Histone H1 Interacts Preferentially with DNA Fragments Containing a Cisplatin-Induced 1,2-Intrastrand Cross-Link

Julia N. Yaneva[a], Elena G. Paneva[a], Siyka I. Zacharieva[b], and Jordanka Zlatanova[c,*]

[a] Department of Gene Regulations, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[b] Department of Immunology, Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[c] Department of Molecular Biology, University of Wyoming, 1000 East University Avenue, Laramie, WY 82071, USA. E-mail: jordanka@uwyo.edu

* Author for correspondence and reprint requests

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Cisplatin [*cis*-diamminedichloroplatinum(II) or *cis*-DDP], but not its stereoisomer transplatin, is suggested to be among the most powerful anticancer agents. It is believed that its therapeutic activity results from its interaction with DNA forming *intra*- and *inter*strand cross-links. During our earlier investigations, we have observed a prominent preference of the linker histone H1 for binding to *cis*-platinated DNA (containing several different cross-links along the DNA fragment) compared with unmodified or transplatin-modified DNA. This report presents our recent experimental data obtained by band-shift analysis on the binding of H1 to a cisplatin-modified synthetic 34 bp DNA fragment containing a single target d(GG/CC) for 1,2 *cis-intra*-platination. Results obtained with another nuclear protein with similar DNA-binding properties, HMGB1, are also presented. The experimental data throw light on the precise preference of histone H1 for binding to different types of cisplatin-created cross-links in DNA.

Key words: Cisplatin, DNA, Histone H1

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II), *cis*-DDP] is an effective chemotherapeutical drug widely used to cure a variety of malignant diseases (Jamieson and Lippard, 1999). The therapeutic effect of the compound is suggested to arise through its covalent linkage with DNA, thus interfering the protein/DNA interactions (Zlatanova *et al*., 1998).

During to the past decade we have been characterizing the interaction of the linker histones (LHs) – H1, H1°, and H5 – with DNA modified by the platinum compounds cisplatin and its therapeutically inactive stereoisomer transplatin. In the case of globally cisplatin-modified DNA fragments (natural and synthetic) containing many and different types of platinum adducts, we observed an apparent preference of these very lysine-rich histones for binding to cisplatin-damaged DNA compared with the same unmodified or transplatin-modified DNA fragment (Yaneva *et al*., 1997; Paneva *et al.*, 1998). These experiments, however, did not address any preference that H1 may have for binding to particular type(s) of cisplatin-created adducts in DNA. That is why it was intriguing to investigate the nature of interaction of LH with DNA containing a single particular type of cisplatin modification: the predominantly formed intrastrand 1,2(dGpG, dApG) and 1,3-(dGpXpG), and interstrand (dGpC/dCpG) cross-links (CLs). Surprisingly, in our recent work we did not observe any preference of the histones H1, H5 or its globular domain GD5 for interaction with cisplatin-damaged DNA bearing interstrand cross-links (Yaneva *et al*., 2006). Our efforts are now concentrated on detailing the binding affinity of LHs to different kinds of DNA intrastrand CLs created by cisplatin: 1,2(dGpG, dApG) and 1,3-(dGpXpG).

In this report, we present data on the interaction of linker histone H1 with a synthetic 34 bp DNA fragment, designed specifically to contain a unique site d(GG/CC) for a single intrastrand cisplatin-induced cross-link. Comparative data with another nuclear protein showing similar DNA-binding properties as LHs – high-mobility group box protein 1 (HMGB1) – are also presented.

