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Recognition of Platinum−DNA Damage by Poly(ADP-ribose) Polymerase-1†

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ABSTRACT: Poly(ADP-ribose) polymerase-1 (PARP-1) was recently identified as a platinum−DNA damage response protein. To investigate the properties of binding of PARP-1 to different platinum−DNA adducts in greater detail, biotinylated DNA probes containing a site-specific cisplatin 1,2-d(GpG) or 1,3-d(GpTpG) intrastrand cross-link or a cisplatin 5′-GC/5′-GC interstrand cross-link (ICL) were utilized in binding assays with cell-free extracts (CFEs) in vitro. The activated state of PARP-1 was generated by treatment of cells with a DNA-damaging agent or by addition of NAD^+ to CFEs. PARP-1 binds with a higher affinity to cisplatin-damaged DNA than to undamaged DNA, and the amount of protein that binds to the most common cisplatin−DNA cross-link, 1,2-d(GpG), is greater than the amount that binds to other types of cisplatin−DNA cross-links. Both DNA damage-activated PARP-1 and unactivated PARP-1 bind to cisplatin-damaged DNA, and both automodified PARP-1 and cleaved PARP-1 bind to cisplatin−DNA lesions. The role of poly(ADP-ribose) (pADPr) in mediating binding of PARP-1 to platinum damage was further investigated. The extent of binding of PARP-1 to the cisplatin 1,2-d(GpG) cross-link decreases upon automodification, and overactivated PARP-1 loses its affinity for the cross-link. Elimination of pADPr facilitates binding of PARP-1 to the cisplatin 1,2-d(GpG) cross-link. PARP-1 also binds to DNA damaged by other platinum compounds, including oxaliplatin and pyriplatin, indicating protein affinity for the damage in an adduct-specific manner rather than recognition of distorted DNA. Our results reveal the unique binding properties for binding of PARP-1 to platinum−DNA damage, providing insights into, and a better understanding of, the cellular response to platinum-based anticancer drugs.

Platinum-based chemotherapy has been widely used in the clinic for more than 30 years. Three FDA-approved platinum anticancer drugs, cisplatin, oxaliplatin, and carboplatin, either alone or in combination with other anticancer drugs, have been used to treat numerous patients with a variety of cancers (*1*). These bifunctional platinum drugs attack DNA to form both intrastrand and interstrand cross-links (ICLs)[1] that block transcription and trigger apoptosis (*2*). Cisplatin forms 1,2-d(GpG) cross-links as the major adduct, with 1,3-d(GpTpG) cross-links and ICLs appearing less frequently (*3*). Very recently, a monofunctional platinum compound, *cis*-diammine(pyridine)chloroplatinum(II) [cDPCP or pyriplatin (Figure 1)], exhibited anticancer activity against colorectal cancer cells overexpressing an organic cation transporter and a significantly different mechanism of transcription inhibition of RNA polymerase II compared to that of cisplatin (*4*, *5*).

Poly(ADP-ribose) polymerases (PARPs) make up a family of proteins present in eukaryotes. PARP-1 is the most studied PARP, playing pivotal roles in DNA replication, transcriptional regulation, and DNA damage repair. PARP-1 is one of the most abundant non-histone nuclear proteins, with 10^5−10^6 copies per cell (*6*). PARP-1 contains three domains. There is a DNA-binding domain (DBD) containing two zinc finger motifs, an automodification domain (AMD) that functions as the target of direct covalent self-modification, and a carboxyl-terminal catalytic domain that polymerizes linear or branched chains of ADP-ribose onto acceptor proteins. PARP-1 utilizes nicotinamide adenine dinucleotide (NAD^+) as the substrate to add pADPr onto itself (automodification) or other acceptor proteins (hetero- or transmodification), including histone H1 and transcription factors. Once polymerized, pADPr is hydrolyzed by poly(ADP-ribose) glycohydrolase (PARG) (*7*).

PARP-1 functions in the DNA damage response. Whereas the catalytic activity of PARP-1 is low under physiological conditions, PARP-1 activity is highly upregulated following DNA damage (*8*). Such PARP-1 activation recruits DNA damage response proteins to the sites of modification. These proteins include XRCC1 and DNA ligase III, which then trigger the base excision repair (BER) pathway. If the DNA is severely damaged, PARP-1 will initiate necrosis by depletion of NAD^+ and ATP in the cells (*9*, *10*).

