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Unwinding of DNA polymerases by the antitumor drug, *cis*-diamminedichloroplatinum(II)

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The helix-turn-helix motifs of the DNA binding domains of human polymerase-α and polymerase-κ are dramatically perturbed upon binding to cisplatin with concomitant release of zinc.

The anticancer drug, *cis*-diamminedichloroplatinum(II) (cisplatin), is widely used for the treatment of a variety of cancers.[1] The platinum compound arrests the cell cycle at S and G2 phases in a dose dependent manner and triggers apoptosis.[2] It is commonly believed that DNA is the cellular target.[3] However, how the DNA–platinum adducts transduce apoptosis remains to be unveiled. Moreover, other biomolecular targets including binding to replication enzymes and transcription factors have yet to be ruled out.[4,5] Recently, we have documented that cisplatin irreversibly binds human polymerase-α, a key replication enzyme, and inhibits the polymerase activity.[6] This platinum binding is associated with the release of Zn(II) from the DNA binding domain of the enzyme. The inhibition of polymerase activity has also been observed for *E. coli* polymerase I, T4 and T7 polymerases.[7,8] We speculated that the platinum binding results in severe structural perturbations of these enzymes which leads to the irreversible inhibition of enzyme activities. In this communication, we present unequivocal evidence for the unwinding of the DNA binding motif of the two polymerases, human polymerase-α (polα) and polymerase-κ (polκ)† upon binding to cisplatin. In particular, structures of Zn(II) and Pt(II) complexes of the putative DNA binding motif (shown below) for these two polymerases were compared. Polymerase-κ is a recently discovered enzyme which is thought to function when a cell detects mutation,[9] a process leading to the initiation of cancer development.

The human polα contains a DNA binding domain near the carboxy terminus.[10] This domain bears the homology with the zinc finger motif of the GATA box,[11] and some other transcription factors. This domain readily binds Zn(II) with the binding constant of $3.91 \pm 0.44 \times 10^4$ M^{-1}, determined from a series of fluorescence spectra‡ of the peptide in variable Zn(II) concentration. The circular dichroism spectra of these Zn(II)-polα complexes exhibit 26% of helix, 19% of β-sheet, 21% β-turns, and 34% non-ordered structure. The peptide mostly exhibits disordered conformation without Zn(II). The divalent metal center is bound to four cysteine residues in polα. Upon adding one equivalent cisplatin to each of the Zn(II)-complexes, a marked decrease in the helical component and a concomitant increase in random coil secondary structures were observed. The three dimensional NMR structures, determined by solving complete relaxation matrices,[12] of polα and an extended polα(polαx) which exhibit a helix at the amino terminus involving ten residues followed by several turns, and a second small helix at the carboxy terminus. An addition of one equivalent cisplatin leads to the binding to two selective cysteine residues at the carboxy terminus as evidenced by noe data and the retention of Zn(II) in the peptides. The binding sites of cisplatin were established from HMQC and NOESY experiments utilizing $^{15}NH_3$. The noe connectivity of this enriched ammonia indicated that the cisplatin is located near two cysteine residues at the carboxy terminus. The NMR structures of the platinum complex determined from noe data exhibit an unwinding of the helix. An addition of the second equivalent of cisplatin completely deligates Zn(II) but does not show further structural changes as determined from the CD and noe data, as has been depicted on Figure 1.

The CD data, shown on Figure 2, indicate that polκ contains a helix turn helix motif (HTH). The existence of this HTH motif was predicted from the amino acid sequences.[9] Zn(II) is tetrahedrally coordinated to three cysteine and one histidine residues. Like in polα, binding of zinc ions to the polκ peptide sequence enhances the α-helical component in the structure (62% α-helix, 0% β-sheet, 38% turns and disordered domains). Furthermore, platination of polκ shows a substantial decrease of helix and with an increase in random coil secondary structures. In particular, the CD spectrum of platinum(II) complex reveals the presence of only 18% of helix with significant increase in disordered structures (78%). However, unlike the polα–cisplatin reaction, an addition of one equivalent of cisplatin completely displaces Zn(II) from polκ. The electrospray mass spectrometric data indicate the existence of two platinum(II)–

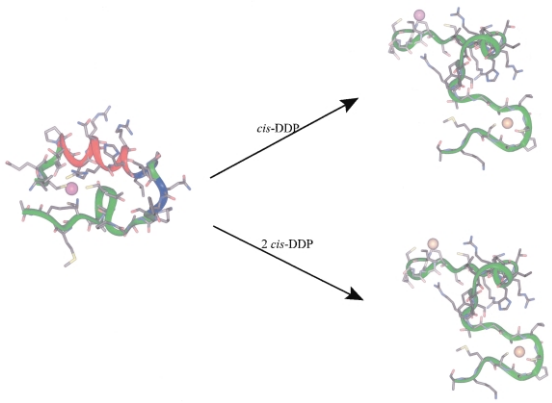

Fig. 1 Structural perturbation of Zn(II)–polα complex upon binding one and two equivalent of cisplatin.

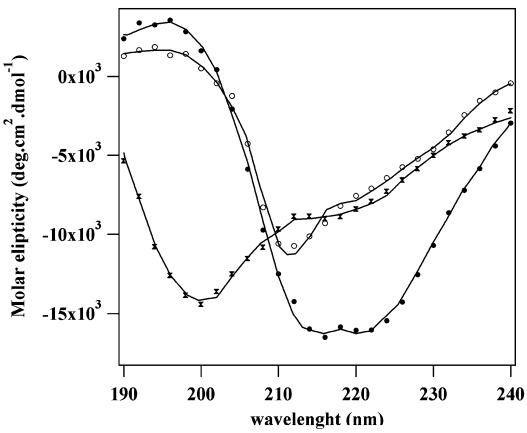

Fig. 2 CD spectra of the DNA binding domain of polκ (open circles), polκ–Zn(II) (closed circles) and polκ–cisplatin (triangles) complexes. The solid lines correspond to simulated spectra.

