oligo and 0.1 μg TRC/HMG2 were mixed with 2 μl 10x annealing

Table 1. Oligonucleotides utilized in these studies. Oligonucleotide sequences are oriented 5′ to 3′. *Altered Sequence* indicates the new codon (underlined bases) in mutant constructs and the new sequence generated at the Sca1 site. Bases in bold type indicate changes in nucleotide sequence. The sequence of each mutant was confirmed by DNA sequence analysis as described in Materials and methods. *Amino acid substitution* indicates the new amino acid (Ser) present at positions 23, 45 and 106 of HMG2. *Sca1 site* indicates the sequence of selection oligonucleotides utilized. This oligonucleotide alters the unique Sca1 site (underlined bases, ↓) present at position (1378–1383) in the expression vector

Position altered	Oligonucleotide	Altered sequence	Amino Acid Substitution
Cys_{23}	CTCTT CCG GC**T**GGTCTGCACGAA	**T**GC → **A**GC	$Cys_{23} \rightarrow Ser_{23}$
Cys_{45}	TCTCTCCGAA**CT**CTTCTTGGAGAA	**T**GT → **A**GT	$Cys_{45} \rightarrow Ser_{45}$
Cys_{106}	ATGTTCAGA**GCT**AAACAGGAAGAA	**T**GC → **A**GC	$Cys_{106} \rightarrow Ser_{106}$
Sca1 site	CTGGTGAGT**ATT**CAA CCA AGTC	AGT↓**A**CT → AGTA**TT**	

buffer (500 m*M* NaCl, 200 m*M* Tris-HCl, pH 7.5, 100 m*M* $MgCl_2$) and H_2O to a final volume of 20 µl. The mixture was placed in boiling water for 3 min and then immediately placed on ice for 5 min.

Synthesis of mutant strand

For mutant strand synthesis, 3 µl of 10x synthesis buffer (66 m*M* Tris-HCl, pH 7.5, 2.5 m*M* deoxynucleotide triphosphate), 4 U of T4 DNA polymerase, 6 U T4 DNA ligase, and dH_2O were added to the annealing reaction mixture for a final volume of 30 µl. The mixture was incubated (37°C, 2 h) and then stopped by the addition of 3 µl stop solution (5 m*M* EDTA, 0.25% SDS). Samples were incubated at 65°C for 5 min, to inhibit ligase activity.

Bacterial transformation and mutant screening

E. coli (BMH 71–18 mut S) was transformed with the synthesis reaction mixture (diluted 1 : 4 with sterile water) by electroporation (2.5 kV; 0.2 cm cuvettes). Transformants were selected on LB-ampicillin plates. The plates were allowed to grow overnight at 37°C, and colonies were pooled by adding 5 ml Luria broth to the plate and shaking (5 min) on a platform shaker. The bacterial suspension was removed from the plate and pelleted by centrifugation. Plasmid DNA was isolated, digested with 20 U Sca 1, and subsequently used to transform competent *E. coli* (DH5α). The transformed bacteria were then plated on LB-ampicillin plates. Since supercoiled plasmid DNA is more efficient than linear DNA at transforming *E. coli*, those constructs resistant to Sca 1 digestion (by virtue of the fact that the Sca 1 restriction site had been altered) would preferentially transform recipient bacteria. Individual colonies were picked, overnight cultures were prepared, and plasmid DNA was isolated, digested with Sca 1 and analyzed on 0.7% agarose gels. Plasmids resistant to Sca I cleavage were sequenced using a CircumVent thermal cycle dideoxy DNA sequencing kit (New England Bio Laboratories, Beverly, Mass.), according to the manufacturer's instructions, to confirm the presence of the introduced mutation (Table 1).