The development of PARP-1 inhibitors as anticancer drugs is an emerging field of drug discovery. Several PARP inhibitors

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[1]Abbreviations: AMD, automodification domain; ADP-HPD, adenosine diphosphate (hydroxymethyl)pyrrolidinediol; BER, base excision repair; cDDP, *cis*-diamminedichloroplatinum(II); cDPCP, *cis*-diammine(pyridine)chloroplatinum(II) or pyriplatin; CFE, cell-free extract; DBD, DNA-binding domain; dsDNA, double-stranded DNA; DOXO, doxorubicin; EMSA, electrophoretic mobility shift assay; ICL, interstrand cross-link; LB, lysis buffer; MMR, mismatch repair; NAD^+, nicotinamide adenine dinucleotide; NER, nucleotide excision repair; PAGE, polyacrylamide gel electrophoresis; pADPr, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP-1, poly(ADP-ribose) polymerase-1; XRCC1, X-ray repair cross complementing 1.

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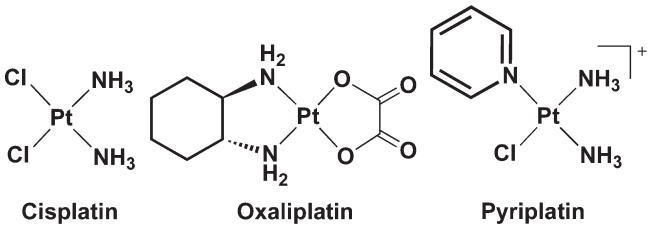

FIGURE 1: Chemical structures of platinum compounds cisplatin, oxaliplatin, and pyriplatin.

have entered into clinical trials for various types of solid tumors, especially BRCA-mutated breast or ovarian cancers (*11*−*13*). Inhibitors targeting the catalytic domain of PARP prevent polymerization of pADPr during DNA damage and thereby block the recruitment of other DNA damage response proteins. PARP-1 inhibition therefore results in synthetic lethality of tumors with genetic lesions in DNA repair pathways such as BRCA1/2 because multiple DNA damage repair pathways are blocked. Because cisplatin treatment of cancer cells results in DNA damage, combination therapy with cisplatin and PARP-1 inhibitors in DNA damage repair-deficient cells could similarly result in symbiotic lethality. The strategy of using cotreatment with PARP-1 inhibitors has made it feasible to overcome platinum-based drug resistance. Thus, PARP-1 inhibitors sensitize specific cancer cell types to cisplatin. For example, the PARP inhibitor AZD2281 sensitizes BRCA2-deficient cells but not BRCA2-proficient cells to cisplatin (*14*).

Recently, we identified PARP-1 as a protein that binds to platinum-damaged DNA using $PtBP_6$, a photoreactive analogue of cisplatin. PARP-1 binds to $PtBP_6$ 1,2-d(GpG) and 1,3-d(GpTpG) intrastrand cross-links and to 5′-GC/5′-GC ICLs (*15*−*17*). The photo-cross-linking efficiency is higher in the presence of PARP-1 inhibitors, suggesting that PARP-1 mediates the binding of damage response proteins (*18*). However, the specificity of PARP-1 binding to different cisplatin−DNA cross-links and the potential binding of PARP-1 to DNA damage by other platinum anticancer compounds have not yet been investigated. Moreover, the effect of automodification of PARP-1 by pADPr to platinum-damaged DNA needs further elucidation.

To provide this information, in the present study we investigate the binding properties of PARP-1 to platinum-damaged DNA in greater detail. PARP-1 binding to a variety of cisplatin-DNA lesions was examined, and the relative affinity of PARP-1 for damaged versus undamaged double-stranded DNA (dsDNA) was studied. We also determined that PARP-1 recognizes oxaliplatin− and pyriplatin−DNA adducts. The effects of PARP-1 automodification on its binding to the platinum damage were further explored. The potential roles of PARP-1 in the platinum−DNA damage response are discussed.

EXPERIMENTAL PROCEDURES

Materials and Methods. All chemicals and solvents were purchased from commercial sources. Analytical and preparative HPLC was performed on an Agilent 1200 HPLC system. Polyacrylamide gel electrophoresis (PAGE) on DNA samples was performed on a Protean II xi Cell from Bio-Rad. SDS−PAGE analysis of protein samples was conducted using a Criterion Cell from Bio-Rad. UV−vis spectra were recorded on an HP 8453 spectrometer. Platinum analysis was performed by flameless atomic absorption spectrometry on a Perkin-Elmer AAnalyst 300 system. Western blots were run using the SuperSignal Western Blot system from Pierce (Rockford, IL). Rabbit anti-PARP-1 polyconal antibody was from Affinity Bioreagents (Rockford, IL). MyOne Streptavidin C1 Dynabeads were purchased from Invitrogen (Carlsbad, CA). Oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). cDPCP and [Pt(*R*,*R*-DACH)Cl_2] (DACH = 1,2-diaminocyclohexane) were kindly provided by K.S. Lovejoy from the Department of Chemistry at the Massachusetts Institute of Technology.