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peptide complexes, one retaining both ammine ligands and the other coordinating to only one ammonia. The relative intensities of these two molecular ions indicate that the former complex is the dominant species. We suspect that the initial platinum(II) binding took place with two cysteine residues followed by a slow release of one ammine ligand due to trans labilization with coordination to the third cysteine.

The implications of the unwinding of these polymerases in platinum anticancer chemistry and beyond are quite intriguing. First, the inhibition of DNA synthesis in S phase at higher cisplatin concentration[2,13] may partly be due to the direct cisplatin binding to the polymerase. The protein binding hypothesis involving polymerases or zinc-finger transcription factors can be supported by a number of observations. First, direct incubations of cisplatin with human polymerase-α, *E. coli* polymerase I, T7 polymerase, and Klenow fragment resulted in the inhibition of DNA synthesis.[6–8] Furthermore, this inhibition of DNA synthesis was accompanied by the release of Zn(II) in the case of polymerase-α. Since the initiation of DNA synthesis by polymerases require binding to template DNA,[14] the unwinding of these enzymes by direct platinum coordination might prohibit the DNA binding. Furthermore, it is documented that only a very small fraction of the administered cisplatin binds the genomic DNA;[15,16] the majority of the drug binds small molecules and proteins, both in extra and intracellular milieu. Moreover, polymerase-β, a repair enzyme,[17] readily bypasses the platinum–DNA lesion in synthesizing the leading strand of DNA.[18,19] Although total protein binding is small,[20] cisplatin binding could be highly selective toward some specific proteins, especially zinc-finger proteins. In fact, as much as 60% of administered cisplatin was found to coordinate to glutathione in leukemia cells.[21] Since the DNA binding domains of the polymerases contain multiple cysteines and since platinum(II) has tremendous affinity toward the sulfur donor atoms,[22,23] we suspect that polymerase binding might contribute to the cisplatin anticancer activity. The implication of severe structural perturbations of the zinc-finger domain by platinum coordination may also be extended to understand the role of transcription factor in cisplatin induced apoptosis. Many transcription factors contain Zn-finger domains with two or more cysteine residues: these classes of proteins might also be affected by cisplatin. The transcription factor binding may also play an important role, since at low concentration of cisplatin, apoptosis is initiated at the G2 phase, a post replication phase. In fact, the expression of the antiapoptotic transcription factor, XAIP, has shown itself to be considerably down regulated by cisplatin in several tumor cell lines.[24] Results from our laboratory also reveal that the expression of servivin, an antiapoptotic transcription factor with a zinc finger motif, is completely abolished by cisplatin within 12 h incubation in Hela cells.[25] It is interesting to note that survivin is involved in the transcription process at the G2 phase.[26] Furthermore, it been shown that platinum(II)–glutathione complexes trigger apoptosis by inhibiting translation processes[27] which certainly require involvement of transcription factors.

Apart from their implications in the anticancer chemistry, present results also point to a valuable direction in therapeutic development with metal complexes targeting enzymes. For example, the replication enzymes can be used as therapeutic targets for both cancer and HIV diseases and therefore efficient metal complexes can be designed to unwind the polymerases.[28]

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Notes and references

† polκ: TGQALVCPVCNVEQKTSDLTLFNVHVDVCLNKSFI-NH_2 polα: ICEEPTCRNRTRHLPLQFSRTGPLCPACMKA-NH_2 polαx: WLICEEPTCRNRTRHLPLQFSRTGPLCPACMKATLQPE-NH_2. Coordinates for the average and ensemble structures of polα and polαx can be found in Protein Data Bank with identifications 1K18, 1K0P and 1N5G.

‡ *Experimental:* Fluorescence spectra were recorded on spectrofluorometers (Photon Technology Alphascan photon-counting or Waters 474 scanning) in HEPES buffer (1.0 mM). The concentration of the peptide was held invariant at 10.0 μM while [Zn(II)] concentrations were varied from 0 to 200 μM. The spectral changes were observed at 305–310 nm due to the emission from the Phe residue. The dissociation constant was determined from the equation: $(F_{P0} - F_{PM})/(F_{P0} - F_X) = 1 + I_D/[Zn^{2+}]$, where the F_{Po} and F_{PM} represent the fluorescence intensities of free peptide, and peptide–metal complex, and F_X is a composition of F_{po} and F_{PM}. The association constant was taken as $1/K_D$.

Circular dichroism spectra of the peptide complexes with 10 to 100 μM concentrations were recorded on JASCO 500 spectrometer in 1.0 mM HEPES (Zn-complex) and phosphate buffers (for platinum complex) at pH 7.0. The components of the secondary structures were analyzed by utilizing 'CONTIN' CD analysis program.[29]

Three dimensional NMR structures of Zn- and Pt-polα complexes were determined from the 2D noe data. The NMR data were collected either on a Bruker 800 MHz or on a 500 Varian MHz instrument. N-15 coordinated ammonia was used for identifying the binding sites of cisplatin to the peptide by HMQC experiments. We were unable to collect necessary noe data to simulate a three dimensional structure for polκ. The lack of large noe data is due to its solubility at pH > 7.5 which leads to rapid exchange of the amide protons thus inhibiting the existence of correlations between amide protons and others.

The electrospray mass spectra were recorded on Bruker's ESQUIRE equipped with the ion trap technology. The molecular ions were detected in the positive ion mode.

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