Expression and purification of recombinant proteins

Recombinant proteins were purified by affinity chromatography on a Ni chelate column [21]. *E. coli* (strain DH5α) was transformed with wild-type and mutant HMG2 expression constructs and selected on LB-ampicillin plates. Bacteria containing each expression construct were grown overnight at 37°C in 10 ml LB broth containing ampicillin (50 µg/ml). The overnight cultures were then used to inoculate 500 ml LB broth containing ampicillin. These cultures were grown to an OD_{600} of 0.7 and induced with 1 m*M* IPTG for 4 h, after which phenylmethylsulfonylfluoride (final concentration 0.5 m*M*) was added to inhibit proteolytic degradation. The bacteria were pelleted by centrifugation (4000 *g*, 10 min, 4°C), the supernatant was removed and the pellets were resuspended in 5 ml loading buffer (50 m*M* sodium phosphate, pH 8, 300 m*M* NaCl, 5 m*M* imidazole, 10 m*M* βME]. Bacteria were disrupted by sonication (1 min, on ice)**.** Triton X-100 was next added to a final concentration of 1% and the samples were placed on a rocker platform (15 min, 4°C). The suspensions were centrifuged (10 000 *g*, 10 min, 4°C) and the supernatants were filtered through a 0.45 µm membrane filter. The filtered lysate was applied to a column containing 1 ml of Ni-NTA Resin (Qiagen, Chatsworth, Calif.), previously equilibrated with loading buffer. (Recombinant proteins bind to the nickel column due to the presence of six His residues present in the fusion protein.) The column was washed with 10 ml loading buffer followed by 10 ml wash buffer (50 m*M* sodium-phosphate, pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole, 10 m*M* βME], and bound protein was eluted with 5 ml Elution buffer (300 m*M* imidazole). The eluted protein was diluted with 2 vols distilled water and applied to a monoQ anion exchange column (0.5 ml bed volume). Recombinant HMG2 proteins eluted from the column with 25 m*M* Tris (pH 7.5) and 325 m*M* NaCl. The yield of protein from the C45S mutant construct was consistently lower than C23S and C106S. Purified recombinant protein was used in binding studies.

Results

The first set of experiments was designed to determine the fate of Pt-DNA adducts following protein binding. Purified HMG protein was incubated with CDDP-DNA. Under these conditions, the HMG–CDDP-DNA complexes are stable in 0.5 *M* NaCl [5]. However, we observed that bound protein could be removed from CDDP-DNA by incubation in KI. Treatment of HMG–CDDP-DNA complexes with 1 *M* KI (50°C, 30 min) resulted in the elution of $> 95\%$ of bound protein (Fig. 1). The CDDP-DNA was subsequently utilized as substrate in a second round of protein binding experiments. We observed that HMG1 and HMG2 bound to CDDP-DNA previously incubated with HMG proteins (Fig. 1). Hence, these results indicate that the Pt-DNA adducts remain following protein binding and removal.

To further assess the effect of protein binding on Pt adducts, CDDP-DNA was incubated with HMG protein, the protein was removed using a commercial DNA

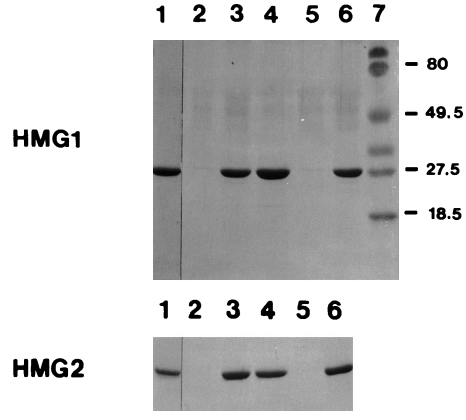

Fig. 1 Binding and elution of HMG1 and HMG2 from CDDP-DNA. *Lane 1* input protein; *lane 2*, protein binding to undamaged DNA; *lane 3*, protein binding to CDDP-DNA; *lane 4*, protein binding to CDDP-DNA pretreated with 1 *M* KI (30 min, 50°C). CDDP-DNA was incubated with HMG1 or HMG2, the protein–DNA complexes were washed and subsequently incubated in KI to remove bound protein. The amount of protein remaining bound to CDDP-DNA following KI treatment is shown in *lane 5*. The CDDP-DNA previously incubated with HMG protein followed by treatment with KI (*lane 5*) was subsequently used as substrate DNA in a second round of binding assays (*lane 6*). *Lane 7*, M_r standards. Numbers on right, M_r in kDa. *Upper gel*, HMG1; *lower gel*, HMG2. Note that KI stripped bound protein from CDDP-DNA (*lane 5*) and HMG1 and HMG2 bound to CDDP-DNA which had been previously incubated with HMG protein (*lane 6*).