Experimental

Two complementary, 34 nucleotides long ss DNA fragments were custom-synthesized on an Applied Biosystem 380B DNA synthesizer using the phosphoramidite technique. Double stranded 34 bp DNA fragment with a unique site (in bold) for 1,2 *cis-intra*-platination 5′-GTTGATTGAT-

Abbreviations: AA, acrylamide; bp, base pairs in DNA; *cis*-DDP, *cis*-diamminedichloroplatinum(II) or cisplatin; CL, cross-link; ds, double-stranded; EDTA, ethylenediamine tetraacetate, sodium salt; EMSA, electrophoretic mobility-shift assay; HMGB1, high-mobility group box protein 1; LH, linker histones; PAGE, polyacrylamide gel electrophoresis; R_b, platinum to nucleotide molar ratio; SDS, sodium dodecyl sulphate; ss, single-stranded; Tris, tri(hydroxymethyl) aminomethane; w/w, weight/weight ratio.

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GTATAT**GG**TCATGTCATGATCAAC-3′ 3′-CAGACTAACTACATATACCAGTACAGTACTAGTTG-5′ was obtained by annealing equal stoichiometric amounts of the complementary ss fragments for 1 h at room temperature, in 50 mM phosphate buffer, pH 6. 8. Following precipitation with ethanol at −20 °C, the ds DNA was dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.3). DNA concentration was calculated using a coefficient of 20.0 ml cm^{-1} mg^{-1} after measuring the optical density at 260 nm. The completeness of the reassociation reaction was verified on native 15% PAGE (Sambrook and Russel, 2001).

Cisplatin was purchased from Sigma (MO, USA). A stock solution (10^{-4} M) was prepared by dissolving the compound in deionized water, and kept at 0 °C. The ds DNA fragment, containing a single defined d(GG/CC) site for the formation of a single 1,2 intrastrand CL, was treated with increasing amounts of cisplatin (the molar platinum to nucleotide ratio, R_b, varying from 0.010 to 0.100) in 10 mM Tris-HCl, pH 7.5, 5 mM NaCl containing 100 μg/ml DNA and *cis*-DDP from the stock. After overnight incubation at 37 °C, the cisplatin-treated DNA was precipitated with two volumes of ethanol at −20 °C.

A native sample of the linker histone H1 from mouse liver nuclei was prepared as described by Banchev et *al.* (1991). HMGB1 was isolated following the procedure of Adachi *et al*. (1990). The protein concentrations were determined spectrophotometrically using extinction coefficients (ml cm^{-1} mg^{-1}) of 1.85 for H1 at 230 nm and 1.0 for HMGB1 at 280 nm. The protein purity was checked on PAGE under denaturing conditions (15% AA, 0.1% SDS) in running buffer containing 0.39 M glycine, 0.05 M Tris-HCl, pH 8.3 (Sambrook and Russel, 2001).

Unplatinated or 1,2 *cis-intra*-CL-containing DNA duplexes were allowed to interact with histone H1 or HMGB1 for 20 min at room temperature in reaction mixtures containing 0.5 μg of DNA, 10 mM Tris-HCl, pH 7.8, 20 mM NaCl, 0.1 mM phenylmethylsulphonyl fluoride (PMSF) and increasing amounts of the protein. The abilities of H1 and HMGB1 to interact with both unplatinated and cisplatin-modified DNA fragments were analyzed by EMSA, on native 15% PAGE (AA to bis-AA 29:1) in TAE running buffer (40 mM Tris-HCl, pH 8.3, 25 mM sodium acetate, 1 mM EDTA). The electrophoresis was performed on 1 mm thick gels at 20 V/cm. Following electrophoresis, the gels were visualized by silver-staining and photographed.

Results and Discussion

The 34 bp DNA fragment (for nucleotide sequence, see Experimental) was designed to contain a single site d(GG/CC) in the middle, for the formation of one 1,2 *intra*-CL upon treatment with cisplatin. On this fragment, cisplatin creates a single covalently bound adduct between N7 of neighbouring guanines on the upper strand. After titration of DNA with cisplatin at different R_b ratios (0.010, 0.025, 0.050, 0.075, and 0.100) the samples were run on native 15% polyacrylamide gels to verify the completeness of the platination procedure. The modified DNA fragments containing the 1,2 *cis-intra*-CLs can be recognized on the gel as a retarded smeared band above the sharp band containing the intact DNA (data not shown). For further experiments, we chose the platinum to nucleotide ratio R_b of 0.025.

