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Brief Communication

Platinum Drug Adduct Formation in the Nucleosome Core Alters Nucleosome Mobility but Not Positioning

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SUMMARY

Nucleosome positioning and reorganization regulate DNA site exposure in chromatin. Platinum anticancer agents form DNA adducts that disrupt nuclear activities, triggering apoptosis. Mechanistic insight would aid in the development of improved therapies to circumvent drug toxicity and resistance. We show that platinum adducts formed by reaction of cisplatin or oxaliplatin with the nucleosome core inhibit histone octamer-DNA sliding but do not cause significant alteration of positioning. Thus, adduct formation reinforces positional preferences intrinsic to the DNA sequence, which indicates that modulation of platinum drug site selectivity by histone octamer association may relate to nucleosome-specific properties of DNA. This sheds light on platinum drug-mediated inhibition of chromatin remodeling in vivo and suggests that adducts can shield their own repair and interfere with genomic activities by directly altering nucleosome dynamics.

INTRODUCTION

The primary pharmacological effect of platinum-based chemotherapeutic agents is mediated through formation of DNA lesions, which interfere with genomic activities, such as transcription, and ultimately trigger apoptosis (Jung and Lippard, 2007). Drug reaction occurs at the N7 nitrogen atoms of purine bases, generating predominantly 1,2 intrastrand cross-links at GG and AG dinucleotides and a minor fraction of GXG 1,3 intrastrand and other DNA-platinum adducts. Despite their widespread application in the treatment of specific cancers, the most successful platinum drugs—cisplatin (cisPt, **1**), oxaliplatin (oxPt, **2**), and carboplatin—elicit severe toxicity and resistance effects (Rabik and Dolan, 2007; Kelland, 2007). The development of improved agents or that of therapeutic tactics exploiting the synergy of multiple drugs administered simultaneously is contingent upon a detailed understanding of the mechanism of action of medicinal compounds within the cell.

An additional level of complexity in the activity of DNA-binding agents arises from the packaging of DNA into chromatin (Kornberg and Lorch, 2007). The basic building block of this dynamic assembly is the nucleosome core particle (NCP), in which ~147 base pairs (bp) of DNA are wrapped in one and two-thirds left-handed superhelical turns around a histone protein octamer (Richmond and Davey, 2003). Adjacent nucleosomes in vivo are connected by typically 10–50 bp of linker DNA. Thus, approximately 83% of genomic DNA is associated with histone octamers (Segal et al., 2006), making the nucleosome core an important drug target. Nonetheless, comparatively little is known about the influence of DNA-drug interaction on chromatin function.

The locations occupied by nucleosomes in vivo are strongly influenced by DNA sequence, and the resulting chromatin architecture modulates genomic activities in a gene-specific and state-dependent manner (Yuan et al., 2005; Segal et al., 2006; Lee et al., 2007; Boyle et al., 2008; Schones et al., 2008). For instance, genomic sequence elements promote nucleosome depletion over a defined region of the promoter, which is flanked by strongly positioned nucleosomes. This provides the basis for regulation of transcription, which, like many genomic processes, including replication and repair, relies on reorganization or transient alteration of nucleosomes to allow factor access to specific DNA sites. Nucleosome displacement at promoters during gene activation (Yuan et al., 2005; Lee et al., 2007; Schones et al., 2008) involves the activity of ATP-dependent chromatin remodeling factors (Li et al., 2007). Moreover, DNA lesion repair can be modulated by nucleosome dynamics and facilitated by remodeling activities (Thoma, 2005; Saha et al., 2006). Therefore, an exogenous agent that perturbs the kinetic balance governing nucleosome position and mobility is likely to affect genomic function.

An earlier study investigated the effect of cisPt on hormone-induced transcription from the mouse mammary tumor virus promoter (Mymryk et al., 1995). Treatment of cells with cisPt led to a substantial decrease in gene expression, accompanied by corresponding reduction in chromatin remodeling and transcription factor binding at the promoter. Although the mechanism of this drug-induced inhibition of chromatin remodeling is not clear, studies have shown that cisPt adducts engineered into naked DNA subsequently reconstituted into nucleosomes can alter histone octamer positioning (Danford et al., 2005; Ober and Lippard, 2008). This suggests that cisPt adduct formation on linker DNA could influence the outcome of nucleosome reorganization processes, which call for histone octamer movement onto regions previously unoccupied by nucleosomes.

