

A phosphorus NMR study of the reaction products of *cis*-diamminedichloroplatinum (II) with a double-helical oligonucleotide and with DNA

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The structural distortion of oligonucleotides upon *cis*-PtCl₂(NH₃)₂{d(T-C-T-C-G-G-T-C-T-C)-N7(5), N7(6)} reveals shifting of 4 phosphorus resonances due to platination. 3 Resonances could be assigned by selective ³¹P-irradiation, showing P(6) (P between the two Gs) to be shifted 1.5 ppm to low field. In the concomitant double strands P(6) is shifted 0.9 ppm to lower field. A similar peak has been observed in platinated salmon sperm DNA (37°C), indicating that Pt-binding to GpG-fragments in DNA is similar to that found for the decanucleotide, so the distortion of DNA might be comparable.

cis-PtCl₂(NH₃)₂ ³¹P NMR Double-helical decanucleotide ³¹P-assignment Salmon sperm DNA

1. INTRODUCTION

The cytotoxic activity of the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) is generally accepted to originate from its reaction with DNA [1]. After reaction of *cis*-DDP with DNA in vitro, a single complex appeared to account for more than 50% of the bound platinum [2]. The conformational properties of this *cis*-Pt(NH₃)₂{d(pGpG)-N7(1),N7(2)} adduct [3] have been studied [4], or incorporated in larger oligonucleotides [5]. An important question deals with the degree of distortion of a DNA double helix after *cis*-DDP binding. Several authors have suggested unwinding of the double helix up to several base pairs [6]. We recently investigated a decamer double strand with a *cis*-Pt(NH₃)₂{d(-GpG-)} part present in the centre of one of the decamers [7]. A proton NMR study of this compound revealed that double-strand formation is destabilized to some extent and that the conformation of the backbone is altered. However, below 28°C the double helix appears to be intact [7].

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The structural distortion of the decamers induced by platinum binding is accompanied by changes in the ³¹P NMR spectra. We describe the assignments of some characteristic ³¹P-resonances and the application of the results to platinated salmon sperm DNA.

2. EXPERIMENTAL

The deoxydecanucleosidenonaphosphates **I**, d(T-C-T-C-G-G-T-C-T-C), and **II**, d(G-A-G-A-C-C-G-A-G-A), were synthesized using an improved phosphotriester approach [8]. As previously described [7], the reaction of **I** with *cis*-DDP yields *cis*-Pt(NH₃)₂{d(T-C-T-C-G-G-T-C-T-C)-N7(5),N7(6)} [3], abbreviated as **I-Pt**. The double strands (**I** + **II**) and (**I-Pt** + **II**) are abbreviated as **III** and **III-Pt**, respectively. 5 mM NMR samples were prepared by dissolving the appropriate amounts of the decamers in D₂O with a trace of EDTA. Salmon sperm DNA was obtained from Sigma. Reaction with the appropriate amounts of freshly dissolved *cis*-DDP was left overnight at 37°C at a concentration of 0.25 DNA mg/ml. After lyophilization, the NMR samples were prepared by dissolving the pro-

duct in 9.5 ml D₂O with 10 mM Tris/HCl (pH 7.0), 100 mM NaCl, 10 mM MgCl₂, 1 mM EDTA and 0.1 ml tetramethylphosphonium bromide (TMPB). ³¹P NMR spectra were recorded at several temperatures on a Bruker WB-200 spectrometer, operating at 81.1 MHz, interfaced with an Aspect-2000 (12 k datapoints) computer for data accumulation. Heteronuclear proton noise decoupling was used throughout. TMPB was used as an internal

reference, shifts are reported relative to 3'5'-cyclic AMP (cAMP) [9]. Generally, 2000 scans for the decamers and 20 000 scans for the DNA samples were accumulated to improve signal-to-noise ratio. Proton spectra were recorded at 500 MHz on a Bruker WM-500. Phosphorus signals were selectively irradiated, using a locally constructed decoupling equipment.