Preparation of Site-Specifically Modified Platinum−*DNA Probes*. Cisplatin-modified 1,2-d(GpG) and 1,3-d(GpTpG) probes were synthesized as reported previously (*19*) and characterized by native PAGE (Figure S1 of the Supporting Information). The cDDP ICL was prepared following published procedures (*17*). For oxaliplatin-modified DNA probes, we used [Pt(*R*,*R*-DACH)Cl_2] as the starting material. A 21 mM aqueous solution of [Pt(*R*,*R*-DACH)Cl_2] was activated by addition of 1.98 equiv of $AgNO_3$ followed by agitation overnight in the dark at room temperature. The suspension was centrifuged. To a solution of 0.21 mM top strand in 10 mM NaH_2PO_4 (pH 6.3), 1.2 equiv of activated [Pt(*R*,*R*-DACH)Cl_2] was added. The reaction mixture was incubated in the dark at 37 °C for 8 h. The reaction was stopped when the solution was frozen. PtDACH-modified top strand DNA was purified by ion exchange HPLC [Dionex DNAPac PA-100, linear gradient, 0.48 to 0.55 M NaCl in 25 mM Tris-HCl (pH 7.4) over 11 min]. After purification, the platinated DNA solution was dialyzed against H_2O and lyophilized. The platination level was confirmed by UV−vis and atomic absorption spectroscopy, which yielded a Pt/DNA ratio of 0.95 ± 0.04. A 5 nmol quantity of the platinated top strand was annealed to 1 equiv of the bottom strand containing a biotin moiety in a buffer comprising 0.25 mM NaCl, 2 mM $MgCl_2$, and 10 mM Tris-HCl (pH 7.4). The duplex was purified by a 12% native-PAGE. The cDPCP-modified duplex was synthesized as described previously (*4*).

Cell Culture and Transfection. HeLa S3 cells were grown in DMEM high glucose supplemented with 10% FBS, 1% penicillin, and streptomycin at 37 °C and 5% CO_2. Transfection was performed as described previously (*20*). Briefly, at the time of transfection, cells were resuspended at a concentration of 1×10^6 cells/mL in the medium without antibiotics. Cell cultures (40 mL) were transfected with 40 μg of eGFP-PARP-1 plasmid using 40 μL of 293fectin transfection reagent (Invitrogen, Carlsbad, CA) overnight. Cells were treated with 0.2 μM latrunculin B for 1 h to disrupt the microfilament network prior to cell lysis. For PARP-1 activation, cells were treated with 10 mM H_2O_2 for 30 min or with 5 μM doxorubicin (DOXO) (Sigma, St. Louis, MO) for 1 h prior to cell lysis.

Cell-Free Extract (*CFE*) *Preparation*. HeLa S3 cells were centrifuged at 400*g* for 3 min at 4 °C. Cell pellets were washed twice with ice-cold PBS prior to resuspension in cell lysis buffer (LB) containing 150 mM NaCl, 50 mM HEPES, 1 mM $MgCl_2$, 0.5% (v/v) Triton X-100, 1 mM DTT, and 1 mM EGTA (pH 7.4), with an EDTA-free protease inhibitor tablet (Roche, Branford, CT). For 1×10^7 cells, a 146 μL quantity of LB was used. The mixture was incubated on ice for 10 min and spun at 5000*g* for 5 min. The supernatant was collected and supplemented with 1 μM cytochalasin D and 1 μM nocodazole. For ARH3 or NAD^+ pretreatment conditions, CFE was incubated with either 0.1 mg/mL ARH3 at 4 °C for 30 min or 1 mM NAD^+ at 4 °C for 30 min before the binding assay (*20*).

PARP-1 Binding Assay. MyOne Streptavidin C1 Dynabeads were washed three times with LB. To a 50 μg quantity of

