Evidence for similar structural changes on binding of platinum anti-tumor agents to DNA and nucleosomes

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Received 15 August 1984

The influence of Pt(II) compounds on the ^{31}P NMR spectra of natural DNA, synthetic polynucleotides, and nucleosomes was investigated. With Pt complexes which are anti-tumor agents, a new peak or shoulder centered at ~1.2 ppm downfield from the untreated DNA signal was observed. When Pt compounds known not to be anti-tumor agents were studied, no such new signal was observed. The most reasonable explanation for the downfield resonance is that it is a consequence of a structural change induced in the DNA by the anti-tumor agent. Since the effect of the Pt compounds on nucleosomes was similar, the same structural change is probably occurring in DNA in solution and in nucleosomes. A nonalternating dG·dC polymer, but not alternating G·C or any A·T polymers, exhibited a similar spectral change and this finding suggests that the structural change in the DNA arises primarily from reaction of Pt anti-tumor agents with adjacent G residues.

DNA-Pt *^{31}P NMR* *Antitumor platinum* *Nucleosome-Pt*

1. INTRODUCTION

Strong evidence exists that the key in vivo molecular target of platinum anti-neoplastic agents is DNA [1]. The specific nature of the lesion is the subject of much current interest and investigation. Guanine bases appear to be attacked first and particularly GC rich regions of polynucleotides appear to be preferential targets [2–6]. Although N7,O6 chelation is espoused by some workers, recent emphasis has been placed on intra-strand crosslinks between adjacent Gs (type I) or Gs separated by one or two intervening bases (type II) [2–6]. These suggestions satisfy the need to explain the relationship between structures and activity of some Pt compounds such as *cis*-$Pt(NH_3)_2Cl_2$ and $PtenCl_2$ (en = ethylenediamine) and the inactivity of other Pt compounds which lack two *cis* leaving groups such as *trans*-$Pt(NH_3)_2Cl_2$ and [PtdienCl]Cl (dien = diethylenetriamine) [1]. The remarkable effectiveness of the Pt anti-tumor agents may arise from an ability to disrupt DNA conformation so severely that excision-repair and DNA-replication enzymes are inhibited [1].

Using ^{31}P NMR, we have discovered a large conformational change induced in DNA by Pt anti-tumor drugs [7] and report here more detailed observations on this effect. The ^{31}P shift we observe is as large as for the B → Z transition of DNA. Evidence is presented for similar distribution induced by the anti-tumor drugs in DNA in solution and in nucleosomes, as well as in a nonalternating dG·dC polymer. Inactive Pt compounds caused slight downfield shifts and broadening of the main resonances indicative of secondary types of binding.

2. MATERIALS AND METHODS

2.1. *Materials*

Salmon sperm and calf thymus DNA were from

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

Worthington and the synthetic polymers from P-L Biochemicals. These were prepared for NMR studies as described previously [7,8]. Solution conditions were generally pH = 7, PIPES buffer (0.01 M PIPES, 10^{-3} M EDTA, 0.1 M $NaNO_3$), $T = 30°C$. Chicken erythrocyte nucleosomes were prepared by a slight modification of the method of [9]. Intact nuclei were briefly digested with micrococcal nuclease. Histone H1 free long chromatin was isolated chromatographically and redigested with micrococcal nuclease. Monomer nucleosomes were isolated using sucrose density gradient centrifugation and dialyzed into a 10 mM Tris, 0.1 mM EDTA, pH 8 buffer. Final preparations, analyzed by DNA and histone gel electrophoresis, T_M, sedimentation, and ^{31}P NMR, were found to be pure intact monomer nucleosomes containing only histones H2A, H2B, H3, and H4.

2.2. *Methods*

DNA samples were lyophilized and redissolved in the desired %D_2O for NMR experiments in PIPES buffer. For NMR studies of nucleosomes a sample of the Tris buffer was lyophilized and redissolved in D_2O. This was added to a nucleosome sample in H_2O–Tris buffer to give a final concentration of nucleosomal DNA-P in the range of 0.01 M with 30–40% D_2O, depending on the dilution. Spectra were obtained on an IBM WP 200SY at 81.01 mHz using ~3000–9000 scans, 90° pulse, 15 s delay, ^1H gated decoupling, and trimethylphosphate reference. In most cases spectra were also obtained on a JEOL FX60Q at 24.15 MHz with similar results, except that peak widths in Hz were ~1/3. In some cases the ^{31}P signals were characterized by a nonlinear least-squares Lorentzian curve deconvolution computer program. Pt compounds were then added to the DNA and nucleosome samples, and after reaction (typically 20–30 h), NMR spectra were again obtained under the same conditions.