We have recently shown that histone octamer association modulates the site selectivity of cisPt and oxPt (Wu et al., 2008), which suggests that the effect of adducts on nucleosome positioning may depend on whether the initial substrate is naked

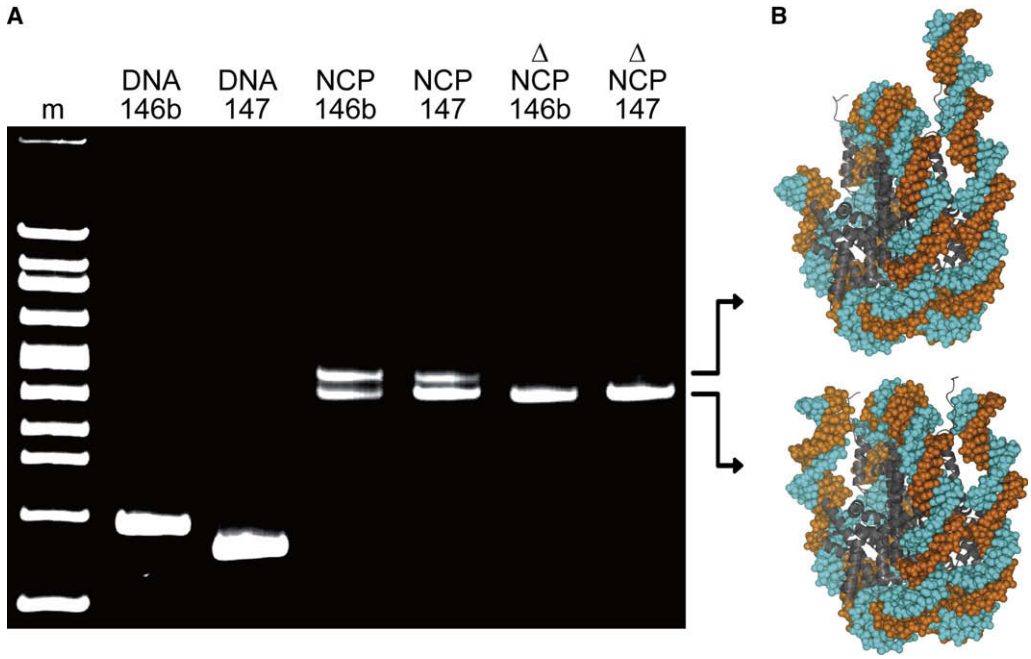

Figure 1. Distinct Translational Positioning and Histone Octamer Sliding in the NCP

(A) Five percent native PAGE analysis of DNA and NCP, 146b and 147. Heat treatment (Δ) promotes histone octamer repositioning to yield exclusively the faster migrating centered form of NCP, in which histone-DNA contacts are maximal. Marker (m) consists of a 100 bp ladder.

(B) View of the NCP with the particle pseudo-twofold axis running vertically through the middle (DNA, colored; histone proteins, gray). The centered (bottom) versus off-centered (top) forms of NCP differ in positioning by a single turn of the double helix.

or nucleosomal DNA. Considering that most platinum drug adducts appear to form and accumulate in nucleosome core regions of the genome (Davey and Davey, 2008), we conducted a study to assess the influence of such adduct formation on nucleosome positioning and mobility.

RESULTS

NCP147 and NCP146b were assembled from recombinant *Xenopus laevis* histones and 147 bp or 146 bp fragments of differing sequence based on human α-satellite DNA (Luger et al., 1999; Davey et al., 2002). These constructs each yield two distinctly positioned species upon reconstitution (Figure 1). The symmetrically aligned, centered form is thermodynamically favored and differs in position by 10 bp relative to the off-centered form (Luger et al., 1999). Thus, the two species share the same rotational phase of DNA with respect to histone octamer and differ only in translational position. Incubation at elevated temperature promotes repositioning of the histone octamer to yield purely centered NCP (see Figure 1).