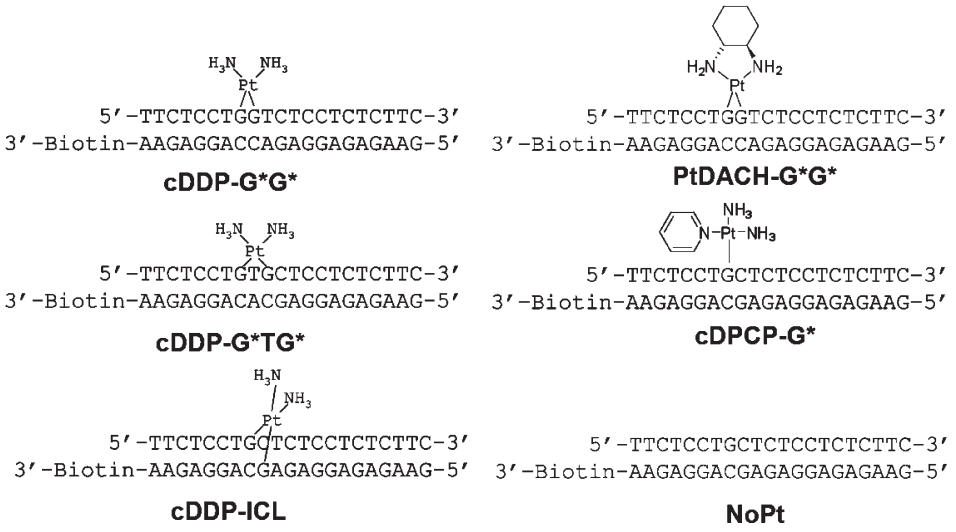

FIGURE 2: Sequences of the 21 bp duplexes containing site-specific platinum damage and biotin moiety.

Dynabeads was added 25 pmol of DNA probes. The mixture was incubated in LB at 25 °C for 30 min to allow binding of the DNA probes. The supernatant was removed with the help of a magnet. Measured amounts of CFE were incubated with the beads at 4 °C for 1 h. The mixture was then washed three times with LB. The protein mixture was released into 15 μL of 1× LDS loading buffer (Invitrogen) by being heated at 95 °C for 5 min. The samples were resolved on precast 4−12% Bis-Tris gels or by 8% SDS−PAGE. For the post-treatment of ARH3, the binding reaction mixture was washed twice with LB and treated with 0.1 mg/mL ARH3 for 30 min at 25 °C. The beads were washed twice with LB, and the protein mixture was released in LDS loading buffer.

RESULTS

Design of DNA Sequences. We chose to investigate 21 bp oligonucleotide duplexes containing site-specific damage by bifunctional or monofunctional platinum compounds (Figure 2). The sequences are identical in each of the probes except for, in the center of the duplex, a cisplatin 1,2-d(GpG) cross-link (cDDP-G*G*), a cisplatin 1,3-d(GpTpG) cross-link (cDDP-G*TG*), a cisplatin ICL (cDDP-ICL), an oxaliplatin 1,2-d(GpG) cross-link (PtDACH-G*G*), or a pyriplatin dG lesion (cDPCP-G*). In this manner, we were able to obtain the best comparison of PARP-1 binding to different kinds of platinum−DNA damage. A no-platinum control probe (NoPt) was also synthesized. Each of the probes contains a biotin moiety for binding to streptavidin-coated magnetic beads.

Synthesis and Characterization of 21 bp DNA Probes. For the synthesis of probes containing site-specific cisplatin/oxaliplatin intrastrand cross-links or a pyriplatin-modified dG, the top strand was platinated and purified first. Platinated top strands were annealed with biotinylated bottom strands to yield the duplexes. The purity of these duplexes was confirmed by a nondenaturing gel (Figure S1 of the Supporting Information). The cisplatin ICL probe was synthesized as described previously (*17*). An analytical denaturing PAGE gel revealing the starting materials and cDDP-ICL is shown in Figure S2 of the Supporting Information. Removal of the ICL by treatment with NaCN confirmed that cross-link results from platinum.

PARP-1 Binding Assay Strategy. A PARP-1 binding assay was performed in vitro using cell-free extracts (CFEs). Biotinylated DNA probes were prebound to streptavidin-coated magnetic beads in LB. The magnetic beads containing the DNA probes with different platinum adducts were incubated with CFE. After incubation, the complexes were washed stringently with LB to remove nonspecifically bound proteins. The DNA−Pt−protein complexes were precipitated and resolved by SDS−PAGE. Identical amounts of each reaction mixture were loaded to quantify the amount of total protein bound to the DNA probe. PARP-1 concentrations in each sample were detected by immunoblotting with a PARP-1 antibody.

There are some advantages of utilizing this PARP-1 binding strategy compared with traditional electrophoretic mobility shift assays (EMSAs) using purified proteins. First, PARP-1 is in a dynamic equilibrium with PARG, and the activity of PARP-1 partially requires its associated proteins. Therefore, use of a CFE is the better way to mimic the cellular environment in vitro. Second, the activation state of PARP-1 can be controlled in this system. For example, the binding of activated PARP-1 to specific platinum adducts can be adjusted by first treating cells with DNA-damaging agents, and the amount of pADPr polymer in CFE can be modified by addition of PARG or NAD^+.