Table 2. Platinum adducts in DNA samples before and after HMG protein binding. Puc 19 plasmid DNA was treated with CDDP ($R_f = 0.4$) for 18 h in 1 m*M* phosphate buffer (pH 7.4), 3 m*M* NaCl and subsequently precipitated by the addition of NaCl and 2 vols ethanol. The DNA was resuspended in TE buffer and utilized as substrate for HMG protein binding. DNA was incubated in TE buffer in the absence or presence of HMG1 (10 μg) and HMG2 (10 μg) for 30 min and deproteinated using a GeneClean II kit (BIO 101, La Jolla, Calif.). The amount of Pt present was determined by atomic absorption spectrometry. Values are average obtained from two determinations

Sample	Treatment[2]	Pt adducts/plasmid
1.	None	12.39 ± 0.37
2.	HMG1 and HMG2	10.23 ± 1.16

purification resin, by direct chloroform extraction, or incubation in 1 *M* KI followed by chloroform extraction. The amount of Pt bound to the DNA was determined by atomic absorption. The amount of Pt present on the DNA was the same before and after incubation with purified HMG1 and HMG2 (Table 2, and data not shown). Hence, protein binding had no effect on Pt adducts under these conditions.

The next series of studies were to determine whether HMG protein binding to CDDP-DNA was reversible. Purified HMG1 and HMG2 were incubated with CDDP-DNA, the protein was stripped from the CDDP-DNA by incubation in 1 *M* KI, dialyzed, concentrated and used in a second round of binding experiments. In these experiments, HMG1 and HMG2, removed from CDDP-DNA, were capable of binding to CDDP-DNA with the same relative affinity as native protein (Fig. 2). Therefore, these results demonstrate that protein binding is a reversible process.

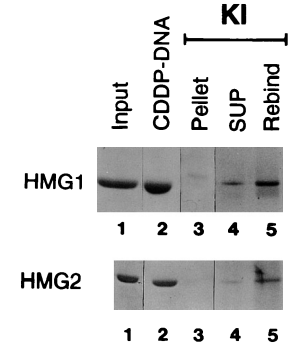

Fig. 2 Rebinding of HMG1 and HMG2 to CDDP-DNA. Purified HMG protein was incubated with CDDP-DNA and removed from CDDP-DNA by incubation in 1 *M* KI (50°C, 30 min). Protein removed from the CDDP-DNA was utilized in a second round of binding experiments (*lane 1*, input protein, *lane 2*, protein binding to CDDP-DNA, *lane 3*, protein remaining bound to CDDP-DNA following incubation in KI, *lane 4* protein recovered from CDDP-DNA following KI incubation, *lane 5* protein stripped from CDDP-DNA was used in a second round of binding experiments; *upper gel* HMG1, *lower gel*, HMG2). Note that protein removed from CDDP-DNA (*lane 4*) rebound to CDDP-DNA (*lane 5*).

In previous study, we demonstrated that thiol-modifying reagents (such as N-ethylmaleimide or Ellman's reagent) inhibit HMG protein binding to CDDP-DNA [5]. In contrast, preincubation of HMG1 or HMG2 with βME has no effect on protein binding [5]. These results suggest that Cys residues are involved in protein binding to CDDP-DNA or that reduced Cys residues maintain the proper protein conformation necessary for adduct recognition.

To more closely examine the role that Cys residues play in the binding of HMG2 to CDDP-DNA, site-directed mutagenesis studies were undertaken. HMG2 contains three Cys residues (Fig. 3) at positions 23, 45 and 106 [21]. For these experiments, each Cys residue was selectively converted to a Ser. Each mutant construct was sequenced to verify the presence of the introduced amino acid substitution. Bacteria were transformed with each construct, and recombinant proteins were purified and utilized in binding studies. The amount of protein binding to CDDP-DNA was quantitated by scanning the stained polyacrylamide gels in an LKB densitometer.

The recombinant wild-type HMG2 binds to CDDP-DNA with the same affinity as native HMG2 from calf thymus (Fig. 4; [21]). Conversion of Cys residue 23 to a Ser had little effect on protein binding. However, replacement of Cys 45 or 106 with Ser resulted in a significant reduction in protein binding. Mutant proteins HMG2C45S and HMG2C106S exhibited

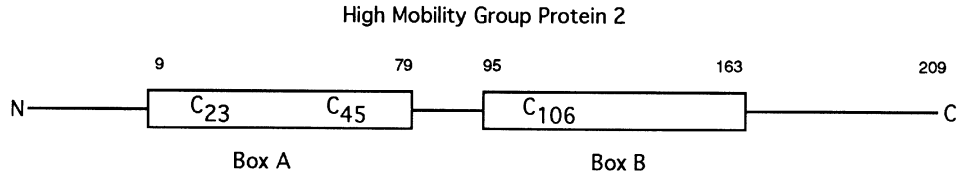

Fig. 3 Schematic diagram of HMG2. The two HMG boxes (*Box A* and *Box B*) are indicated by the boxed regions. The three Cys (*C*) residues at positions 23, 45 and 106 are indicated. Numbers on top of the figure indicate amino acid residues from the N-terminal (*N*) initiation Met (also refer to Table 1 for details of site-specific mutagenesis)