Predominantly off-centered NCP was generated by conducting reconstitution at 4°C. Samples in a buffer of 20 mM K-Cacodylate (pH 6.0) were treated with three different cisPt:NCP or oxPt:NCP stoichiometry for 48 hr at 4°C and subjected to heat shifting to assess propensity for repositioning (Figure 2). Relative to untreated samples, higher stoichiometry of drug treatment results in an increasing reduction in rate of histone octamer repositioning to the centered form. A similar degree of reduction in mobility occurs at a roughly 10- to 20-fold higher stoichiometry of oxPt treatment relative to cisPt.

A much lower DNA reactivity of oxPt compared with cisPt has been documented previously (Woynarowski et al., 1998). To obtain a more in-depth understanding of the drug and DNA sequence dependency of the observed mobility reduction, we used an atomic absorption spectrophotometer to measure platinum content in NCP treated with cisPt or oxPt. The relative reactivity of cisPt versus oxPt displays some variation with DNA sequence (between NCP147 and NCP146b), but on average oxPt yields a similar number of NCP adducts at a 19-fold higher stoichiometry compared with cisPt at 4°C (Figure 3). Removal of histone proteins from NCP samples by extraction also allows quantification of platinum in the DNA fraction alone. The histone versus DNA distribution of platinum adducts displays slight DNA sequence dependence, yet differs substantially between cisPt and oxPt. On average, 73% of the cisPt adducts are associated with the DNA, compared with only 59% of the oxPt adducts. The remaining platinum fraction corresponds mostly to histone methionine adducts, of which H3 Met120 and H4 Met84 are likely to be the primary sites of attack (Wu et al., 2008).

The large difference in the observed reactivity between cisPt and oxPt at 4°C indicates a similar influence of the two adduct types on nucleosome mobility. An effect is apparent from the lowest cisPt and oxPt stoichiometry tested, indicating that as little as one platinum adduct per nucleosome can influence mobility (see Figure 2). In addition, for approximately the same average number of NCP- or DNA-platinum adducts, the two drug types elicit a similar degree of mobility reduction for NCP146b. However, the effect is significantly stronger with fewer oxPt compared with cisPt adducts for NCP147, which thus reveals both DNA sequence and drug dependence for this phenomenon.