Binding of Different Forms of PARP-1 to Cisplatin-Damaged DNA. We first investigated the binding of endogenous PARP-1 to cisplatin−DNA cross-links. Three biotinylated DNA probes were used, cDDP-G*G*, cDDP-G*TG*, and cDDP-ICL. An unplatinated control (NoPt) was included in this assay to differentiate between binding of PARP-1 to damaged and undamaged DNA. Doxorubicin, a DNA-damaging agent, was used to activate PARP-1 in HeLa S3 cells, and the binding of endogenous PARP-1 to the DNA probe was tested (*21*). A PARG inhibitor, adenosine diphosphate (hydroxymethyl)-pyrrolidinediol (ADP-HPD) (*22*), was included during the steps of cell lysing and protein binding to the DNA probe in the PARP-1 binding assay to suppress the endogenous activity of PARG. Increasing amounts of DNA probes bound to magnetic beads were incubated with a constant amount of CFE. Immunoblot results showed that the approximately 115 kDa band is the native form of PARP-1, and the band above 115 kDa corresponds to automodified PARP-1. Both the native form and automodified PARP-1 bind to cisplatin−DNA damage (Figure 3A). There is a larger amount of PARP-1 that binds to cDDP-G*G* than to cDDP-G*TG*, cDDP-ICL, or NoPt. Activated PARP-1 also

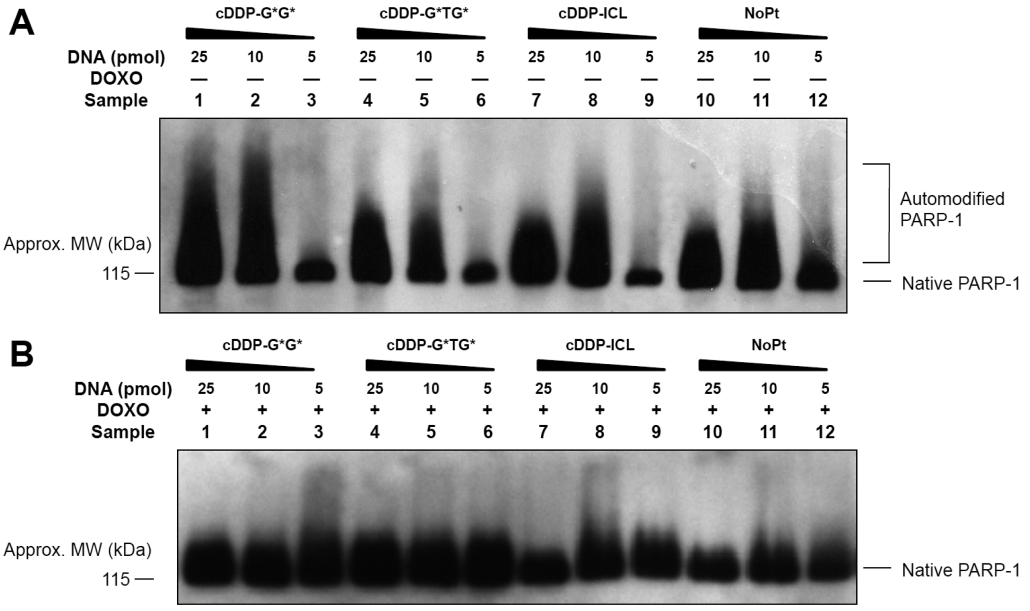

FIGURE 3: Western blot analysis of endogenous PARP-1 binding to different DNA probes. (A) Binding of unactivated PARP-1 to cDDP-G*G* (lanes 1−3), cDDP-G*TG* (lanes 4−6), cDDP-ICL (lanes 7−9), and NoPt (lanes 10−12). (B) Binding of activated PARP-1 to cDDP-G*G* (lanes 1−3), cDDP-G*TG* (lanes 4−6), cDDP-ICL (lanes 7−9), and NoPt (lanes 10−12). A 45 μL quantity of CFE was used in all lanes. The samples were resolved via 8% SDS−PAGE and detected using a PARP-1 antibody.

binds to cisplatin-damaged DNA probes (Figure 3B). More activated PARP-1 binds to cisplatin−DNA intrastrand cross-links than to a cisplatin ICL or a native DNA duplex. We also transfected HeLa S3 cells with the eGFP-PARP-1 plasmid to obtain CFEs containing overexpressed PARP-1, and the binding assay was conducted in the absence of ADP-HPD (*20*). The result indicated that overexpressed PARP-1 also binds to cDDP-G*G*, cDDP-G*TG*, and cDDP-ICL (Figure S3 of the Supporting Information). Cleaved PARP-1, corresponding to the bands below the native eGFP-PARP-1 band around 140 kDa, retains its binding affinity for cisplatin−DNA cross-links. Including all the native, automodified, and cleaved forms, the binding affinity of PARP-1 for a cisplatin 1,2-d(GpG) cross-link is the greatest.