The interaction of histone H1 with either the unmodified DNA fragment or the fragment bearing a single 1,2 *cis-intra*-adduct was analyzed by means of EMSA. In this approach, the DNA complexed with the protein of interest might be detected as a retarded (shifted) band(s) in electrophoretic gels. Usually, at higher protein to DNA ratios, the linker histones aggregate the DNA in

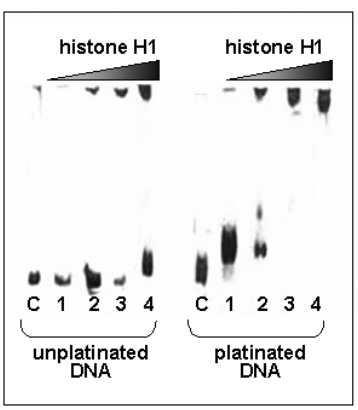

Fig. 1. Electrophoretic analysis of histone H1 interaction with 34 bp DNA fragments: unmodified (left) and 1,2 *cis-intra*-modified (R_b 0.025, right). Increasing amounts of histone H1: 0.25 (1); 0.50 (2); 0.75 (3) and 1.0 μg (4) were allowed to form complexes with 0.5 μg DNA; then the samples were fractionated on native 15% PAGE. Controls C_1 and C_2 contain intact and 1,2 *cis-intra*-modified naked DNA, respectively. For more information, see the text.

complexes that do not enter the gel (remain at the start position). The fragments of choice (unmodified or 1,2 *cis-intra*-modified) were incubated with increasing amounts of mouse liver histone H1 and the samples were run on native PAGE (Fig. 1). Visual inspection of the electrophoretic patterns showed that the cisplatin-modified DNA molecules were retarded almost completely even at a protein to DNA ratio of 0.5 (w/w) while the complex formation with the unmodified DNA was less effective even at a ratio of 2. 0. The cisplatin-induced 1,2*intra*-bifunctional adducts cause local distortions (bending and unwinding) in the DNA double helix: the unwinding angle was calculated to be 13°, and the bending angle –33° (Bellon *et al.*, 1991). Obviously, DNA molecules bearing this type of CLs are preferred partners for interaction with H1 over unmodified DNA. It should be noted that H1 binding by itself induces unwinding and DNA bending; thus, pre-existing distortions in the DNA that mimic the DNA conformation in H1/DNA complexes would be expected to be preferred H1 partners.

Since high-mobility group box nuclear proteins HMGB1 and HMGB2 display similar DNA-binding properties as H1 and interact preferentially with both *cis-intra-* and *cis-inter*-modified DNAs (reviewed by Jamieson and Lippard, 1999), we have also checked, under the conditions of our experiments, the affinity of HMGB1 for interaction with the same fragments (unmodified and 1,2 *cis-intra*-modified). Fig. 2 demonstrates that under the conditions of our assay HMGB1 interacted with 1,2 *cis-intra*-modified DNA more effectively than with unmodified DNA. The retarded complexes of the protein with the platinated DNA are visible as a band shift on the gel even at a w/w protein/DNA ratio of 1.0 (Fig. 2, right), while shifted bands with unmodified DNA are barely observed at a ratio of 2.5 (Fig. 2, left). Similar results with other fragments bearing 1,2 *cis-intra*-CLs were reported earlier (Kane and Lippard, 1996; Dunham and Lippard, 1997). We have previously observed that the linker histone H1 possesses greater than HMGB1 affinity for recognizing and binding to another synthetic 34 bp DNA fragment with many different sites for *cis*-platination, both *intra-* and *inter-* (Yaneva *et al.*, 1997).