3. RESULTS AND DISCUSSION

Spectra for calf thymus and salmon sperm DNA, as examples of our results, are shown with $PtenCl_2$ and *trans*-$Pt(NH_3)_2Cl_2$ in fig.1. In the presence of active Pt anti-tumor drugs a downfield shoulder (approximately 3.0 PPM) is seen at near neutral pH and 30°C. Increasing the temperature

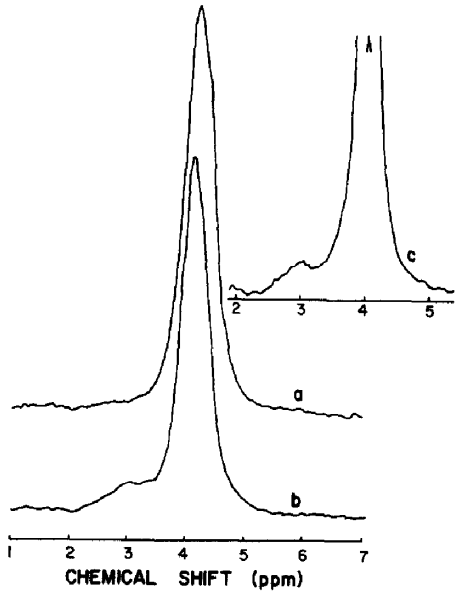

Fig.1. ^{31}P NMR spectra at 60°C of calf thymus (a and b) and salmon sperm (c) DNA with $PtenCl_2$ (b and c) and *trans* $Pt(NH_3)_2Cl_2$ (a). PIPES buffer (section 2) was used with 0.1 M $NaNO_3$ (a and b) or with 7 mM $NaNO_3$ (c). Spectra were taken at 24.15 MHz (a and b) or 81.01 MHz (c). The ratio of Pt/P is 0.2 in all spectra.

(>50°C) or pH (>9) sharpens the peaks and increases resolution without causing significant chemical shift changes. The downfield peak can easily be seen in fig.1(b,c), for example, with both calf thymus and salmon sperm DNA samples. These spectra were obtained at ~60°C to sharpen the peaks and enhance resolution. Of all of the compounds utilized, only the anti-tumor agents caused the downfield peak shown in fig.1. The temperature-enhanced resolution is probably related to increased mobility of the PO_4 [10,11]. Spectra for salmon sperm DNA, unreacted, and reacted with $PtenCl_2$ and *trans*-$Pt(NH_3)_2Cl_2$ are shown at 30°C for reference in fig.2. We have observed the downfield peak in a variety of DNA samples from pH 6–9, ionic strength from 0.02 to 0.1, temperature from 25–70°C, with $PtenCl_2$, *cis*-$Pt(NH_3)_2Cl_2$, Pt(cyclohexanediamine)Cl_2 (both (+) and (–) forms), and at a variety of field strengths. Increasing the Pt/P ratio above 0.2 causes increased broadening of the peaks, loss of resolution, and no apparent increase in the downfield peak. As shown in table 1, we also see the new peak with a nonalternating G·C polymer. The peak is not seen with the inactive *trans*-$Pt(NH_3)_2Cl_2$ or

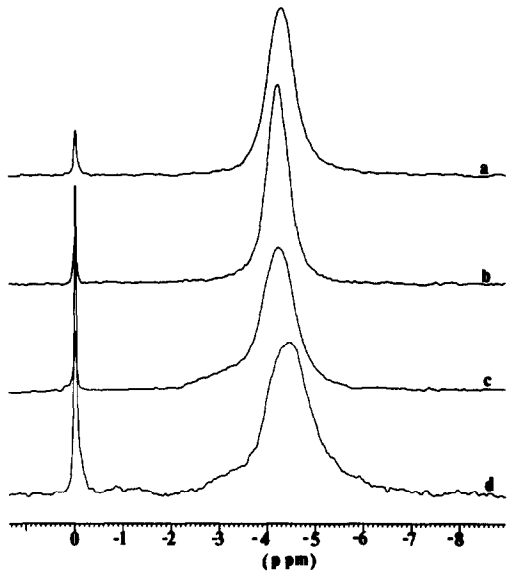

Fig.2. ^{31}P NMR spectra at 30°C and 81.08 MHz for salmon sperm DNA (a–c) and nucleosomes (d). Spectrum (a) is for DNA reacted with *trans*-$Pt(NH_3)_2Cl_2$, (b) is unreacted DNA, and (c) and (d) are for reaction of DNA and nucleosome samples with $PtenCl_2$, respectively. The ratio of Pt/P is 0.2 for spectra (a), (c), and (d); other experimental conditions are given in section 2.

[PtdeinCl]Cl with any of the DNA samples. We have also not observed the downfield peak in any polymer containing A·T base pairs or in the alternating d(G–C) polymer.