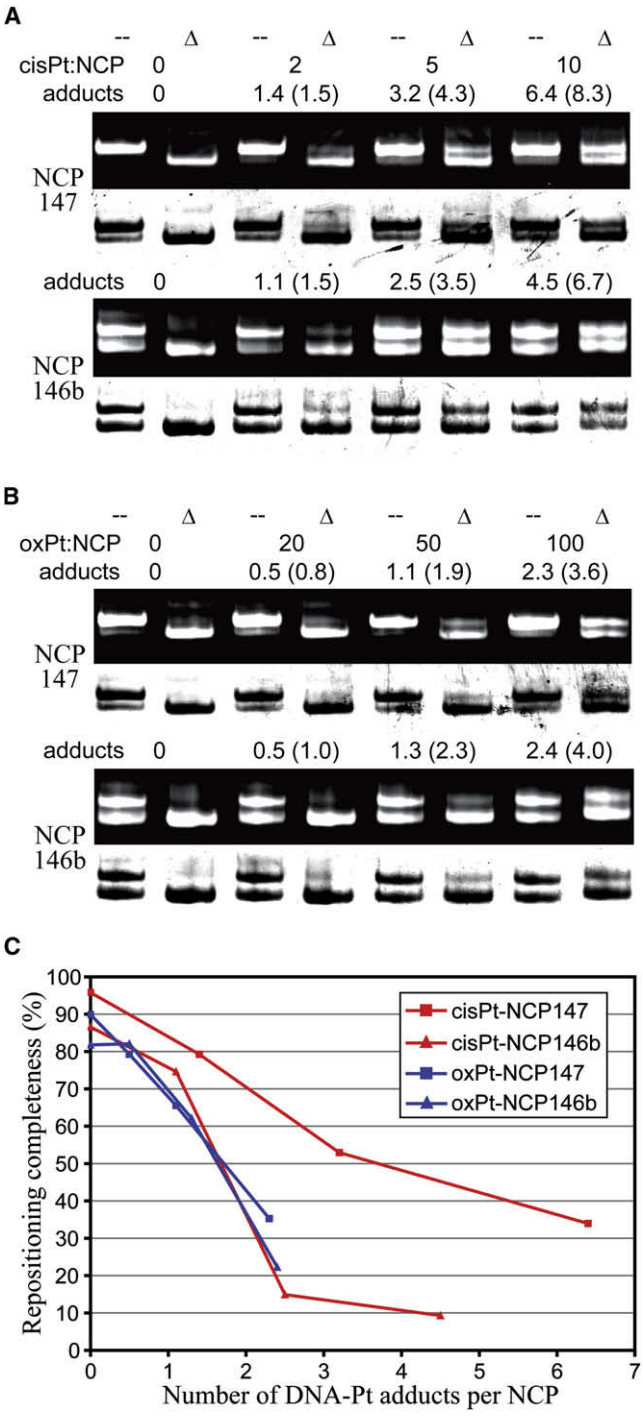

Figure 2. Native PAGE Analysis of Platinum Drug-Treated NCP Reveals the Influence of Adducts on Nucleosome Positioning and Mobility

(A and B) NCP was treated with different cisPt (A) or oxPt (B) stoichiometry (drug:NCP) and subjected to thermal incubation for 20 min at 37°C (NCP147) or 1 hr at 50°C (NCP146b; –/Δ, before/after heat treatment). Gels were stained with ethidium bromide (dark background) and Coomassie blue (light background). The upper and lower bands are off-centered and centered NCP, respectively. Average number of platinum adducts on the DNA or NCP (in parentheses) was determined by atomic absorption spectrophotometry (see text and Figure 3A for details).

(C) Band densitometry analysis of ethidium bromide-stained gels in (A) and (B) showing extent of repositioning (1 – [percent off-centered NCP after heat treatment/percent off-centered NCP before heat treatment]) relative to number of DNA-platinum adducts.

Having established that a substantial fraction of the platinum adducts from cisPt or oxPt treatment of NCP are associated with histone proteins raised the possibility that the observed inhibition of nucleosome sliding arises from histone-platinum-DNA cross-links. If formed, such cross-links could effectively "cement" the histone octamer in its initial position. However, reducing agent-free SDS-PAGE analysis of drug-treated NCP indicates the absence of significant protein-DNA cross-linking (see Figure S1 available online). Moreover, subjection of cisPt- or oxPt-treated NCP147 to extended heat incubation results in essentially complete repositioning to the centered form (Figure 4). For NCP146b, which requires much higher temperature for repositioning, the core particle begins to degrade or dissociate after extended heat treatment. Nonetheless, long-term incubation, short of substantial dissociation, yields primarily the centered form of drug-treated NCP146b. Therefore, platinum adducts residing on DNA or histone give rise to an increased energy barrier toward histone octamer-DNA sliding.

Electrophoretic mobility shift assay is highly sensitive to changes in DNA conformation, such that even differences in orientation of the protruding DNA arm of off-centered NCP can be resolved (Luger et al., 1999). In the present study, we observe no significant degree of alteration in electrophoretic mobility of either the off-centered or centered forms of drug-treated NCP relative to native samples (Figure 2). This encompasses both before and after heat treatment, as well as the extended thermal incubation trials, which yield predominantly centered form having electrophoretic migration rate equivalent to native NCP (Figure 4). Thus, although substantial conformational changes are apparent for the DNA extracted from drug-treated NCP (Figure 3B), platinum adduct formation in the nucleosome core does not result in any gross structural alterations, even when the adduct number exceeds, on average, eight per NCP. Furthermore, previous investigations on cisPt-derivatized DNA reconstituted into nucleosomes have indicated that adducts can profoundly alter histone octamer positional preference, causing inversion of rotational phase by 5 bp and up to 20 bp changes in translational setting (Danford et al., 2005; Ober and Lippard, 2008). The systematic absence of differences in gel mobility arising from adduct formation observed here indicates the lack of pronounced change in positional preference upon platinum drug reaction with nucleosomes.