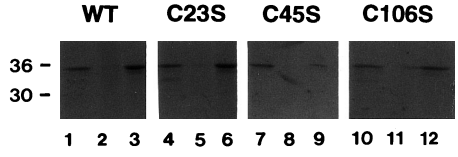

Fig. 4 Binding of Mutant HMG2 proteins to CDDP-DNA. Wild type and mutant HMG2 proteins were incubated with DNA or CDDP-DNA, bound protein was eluted and analyzed on SDS-polyacrylamide gels. Input protein, Lanes 1, 4, 7 and 10. Ten times as much protein present in each input lane was utilized in binding reactions with untreated DNA (lanes 2, 5, 8 and 11) and CDDP-DNA (lanes 3, 6, 9 and 12). Lanes 1–3, wild type (WT) *r*HMG2, lanes 4–6, HMG2C23S, Lanes 7–9, HMG2C45S, Lanes 10–12, HMG2C106S. Under the conditions used for these experiments, binding of WT *r*HMG2 to CDDP-DNA was $20 \pm 3\%$ of input protein. Note that HMG2C45S and HMG2C106S have reduced affinity for CDDP-DNA. Results presented are those obtained from a representative experiment.

$25 \pm 10\%$ and $50 \pm 8\%$ binding to CDDP-DNA relative to control, respectively (Fig. 4).

Discussion

CDDP is a clinically effective chemotherapeutic agent utilized in the treatment of a variety of human cancers. We and others have reported that HMG1 and HMG2 bind with high affinity to CDDP-DNA [17, 25]. In the current study, we further characterized protein binding.

In these studies, we observed that CDDP-DNA can be re-utilized as substrate for protein binding studies (Fig. 1), and further, atomic absorption spectrometry of the samples indicated that the same amount of Pt was present on the DNA before and after protein binding (Table 2). We also observed that bound protein could be removed from CDDP-DNA and subsequently utilized in a second round of binding experiments (Fig. 2). Hence, these results indicate that: (1) the Pt-DNA adducts remain intact following the binding and removal of HMG1 and HMG2 from CDDP-DNA, and (2) HMG1 and HMG2 are capable of reversibly binding to CDDP-DNA.

Site-specific mutagenesis studies indicated that specific Cys residues play an essential role in protein binding. Substitution of Cys 45 or 106 with Ser residues resulted in a significant reduction in protein binding (Fig. 4). These results are in agreement with previous work demonstrating that thiol-modifying reagents reduce HMG protein affinity for CDDP-DNA [5].

The precise mode of binding of HMG2 with CDDP-DNA is not known. HMG1 and HMG2 bind to the minor groove in DNA and recognize bent DNA structures [4, 13, 15, 20]. CDDP forms intrastrand adducts between adjacent purines in genomic DNA. The resultant adducts (*cis*-GpG and *cis*-ApG) result in the formation of DNA structures containing a 32–34° bend towards the major groove [3, 9]. The high affinity binding of HMG proteins to CDDP-DNA probably results from the fact that platinum binding mimics the bent or kinked DNA structure which is recognized by HMG box-containing proteins [4, 13, 15]. In previous study, we demonstrated that HMG boxes A and B in HMG2 are required for high-affinity protein binding to CDDP-DNA [21]. Cys 45 and 106 reside in HMG box A and box B, respectively [21]. Once HMG2 binds to the bent structure resulting from *cis*-Pt-DNA adduct formation, the free thiol groups on these Cys residues could stabilize protein binding by interacting with the Pt adduct.

Potentially relevant to our finding that Cys residues play an important role in HMG protein binding are observations that tumor cells resistant to CDDP toxicity have increased levels of glutathione [23, 28] and glutathione S-transferase activity [14]. Further, depletion of intracellular glutathione with buthionine sulfoximine has been shown to potentiate the cytotoxic activity of CDDP [23, 28]. Alterations in intracellular reduced glutathione (GSH) levels would be expected to alter the cellular redox environment. For example, cells with lower GSH levels would presumably be under increased oxidative stress, resulting in oxidation of protein thiols in HMG1 and HMG2 and decreased binding to CDDP-DNA. In contrast, cells with high GSH levels would have a stronger reductive environment, serving to reduce protein thiols and in the case of HMG1 and HMG2, could result in increased protein binding to CDDP-DNA.