Several observations about the new peak are noteworthy. (i) The signal is remarkably far downfield and the change in shift ($\Delta\delta$) is as large as in the B to Z conversion of DNA [12]. (ii) The T_1 value and linewidth for both the shoulder and the main peak are similar at a given field strength. (iii) Both peaks are shifted upfield similarly (~0.15 ppm) by Mg^{2+} ($r = 0.2$). (iv) The area of the shoulder is ~5% at $r = 0.2$ with salmon sperm and calf thymus DNA.

For the following reasons, we feel that the shoulder most probably indicates a Pt-induced structural distortion in the DNA. (i) $\Delta\delta$ is too large for an inductive effect of the Pt bound to the heterocyclic bases [13]. The non-anti-tumor Pt compounds studied would have similar inductive effects, yet the new peak was not observed. Inductive effects through A should be comparable to those through G or C but the shoulder is not seen in AT polymers. (ii) $\Delta\delta$ is within the range expected for direct Pt to phosphate O binding [14]. However, such binding is known to be very weak and

Table 1
Summary of ^{31}P NMR studies[a]

Type of DNA	Pt compound[c]	Main signal, ppm (width, Hz)	Downfield signal, ppm[d] (width, Hz)
salmon sperm[b]	none	4.21 (41)	–
salmon sperm[b]	$PtenCl_2$	4.22 (63)	2.95 (55)
salmon sperm[b]	*trans*-$Pt(NH_3)_2Cl_2$	4.24 (53)	–
polydG·polydC	none	4.12 (25)	–
polydG·polydC	$PtenCl_2$	4.10 (57)	~3.4
polydG·polydC	*trans*-$Pt(NH_3)_2Cl_2$	4.24 (87)	–
poly(dGdC)	none	4.15 (45)	–
poly(dGdC)	$PtenCl_2$	4.05 (87)	–
poly(dGdC)	*trans*-$Pt(NH_3)_2Cl_2$	4.20 (86)	–
nucleosomes	none	4.39 (58)	–
nucleosomes	$PtenCl_2$	4.39 (90)	2.96 (50)
nucleosomes	*trans*-$Pt(NH_3)_2Cl_2$	4.42 (81)	–

[a] 81.01 mHz, see section 2 for more details
[b] Very similar results were obtained with calf thymus DNA samples
[c] Reaction time 24–48 h
[d] Values obtained by a nonlinear least-squares Lorentzian curve deconvolution program

can account for a small percentage of the bound Pt at best. Our data indicate that about one-third of the Pt could be involved in causing the shift. In addition, such binding does not readily explain the absence of a shoulder in AT polymers unless a chelate between G and a phosphate O were involved. However, the similar influence of Mg^{2+} on both signals makes the presence of positive charge near the phosphate O appear unlikely. ^1H NMR studies with oligonucleotides (and polyI·polyC) have provided strong evidence for the binding of both available coordination sites on Pt to heterocyclic bases [3,15,16]. The only other well-established explanations for such pronounced $\Delta\delta$ include irreversible cleavage of the phosphodiester linkage and changes in bond and torsion angles at P [12]. However, the downfield peak does not shift significantly between pH 6 and 9 and addition of CN^- to displace the bound Pt yields the spectrum of pre-Pt-treated DNA. Therefore, no irreversible change has occurred. The only feasible explanation remaining for the downfield peak is a guanine-specific reaction and resulting structural change in the DNA which alters bond and/or torsion angles at phosphorus.

Only a few studies of Pt anti-tumor compounds have utilized nucleosomes, which are better models for DNA in vivo [6]. We find that the main ^{31}P NMR signal of Pt-treated nucleosomes is still ~0.2 ppm upfield from the free DNA signal (table 1 and fig.2d). Complexes of DNA with positive cations, such as histones, Mg^{2+} [7], and tetralysine [8], give such upfield shifts. Therefore, most of the DNA in Pt-treated nucleosomes must remain complexed with histones. However, the new downfield peak observed with $PtenCl_2$ is almost identical in shift, width, and relative intensity with this signal in free DNA. This similarity may indicate that the DNA–histone interactions are broken or very weak near the Pt-reacted sites which give rise to the new peak. It is possible that these severe distortions are significant in the action of the anti-tumor compounds. No similar distortions are found with inactive *trans*-$Pt(NH_3)_2Cl_2$ (table 1).

These results and those obtained on a decanucleotide in the accompanying paper [17], provide strong evidence for primary reaction of Pt antitumor agents at adjacent G residues on DNA strands. This reaction then leads to a conformational change and large downfield shift of DNA phosphate resonances in ^{31}P NMR spectra. We have shown that a similar reaction and conformational change can occur in nucleosomes. Studies are in progress to identify the exact nature of the conformational change.

ACKNOWLEDGEMENTS

The support of NIH grants GM 29222 (LGM) and GM 30267 (WDW) and a loan of K_2PtCl_4 from Johnson-Matthey is gratefully appreciated.

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