More PARP-1 Binds to a 1,2-d(GpG) Cross-Link Than to Unplatinated DNA. We studied the binding of endogenous PARP-1 to a cisplatin 1,2-d(GpG) cross-link in more detail. In this assay, a constant amount of the DNA probe was incubated with increasing amounts of CFE. After the protein bound to the probes, the complexes were washed stringently with LB, and the samples were treated with ARH3, another pADPr glycohydrolase, to eliminate the pADPr polymer (*23*). The activity of ARH3 is not affected by ADP-HPD. Therefore, all ARH3-treated forms of PARP-1 that bind to the cisplatin−DNA damage will migrate at 115 kDa on an SDS−PAGE gel. Figure 4 shows the binding of PARP-1 to a cisplatin 1,2-d(GpG) cross-link and an unplatinated control. When the protein−Pt−DNA complex was not treated with ARH3, the automodified PARP-1 binds to DNA (Figure 4A, lanes 1−3 and 7−9; Figure 4B, lanes 1−3 and 7−9), and treatment of ARH3 results in a single band (Figure 4A, lanes 4−6 and 10−12; Figure 4B, lanes 4−6 and 10−12). More PARP-1 binds to a cDDP 1,2-d(GpG) cross-link than to an unplatinated control, when both unactivated and activated protein samples were utilized (Figure 4A, lanes 1−3, and Figure 4B, lanes 1−3; Figure 4A, lanes 7−9, and Figure 4B, lanes 7−9). When PARP-1 is activated, less protein binds to the platinum−DNA damage (Figure 4A, lanes 1−3 and 7−9). Quantification of the gel illustrating the binding difference is presented in Figure 4C.

PARP-1 Binds to Different Platinum Lesions. The binding of PARP-1 to different platinum−DNA lesions was studied. To better quantitate the amount of nonmodified PARP-1 that binds, we used low concentrations of CFE so that only the native form of the bound protein is detectable. cDDP-G*G*, PtDACH-G*G*, and cDPCP-G* DNA probes were included to elucidate the binding affinity of endogenous PARP-1. PARP-1 binds to the three DNA probes modified by different platinum compounds: cisplatin, oxaliplatin, and cDPCP (Figure 5A). Compared to the activated PARP-1, more unactivated PARP-1 bound to damaged DNA (Figure 5B). There is a significantly greater amount of proteins that bind to platinated DNA than the proteins that bind to the unplatinated control (Figure 5B).

Overactivation of PARP-1 Inhibits Its Binding to Cisplatin-Damaged DNA. We further examined the role of pADPr in the binding of PARP-1 to cisplatin-damaged DNA. HeLa S3 cells were treated with and without doxorubicin, and then CFEs were generated. Prior to binding to the DNA probes, the CFE was pretreated with either NAD^+ to overactivate PARP-1 or with ARH3 to eliminate pADPr. In agreement with the results mentioned above, less activated PARP-1 bound to cisplatin-modified DNA (Figure 6, lanes 2, 4, and 6) compared to the unactivated protein (Figure 6, lanes 1, 3, and 5). Additionally, more PARP-1 bound when the samples were pretreated with ARH3 (Figure 6, lanes 3 and 4), whereas overactivation of PARP-1 by NAD^+ addition significantly decreased the binding affinity of the protein (Figure 6, lane 6).

DISCUSSION

In the presence of DNA damage, PARP-1 is activated to catalyze the NAD^+-dependent addition of a pADPr polymer to receptor proteins, including PARP-1 itself, resulting in either damage repair or cell death (*8*). PARP-1 also plays a role in transcription regulation by modulating chromatin structure, acting as an enhancer-binding factor, functioning as a transcriptional coregulator, or serving as a component of insulators (*24*). PARP-1 plays a role in the repair of other types of DNA lesions,