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References

1. Alberts DS, Garcia D, Mason-Liddil N (1991) Cisplatin in advanced cancer of the cervix: an update. Semin Oncol 18:11–24

2. Andrews PA, Howell SB (1990) Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. Cancer Cells 2:35–43
3. Bellon SF, Lippard SJ (1990) Bending studies of DNA site-specifically modified by cisplatin, trans-diamminedichloroplatinum (II) and cis-$[Pt(NH_3)_2(N3\text{-cytosine})Cl)]^+$. Biophys Chem 35:179–188
4. Bianchi ME, Falciola L, Ferrari S, Lilley DMJ (1992) The DNA binding site of HMG1 is composed of two similar segments (HMG boxes), both of which have counterparts in other eukaryotic regulatory proteins. EMBOJ 11:1055–1063
5. Billings PC, Davis RJ, Engelsberg BN, Skov KA, Hughes EN (1992) Characterization of High mobility group protein binding to cisplatin-damaged DNA. Biochem Biophys Res Comm 188:1286–1294
6. Billings PC, Engelsberg BN, Hughes EN (1992) Proteins binding to cisplatin damaged DNA in human cell lines. Cancer Invest 12:597–604
7. Bustin M, Lehn DA, Landsman D (1990) Structural features of the HMG chromosomal proteins and their genes. Biochem Biophys Acta 1049:231–243
8. Chow CS, Whitehead, JP Lippard SJ (1994) HMG domain proteins induce sharp bends in cisplatin-modified DNA. Biochemistry 33:15124–15130
9. Chu G (1994) Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J Biol Chem 269:787–790
10. Clark JR, Dreyfuss, AI (1991) The role of cisplatin in treatment regimens for squamous cell carcinoma of the head and neck. Semin. Oncol. 18:34–48
11. Deng WP, Nickoloff JA (1992) Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. Anal. Biochem 200:81–88
12. Einhorn LH (1991) Treatment of testicular cancer: a new and improved model. J. Clin. Oncol. 8:1777–1781
13. Falciola L, Murchie AIM, Lilley DMJ, Bianchi ME (1994) Mutational analysis of the DNA binding domain A of chromosomal protein HMG1. Nucl Acids Res 22:285–292
14. Godwin AK, Meister, A, O'Dwyer PJ, Huang CS, Hamilton TC, Anderson ME (1992) High resistance to cisplatin in human ovarian cancer is associated with marked increase in glutathione synthesis. Proc Natl Acad Sci USA 89:3070–3074
15. Grosschedl R, Giese K, Pagel J (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet 10:94–100
16. Huang JC, Zamble DB, Reardon JT, Lippard SJ, 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 USA 91:10394–10398
17. Hughes EN, Engelsberg BN, Billings PC (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
18. Jantzen HM, Admon A, Bell SP, Tjian R (1990) Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. Nature 344:830–836
19. Laemmli UK (1970) Cleavage of structural proteins during the assembly of bacteriophage T4. Nature 227:680–685
20. Landsman D, Bustin M (1993) A signature for the HMG-1 box DNA-binding proteins. Bioessays 15:539–546
21. Lawrence DL, Engelsberg BN, Farid RS, Hughes EN, Billings PC (1993) Localization of the binding region of high mobility group protein 2 to cisplatin-Damaged DNA. J Biol Chem 268:23940–23945
22. Leichman L, Berry BT (1991) Experience with cisplatin in treatment regimens for esophageal cancer. Semin Oncol 18:64–72
23. Ozols RF (1990) New approaches to management of ovarian cancer. Adv Oncol 6:9–17
24. Ozols RF, Young RC (1991) Chemotherapy of ovarian cancer. Semin Oncol 18:222–232
25. Pil PM, Lippard SJ (1992) Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. Science 256:234–237
26. Raghavan D, Pearson B, Watt WH, Mameghan H, Langdon P, Eisinger D, Wines R (1991) Cytotoxic chemotherapy for advanced bladder cancer: cisplatin containing regimens. Semin Oncol 18:56–63
27. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell BR, Goodfellow PN (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240–244
28. Timmer-Bosscha M, Mulder NH, Vries EGE de (1992) Modulation of cis-diamminedichloroplatinum (II) resistance: a review. Br J Cancer 66:227–238
29. Turrisi III AT (1991) The integration of platinum and radiotherapy in the treatment of lung cancer. Semin Oncol 18:81–87