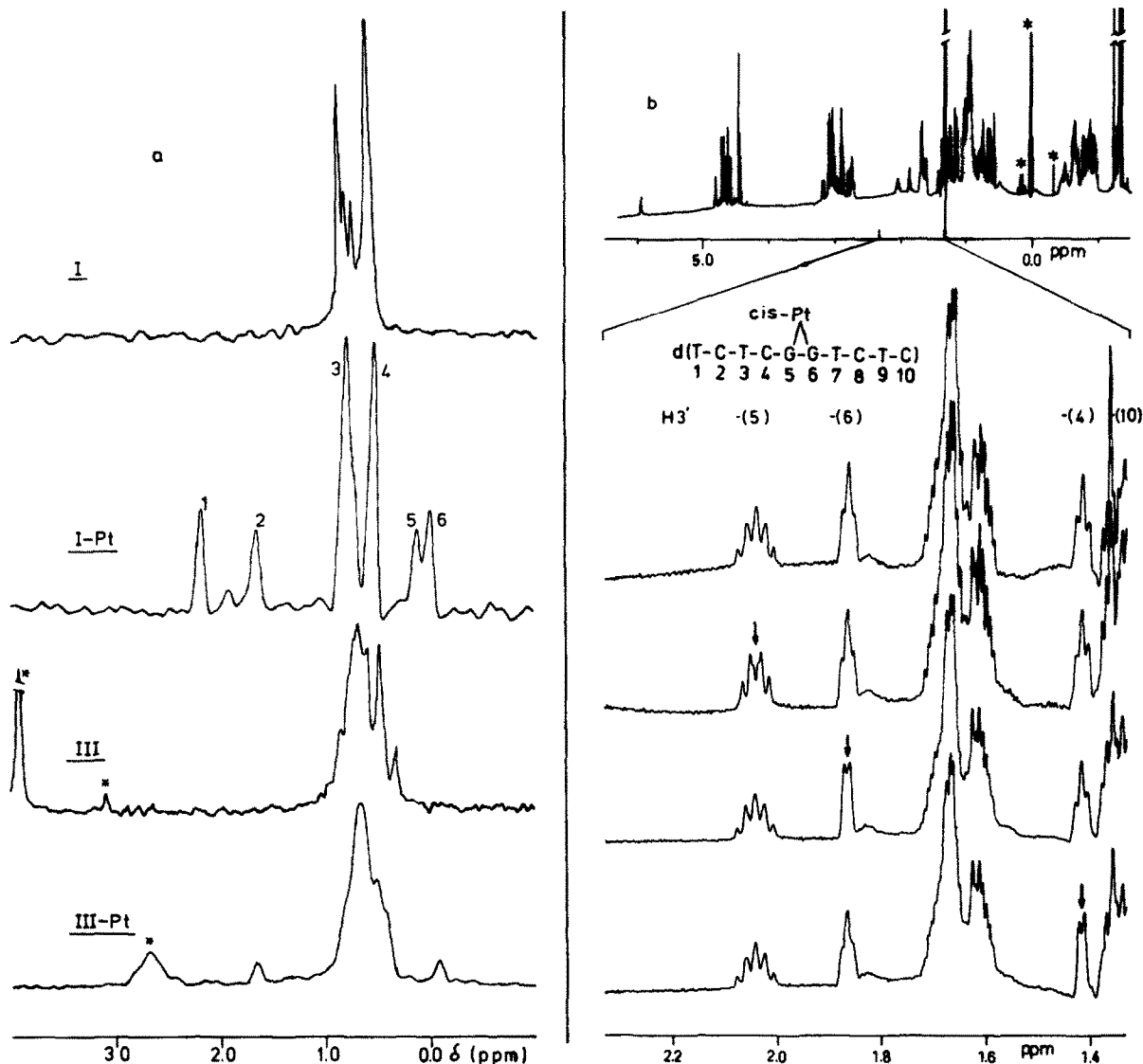


Fig.1. (a) ³¹P NMR spectra of I, I-Pt, III and III-Pt. Reference: cAMP. Impurities (most likely free phosphate) are indicated with an asterisk. (b) Top lane: ¹H NMR spectrum of I-Pt; second lane: expanded region with H₃' protons; third-fifth lane: ¹H NMR spectra with ³¹P selectively irradiated at peaks 1, 4 and 6, respectively. Chemical shift reference is N(Me)₄NO₃, spectra are recorded at 500 MHz.