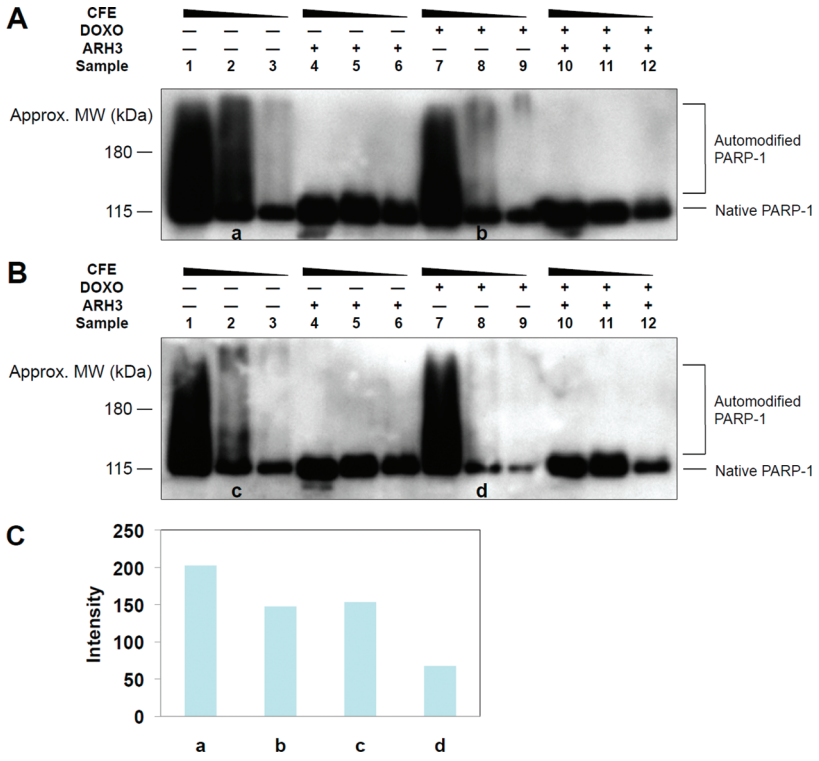

FIGURE 4: Western blot analysis of endogenous PARP-1 binding to cDDP-G*G* (A) and NoPt (B). The samples were resolved via 8% SDS−PAGE and detected using a PARP-1 antibody. Amount of CFE used: lanes 1, 4, 7, and 10 in panels A and B, 60 μL; lanes 2, 5, 8, and 11 in panels A and B, 15 μL; lanes 3, 6, 9, and 12 in panels A and B, 5 μL. (C) Quantification of the gels: lane a, unactivated PARP-1 binding to cDDP-G*G* (lane 2 in panel A); lane b, activated PARP-1 binding to cDDP-G*G* (lane 8 in panel A); lane c, unactivated PARP-1 binding to NoPt (lane 2 in panel B); lane d, activated PARP-1 binding to NoPt (lane 8 in panel B).

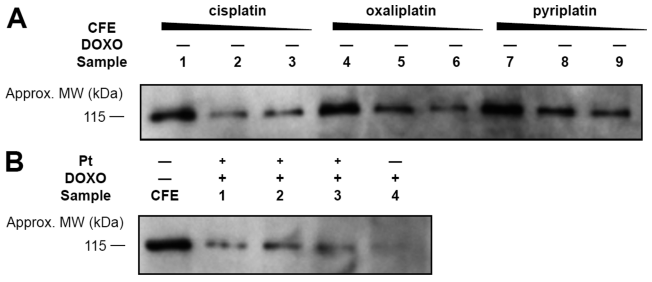

FIGURE 5: Western blot analysis of endogenous PARP-1 binding to different kinds of platinum−DNA damage. (A) Binding of unactivated PARP-1 to cDDP-G*G* (lanes 1−3), PtDACH-G*TG* (lanes 4−6), and cDPCP-G* (lanes 7−9). Amount of CFE used: lanes 1, 4, and 7, 5 μL; lanes 2, 5, and 8, 2 μL; lanes 3, 6, and 9, 1 μL. (B) Binding of activated PARP-1 to cDDP-G*G* (lane 1), PtDACH-G*TG* (lane 2), cDPCP-G* (lane 3), and NoPt (lane 4). A 5 μL quantity of CFE was used. The samples were resolved via 8% SDS−PAGE and detected with a PARP-1 antibody.

including pyrimidine dimers, and a role for PARP-1 in platinum−DNA damage recognition has been recently discovered by a photo-cross-linking strategy (*15*−*17*).

It is argued that automodified PARP-1 bound to DNA can still be cross-linked under the photo-cross-linking condition published previously (*15*). The assumption was that PARP-1 dissociates from the platinum damage only after heavy automodification, and insufficient levels of modification of PARP-1 may result in its shielding of the damage from repair (*25*). Our results clearly show that both native PARP-1 and automodified PARP-1 bind to platinum−DNA lesions, with less of the automodified form binding to the damage. However, when PARP-1 is overactivated by NAD^+, very small amounts of protein bind to

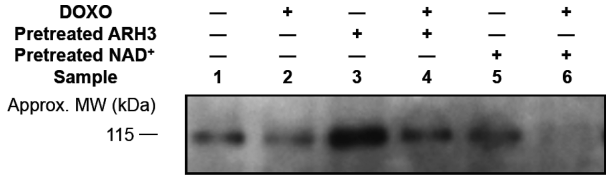

FIGURE 6: Western blot analysis of PARP-1 binding to cDDP-G*G*. A 5 μL quantity of CFE was used. The samples were resolved via 8% SDS−PAGE and detected with a PARP-1 antibody.

the cisplatin 1,2-d(GpG) lesion (Figure 6, lane 6). The fact that activated PARP-1 still binds to platinum−DNA damage in our experiment is in agreement with the model in which, under physiological conditions, when DNA is damaged by platinum drugs and PARP-1 is activated, the protein shields the damage from repair until the downstream pathways are activated.