DISCUSSION

The association of minor groove binding polyamides has been observed to suppress histone octamer-DNA sliding in the nucleosome (Suto et al., 2003). In addition, substitution of rigid DNA sequence elements into the nucleosome core, such as long poly(A) tracts, also results in a decrease in nucleosome mobility (Bao et al., 2006). Our findings on the effect of platinum adducts suggests that DNA cross-linking underlies the reduction in nucleosome mobility. Although platinum adducts on methionine residues could potentially influence nucleosome dynamics, the mobility reduction apparently stems from a decrease in DNA

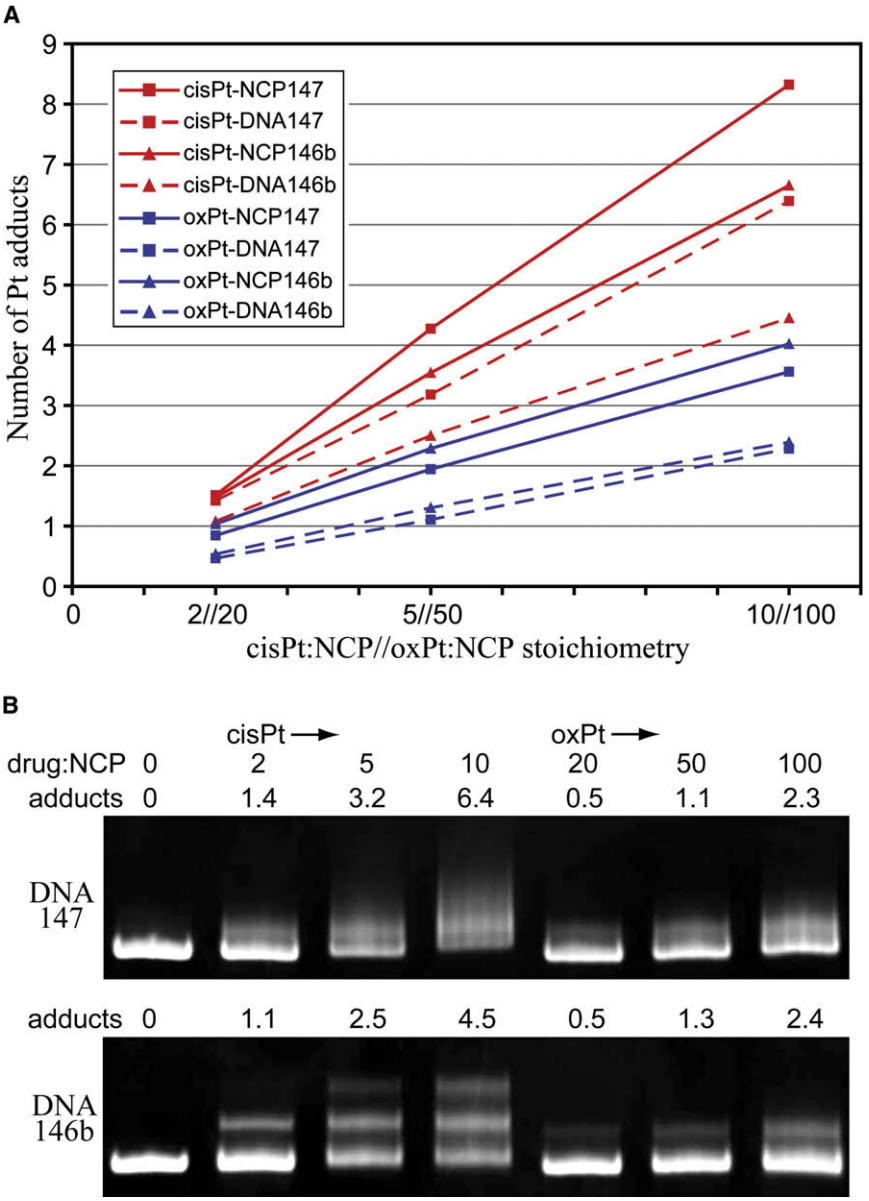

Figure 3. Quantification of Platinum Adducts in the NCP

(A) A Hitachi Z-2000 polarized Zeeman atomic absorption spectrophotometer was used to measure platinum content of cisPt- or oxPt-treated NCP. For determining average number of adducts associated with the DNA (dashed lines), histone proteins were removed by incubating NCP with 2M NaCl at 55°C for 1 hr, followed by phenol-chloroform extraction and ethanol precipitation and resuspension of the DNA. Values correspond to the average of three measurements, with an overall root-mean-square deviation of 7%.