3. RESULTS AND DISCUSSION

3.1. Decamer single strands

^{31}P spectra of **I** and **I-Pt** are shown in fig. 1a. In B-DNA-like single or double-stranded oligonucleotides, ^{31}P resonances occur generally between 0.3 and 0.9 ppm, relative to cAMP [9,10]. The phosphorus atoms of the single-stranded **I** indeed resonate in this range. In **I-Pt**, 4 of the resonances are spread outside the common range. Our assignment method followed [11]: the ^1H spectrum is recorded under simultaneous selective irradiation of a ^{31}P resonance.

With the aid of 1- and 2-dimensional NMR experiments, using the nuclear Overhauser effect, several protons of **I-Pt** could be assigned (unpublished). The proton spectrum of **I-Pt** and the expanded part of the H3' protons are shown in fig. 1b (top two lanes). Four H3' proton resonances are well resolved. The rightmost one, at 1.38 ppm, is partly hidden underneath a side band of the HDO resonance. However, it lacks a ^1H - ^{31}P coupling and can be assigned to H3' (10). Fortunately, the 3 remaining protons allow the assignments of the central 3 phosphorus atoms. The irradiation experiments, also shown in fig. 1b, reveal peak 1 of the ^{31}P spectrum of **I-Pt** (see fig. 1a) to be P(6), peak 4 to contain P(7) and peak 6 to be P(5).

Referring to the phosphorus spectrum of **I-Pt**, now two of the 4 resonances (P(5) and P(6)) observed outside the 0.3–0.9 ppm range, are assigned. The fact that two other resonances besides P(5) and P(6) are observed outside this range perhaps indicates that platinum binding to this single strand affects its structure more than the central 4 residues.

3.2. Decamer double strands

^{31}P NMR spectra of **III** and **III-Pt** are shown in fig. 1a (third and fourth lanes). The unplatinated decamer double strand (**III**) exhibits all ^{31}P resonances in between 0.3 and 0.7 ppm relative to cAMP, which is a feature of normal double-stranded DNA. In contrast, the platinated double strand **III-Pt** exhibits, besides the large peak at 0.6 ppm, one resonance at 1.5 and one at -0.1 ppm. The proton spectrum of **III-Pt** could be partly assigned (unpublished) and H3'(5) appears to be well resolved. This allows the assignment of the low-field resonance to P(6) (not shown).

Comparing **I-Pt** and **III-Pt**, a large chemical shift difference (0.6 ppm at 25°C) is apparent between P(6) in the single and double strand. Besides this, two phosphorus resonances revert to 'normal' chemical shift values going from the single to the double strand. So it can be concluded that structural consequences of platinum binding appear less severe in a double helix compared to a single strand. To investigate the differences between **I-Pt** and **III-Pt** more thoroughly, the temperature dependency of the ^{31}P chemical shifts was studied; results are shown in fig. 2. The phosphorus resonances of the double-strand **III-Pt** clearly exhibits S-shaped curves, reflecting the melting of the duplex (melting transition midpoint of $\sim 37^\circ\text{C}$).

At higher temperature ($T > 60^\circ\text{C}$) the resonance of P(6) in **I-Pt** and in **III-Pt** clearly overlap, confirming the assignment of this phosphorus resonance. The approach of the upfield-shifted resonance in **III-Pt** at $T > 60^\circ\text{C}$ to the resonance of P(5) in the single strand, indicate this phosphorus reson-