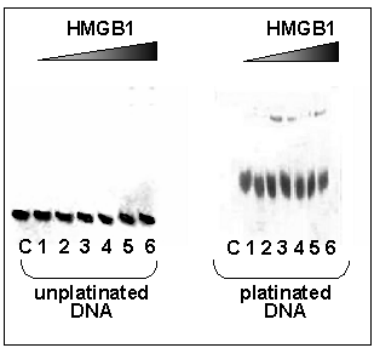

Fig. 2. Titration of 0.5 μg of 34 bp DNA fragment: unmodified (left) and 1,2 *cis-intra*-platinated (R_b 0.025, right) with increasing amounts of HMGB1. The protein to DNA ratios (w/w) from left to right are: 0.5 (1); 1.0 (2); 1.5 (3); 2.0 (4); 2.5 (5); 3.0 (6). The samples were fractionated on 15% PAGE. Controls C_1 and C_2 show the migration of unmodified and cisplatin-modified DNA in the absence of protein.

Our present results indicate that the linker histone H1 recognizes and binds more effectively to DNA bearing 1,2 *intra* d(GpG) cisplatin adducts than to unmodified DNA. To our knowledge, this is the first report of DNA damage recognition by a histone molecule, involving a specific type of cisplatin-induced DNA lesion. Although the biological importance of H1 binding to certain cisplatin adducts is still unclear (Zlatanova *et al.*, 1998), it is possible that the cell handles existing *cis-intra*-DNA cross-links differently from such cross-links bound by proteins. Such a scenario has been recently suggested by Chvalova *et al.* (2007) who demonstrated that DNA containing cisplatin-induced cross-links can be replicated by translesion DNA synthesis, whereas H1 bound to such cross-links completely inhibited such synthesis.

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Adachi Y., Mizuno S., and Yoshida M. (1990), Efficient large-scale purification of non-histone chromosomal proteins HMG1 and HMG2 by using polybuffer exchange. J. Chromatogr. **530**, 39–46.

Banchev T., Srebreva L., and Zlatanova J. (1991), Purification of histone H1° and its subfractions under nondenaturing conditions. Biochim. Biophys. Acta **1073**, 230–232.

Bellon S. F., Coleman J. H., and Lippard S. J. (1991), DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug *cis*-diamminedichloroplatinum(II). Biochemistry **30**, 233–240.

Chvalova K., Brabec V., and Kasparkova J. (2007), Mechanism of the formation of DNA-protein cross-links by antitumor cisplatin. Nucleic Acids Res. **35**, 1812–1821.

Dunham S. U. and Lippard S. J. (1997), DNA sequence context and protein composition modulate HMG-domain protein recognition of cisplatin-modified DNA. Biochemistry **36**, 11428–11436.

Jamieson E. R. and Lippard S. J. (1999), Structure, recognition, and processing of cisplatin-DNA adducts. Chem. Rev. **99**, 2467–2498.

Kane S. A. and Lippard S. J. (1996), Photoreactivity of platinum(II) in cisplatin-modified DNA affords specific cross-links to HMG domain proteins. Biochemistry **35**, 2180–2188.

Paneva E. G., Spassovska N. C., Grancharov K. C., Zlatanova J. S., and Yaneva J. N. (1998), Interaction of histone H1 with *cis*-platinum-modified DNA. Z. Naturforsch. **53c**, 135–138.

Sambrook J. and Russel D. W. (2001), Molecular Cloning. A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, New York.

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

Yaneva J. N., Paneva E. G., Zacharieva S. I., and Zlatanova J. S. (2006), Linker histones do not interact with DNA containing a single interstrand cross-link created by cisplatin. Z. Naturforsch. **61c**, 879–883.

Zlatanova J., Yaneva J., and Leuba S. (1998), Proteins that specifically recognize *cis-Pt*-damaged DNA: a clue to anticancer activity of cisplatin. FASEB J. **12**, 791–799.