(B) Five percent native PAGE analysis of DNA extracted from cisPt- or oxPt-treated NCP147 and NCP146b. Drug stoichiometry and average number of platinum adducts per DNA molecule are given.

flexibility conferred by cross-linking. In view of the capacity of the nucleosome core to accommodate platinum lesions without large structural changes (Wu et al., 2008), adduct formation may permit native-like histone-DNA interactions while reducing DNA mobility through suppression of twisting and looping distortions.

UV-induced pyrimidine-pyrimidine cross-links formed on naked DNA give rise to alterations in positioning when reconstituted into nucleosomes, such that the lesions are preferentially oriented away from the histone octamer (Thoma, 2005). Conversely, UV cross-link formation in the nucleosome does not result in positional change; rather, lesions are preferentially generated at locations facing away from the histone octamer. Thus, histone octamer association modulates lesion formation by systematically altering the energetics of cross-link formation, which consequently complements the positioning preference of the DNA. This may be a phenomenon common to many types of lesion formation in the nucleosome, which give rise to conformational distortions in the DNA. We find that an analogous scenario occurs with platinating compounds, in that cross-links formed on naked DNA can change histone octamer positioning (Danford et al., 2005; Ober and Lippard, 2008), whereas lesion formation in the nucleosome core does not alter positioning. In fact, by inhibiting histone octamer sliding, platinum adducts are reinforcing intrinsic positional preferences.

A modulation of platinum adduct formation by histone octamer association (Wu et al., 2008), which strengthens but does not alter nucleosome positioning suggests the capacity of the nucleosome to permit cross-link formation where it can most readily accommodate histone-imposed conformational restraints. Previous work has shown that 1,3 GTG or 1,2 GG cisPt cross-links formed on naked DNA assume positions in the nucleosome where the major groove faces inward, toward the histone octamer (Ober and Lippard, 2007, 2008). Consistent with this, we have observed one of the primary sites of cisPt attack on naked DNA, corresponding to a location where the major groove faces away from histone octamer, to be substantially protected in the NCP (Wu et al., 2008). At several other locations, adduct formation appears enhanced relative to naked DNA. These preferred positions of attack in the nucleosome may be those that best accommodate DNA conformational restraints and have the potential to strengthen positional cues.

SIGNIFICANCE

The present findings shed light on the physiological consequences of platinum drug reaction with chromatin, indicating that adduct formation may preserve and reinforce