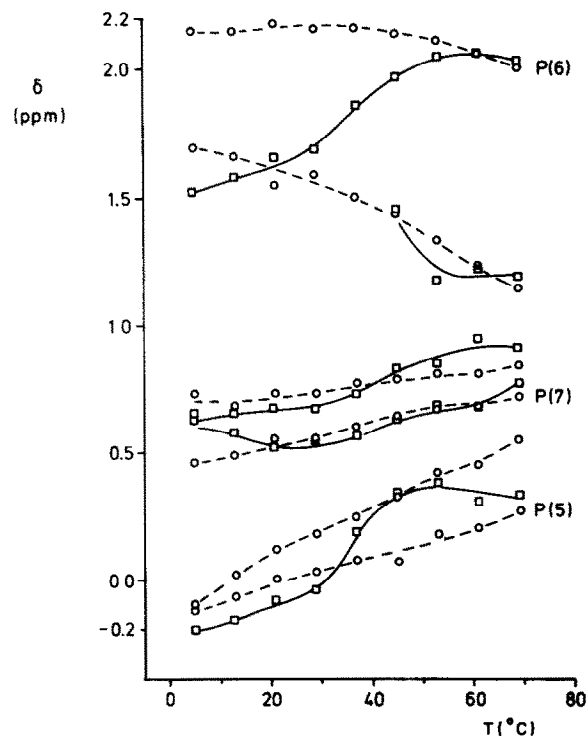


Fig. 2. Temperature vs chemical shift profiles of **I-Pt** (○) and **III-Pt** (□). The curves between 0.5 and 0.9 ppm refer to more than a single phosphorus resonance.

ance to originate from P(5) in the double strand as well.

3.3. Salmon sperm DNA

Authors in [12] showed ^{31}P spectra of platinated DNA in which a shoulder at low field was detected. They proposed that this lowfield resonance originates from the phosphorus in a *cis*-Pt(NH₃)₂{d(-GpG-)-N7,N7} chelate [13,14]. Our oligonucleotide results unambiguously confirm this proposal.

The difference in chemical shift of P(6) in I-Pt and III-Pt at ambient temperature offers the possibility to see whether or not the Pt-GpG moiety in DNA is double stranded at 37°C. In fig.3, the spectra of free and platinated salmon sperm DNA ($r_b = 0.2$ and 0.05 , r_b is the amount of platinum per base) are shown. The chemical shifts of the small low-field peaks amount to 1.73 and 1.68 ppm (r_b or 0.2 and 0.05 , respectively). This value of approx 1.70 ppm fits in the temperature chemical shift profile of P(6) in III-Pt (fig.2) at approx. 30°C. At

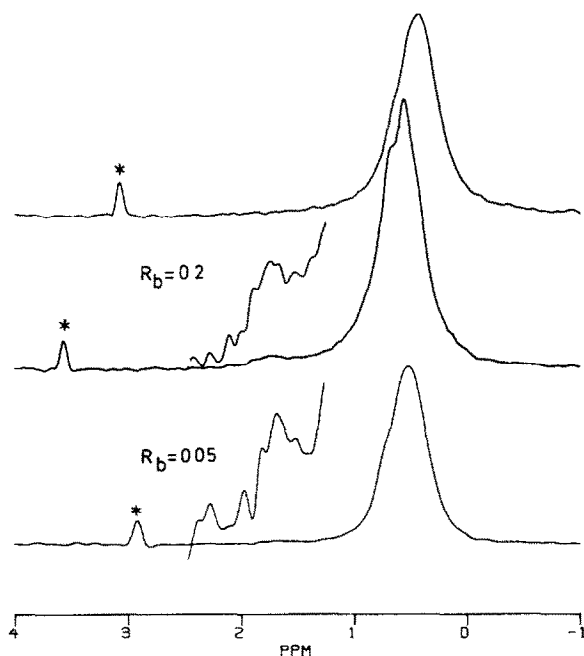


Fig.3. ^{31}P NMR spectra of 'free' DNA (top lane) and platinated DNA ($r_b = 0.2$ and 0.05 in second and third lanes, respectively). The part between 1.5 and 2.5 ppm is expanded, showing the small low field peak. Impurities (most likely inorganic phosphate) are indicated with an asterisk.

this temperature III-Pt still is double stranded [7] and the imino protons in base pairs C(4)·G(17) and G(6)·C(15) are clearly observed. However, the imino-proton resonance of base pair G(5)·C(16) is just broadened beyond detection. This indicates either complete or partial melting of base pair G(5)·C(16), depending on the exchange rate with water protons and on the opening/closing rate of the duplex [15].

Extrapolating the results of the single- and double-stranded decamer model compounds to DNA, it is concluded that *cis*-DDP binding to a GpG fragment in DNA results in relatively small distortion in which, even at 37°C, in the outmost case one base pair is dissociated.

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