Numerous reports support the notion that PARP-1 binds to specific regions of DNA in a sequence-dependent manner. PARP-1 itself has an affinity for dsDNA in vitro (*26*−*28*). PARP-1 also recognizes other forms of DNA, including negatively supercoiled matrix attachment sequences and structurally complex cruciform DNA (*29*, *30*). The affinity of unmodified dsDNAs for PARP-1 is lower than the affinity of end-blocked dsDNA in the trans-poly(ADP-ribosylation) reaction according to an in vitro kinetic study, and the affinity of both unmodified and modified dsDNA for PARP-1 was much lower in an autopoly(ADP-ribosylation) reaction (*31*). Our results indicate that PARP-1 has an affinity for both unmodified and platinum-damaged dsDNA. More PARP-1 binds to the platinum-damaged dsDNA, indicating the role of PARP-1 in the cellular response to platinum damage.

A previous report showed that addition of the PARP inhibitor CEP-A increased the level of photo-cross-linking of proteins to a DNA probe containing a $PtBP_6$ 1,2-d(GpG) cross-link in NTera2, BxPC3, and U2OS cells, but not in HeLa cells. In addition, PARP-1 inhibitors did not sensitize HeLa cells to cisplatin (*18*). Our results here further confirm that PARP-1 automodification attenuates its activity in platinum−DNA damage recognition in HeLa S3 cells. When PARP-1 is moderately activated, the protein binds to cisplatin-damaged as well as undamaged DNA in smaller amounts, and overactivated PARP-1 does not bind to the lesion. The amount of PARP-1 binding to a cisplatin 1,2-d(GpG) cross-link is the largest compared to amounts of other types of cisplatin−DNA lesions, which is consistent with the previous finding that a 1,2-d(GpG) cross-link more efficiently activates the protein (*18*).

Oxaliplatin has been approved by the FDA for the treatment of colorectal cancer, and it is biologically active against some cisplatin-resistant tumor types (*32*). The mechanism of oxaliplatin−DNA adduct repair is slightly different from that of cisplatin−DNA adduct repair. For example, although nucleotide excision repair (NER) cannot discriminate between oxaliplatin− and cisplatin−DNA adducts, mismatch repair (MMR) does not recognize oxaliplatin−DNA cross-links (*33*, *34*). A number of proteins have been identified that bind to oxaliplatin−DNA adducts, but there is no relationship between PARP-1 and oxaliplatin-damaged DNA. In this work, we demonstrate that PARP-1 binds to a dsDNA containing a site-specific oxaliplatin 1,2-d(GpG) cross-link. Therefore, PARP-1 may also play a role in the recognition and processing of oxaliplatin−DNA adducts.

cDPCP, or pyriplatin, shows promising biological activity against colorectal cancer cells, blocks the transcription by RNA polymerase II, and can escape from DNA damage repair pathways (*4*, *5*). The X-ray structure of a cDPCP−DNA adduct revealed that the damage introduces substantially less distortion into the DNA duplex compared to a cDDP−DNA adduct (*4*). The pyridine moiety in cDPCP faces the 5′-end of the platinated strand, allowing formation of a hydrogen bond between the NH_3 ligand trans to pyridine and O6 of the guanosine residue. However, little is known about damage response proteins that might bind to a cDPCP−DNA lesion. Our results indicate that PARP-1 binds to a dsDNA damaged by cDPCP. Taken together with the fact that PARP-1 also binds to dsDNA damaged by other platinum drugs, especially one containing a cisplatin 1,2-d(GpG) cross-link, and that less PARP-1 binds to a cisplatin ICL, which also contains a distorted structure, it is likely that the PARP-1 binds to the damage in an adduct-specific manner rather than recognizing a distortion in the DNA.

In conclusion, the in vitro PARP-1 binding assays have established the specific role of PARP-1 in platinum−DNA damage response. PARP-1 differentiates between normal and platinum-damaged DNA, and the protein has a higher affinity for cisplatin 1,2-d(GpG) cross-links than for other types of cisplatin−DNA cross-links. PARP-1 recognizes not only cisplatin lesions but also those of oxaliplatin and pyriplatin. Automodification of PARP-1 significantly attenuates its binding to the platinated DNA, and elimination of pADPr facilitates binding of the protein to the cisplatin−DNA cross-link. Our results further shed light on platinum−DNA damage recognition, with the implication that PARP-1 may shield the lesion from repair and trigger a cytotoxic response. Future studies on downstream effects that follow automodified PARP-1 binding to the platinum lesion will provide an even more detailed picture of the exact role of this protein as a platinum damage-response factor in the cell.

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SUPPORTING INFORMATION AVAILABLE

Autoradiograph of the 21 bp DNA duplexes (Figure S1), autoradiograph of the cDDP-ICL (Figure S2), and Western blot analysis of overexpressed eGFP-PARP-1 binding to different DNA probes (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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