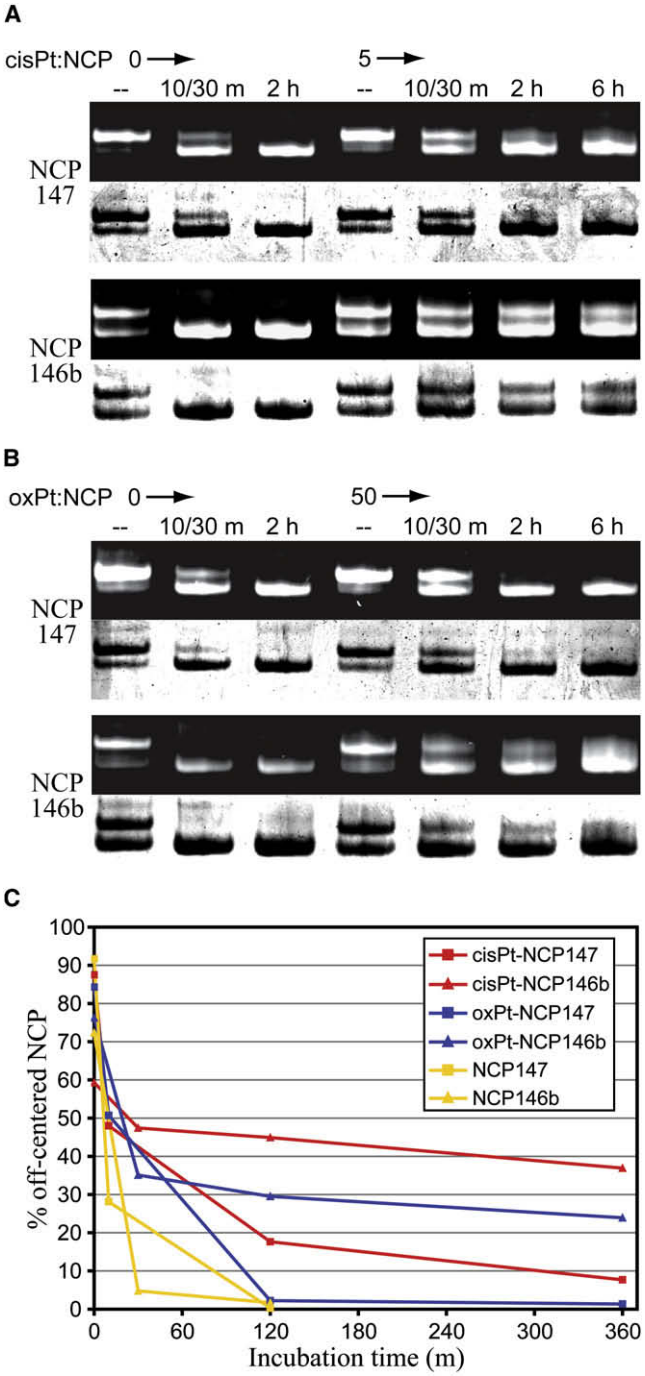

Figure 4. Native PAGE Analysis of Platinum Drug-Treated NCP Showing the Influence of Adducts on Nucleosome Positional Preference

(A and B) Samples were treated with 5:1 cisPt:NCP (A) or 50:1 oxPt:NCP (B) stoichiometry and subjected to thermal incubation at 37°C (NCP147) or 50°C (NCP146b) for up to 6 hr (–, before heat treatment). The first time point for NCP146b is 30 min, as opposed to 10 min for NCP147, due to the lower intrinsic mobility of the former. Gels were stained with ethidium bromide (dark background) and Coomassie blue (light background).

(C) Band densitometry analysis of ethidium bromide-stained gels in (A) and (B) showing positioning as a function of incubation time. Note that the upward smearing of centered NCP bands resulting from core particle disassociation upon extended thermal incubation gives rise to an overestimation of the off-centered NCP fraction.

nucleosome positioning. The reduction in nucleosome mobility conferred by cisPt or oxPt adducts may contribute to the pharmacological activity of this class of agent, and suggests that the inhibition of chromatin remodeling found in vivo for cisPt treatment (Mymryk et al., 1995) may arise in part via direct alteration of nucleosome dynamics.

DNA-adduct levels in cancer patients treated with platinum drugs, up to 1 per 4200 bp for cisPt regimens, are lower than those observed for in vitro studies (Kloft et al., 1999; Pieck et al., 2008). However, the distribution of cisPt and oxPt adducts in cellular DNA is markedly nonuniform (Woynarowski et al., 1998; Davies et al., 2000), and recent work suggests that adducts may accumulate disproportionately in the nucleosome core regions of the genome (Wu et al., 2008; Davey and Davey, 2008). Therefore, the observed DNA sequence and drug dependence of nucleosome mobility modulation indicates that specific elements in the genome may be differentially affected. Furthermore, because histone octamer association inhibits the repair of platinum adducts (Wang et al., 2003), it appears that adducts may contribute to shielding their own repair by hindering dynamic site exposure. As such, the discovery of agents that could markedly suppress mobility of nucleosomes at vulnerable genomic elements may hold therapeutic promise by hampering DNA repair processes.

SUPPLEMENTAL DATA

Supplemental Data include one figure and can be found with this article online at http://www.chembiol.com/cgi/content/full/15/10/1023/DC1/.

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