^{31}P and ^{1}H NMR Spectroscopic Studies of Platinum Adducts of Poly(I)·Poly(C). The Antitumor Agent *cis*-$Pt(NH_3)_2Cl_2$ Forms an N7,N7 Bis-Adduct

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Abstract: The ^{1}H NMR spectral changes at 360 MHz and elevated temperatures induced in the spectrum of poly(I)·poly(C) on low-temperature treatment with *cis*-$Pt(NH_3)_2Cl_2$ were investigated. The resulting platinated polymer, poly(I)·poly(C)·*cis*-Pt, was also examined with ^{31}P NMR spectroscopy as were adducts formed with $Pt(en)Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$, and [Pt(dien)Cl]Cl (dien = diethylenetriamine, en = ethylenediamine). The ^{1}H NMR spectrum of the $Pt(en)Cl_2$-treated polymer was also investigated and found to be qualitatively similar to that of poly(I)·poly(C)·*cis*-Pt. Contrary to published work on the latter platinated polymer, we conclude that most of the Pt is attached to two adjacent I residues via N7 of the base and is, therefore, a bis-adduct. This conclusion was supported both by our reinterpretation of earlier published studies and by additional experiments. In particular, in evaluating the downfield H8 resonances of N7-platinated bases, we allowed for the loss in H8 signal area (ca. 34%) expected from the chemical shift anisotropy of ^{195}Pt and the H8–Pt coupling at high field (360 MHz). With this type of correction, two H8 signals per Pt were found to be shifted downfield. Additional evidence for such a species includes equal intensity of the two major downfield H8 signals (at 8.99 and 8.82 ppm, 80 °C, pH 7.1) as expected for a bis-adduct, an NOE of the expected magnitude between these two resonances, and the absence of spectral changes on treatment with imidazole. Other features supporting N7 platination include evidence for a decreased pK_a of N1H of the platinated base of I, the facile exchange of H8 for D, and the absence of significant line broadening of the H8 signal by added Cu^{2+}. With similar methods, we assign a signal at ~7.9 ppm to an *upfield*-shifted H8 resonance. The ^{31}P NMR spectrum of poly(I)·poly(C) platinated with $Pt(en)Cl_2$ or $Pt(en)(H_2O)_2^{2+}$ contains two signals of roughly equal intensity attributable to the polyI strand. These signals are assigned to the PO_4 groups between the platinated I residues in a bis-adduct and the PO_4 group between complexes. The ^{31}P NMR signal for the polyC strand is sharp and corresponds closely to that for poly(C), unplatinated. This finding, along with the disappearance on treating poly(I)·poly(C) with $Pt(en)Cl_2$ of the N1H ^{1}H NMR signal in H_2O (90%)/D_2O (10%) observed by using the Redfield pulse method, demonstrates essentially complete disruption of the duplex. In contrast, changes in both the N1H ^{1}H NMR signal and in the ^{31}P NMR spectra of poly(I)·poly(C) on treatment with Pt complexes lacking cis leaving group (*trans*-$Pt(NH_3)_2Cl_2$, [Pt(dien)Cl]Cl) were consistent with preservation of the duplex with comparatively less dramatic structural modifications. Similarly, the ^{31}P NMR spectrum of poly(A)·poly(U) was only broadened somewhat by $Pt(en)Cl_2$. These comparative changes parallel, in several respects, the NMR spectral changes observed on treating DNA, nucleosomes, and oligonucleotides with these and related Pt compounds. The configuration of the bis-adducts in the poly(I)·poly(C) reactions must be similar to that observed with oligonucleotides. In contrast, the treatment of polyI (single stranded) with $Pt(en)Cl_2$ led to a different ^{31}P NMR spectrum, possibly due to the formation of cross-links between remote bases on the same or different strands.

Preliminary studies which indicated that Pt antitumor agents caused the appearance of a new peak ca. 1 ppm downfield from the ^{31}P NMR signal of DNA have recently been extended to confirm this observation[2,3] and to demonstrate similar signals in the ^{31}P NMR spectrum of other macromolecules such as nucleosomes and poly(dG)·poly(dC).[2] Inactive agents such as *trans*-$Pt(NH_3)_2Cl_2$ and [Pt(dien)Cl]Cl (dien = diethylenetriamine) do not induce downfield signals and the signals were not observed in other synthetic polynucleotides (poly[d(GC)], poly(dA)·poly(dT) and poly[d(AT)]).[2] Extensive studies of ^{31}P NMR spectral changes with defined sequence deoxyoligonucleotides confirm the appearance of this downfield signal only when two adjacent G residues are present and also only when the Pt compound has two cis leaving ligands.[3-5]

In macromolecules, the width and position of the ^{31}P signal does contain useful information,[6] but in comparison to ^{1}H NMR spectroscopy, it is desirable to broaden background data on the effects of metal species on ^{31}P NMR signals of polynucleotides. For example, several features of the ^{31}P NMR changes in DNA and polydG·polydC remain to be fully understood.[2] In particular, with polydG·polydC, the area of the downfield signal is less than expected on the basis of the anticipated bifunctional mode of binding and the expected shift of the signal of one ^{31}P moiety per each Pt species reacted. Also, whereas no temperature dependence is observed for the ^{31}P NMR downfield peak of polynucleotides,[7] such changes are found in oligonucleotides,[5] perhaps due to the weaker duplexes formed by the shorter molecules. Finally, although deconvolution of broad ^{31}P NMR signals is sometimes difficult, the area of the downfield peak may not be a simple function of GG frequency in different DNA's.[7]

We felt that, although polynucleotides have some disadvantage in handling and in spectral resolution compared to oligonucleotides, a more extensive study of a polynucleotide could be helpful in resolving some anomalies. Since poly(dG)·poly(dC) itself had some puzzling behavior and is both expensive and difficult to manipulate due to aggregation effects, poly(I)·poly(C), a polyribonucleotide duplex, seemed like a good choice for further study. The interaction of this material with *cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$, and [Pt(dien)Cl]Cl has been thoroughly examined by standard biophysical methods (CD, UV, centrifugation, melting temperatures, etc.) as well as by ^{1}H NMR spectroscopy.[8-13] We

(1) Wilson, W. D.; Heyl, B. L.; Reddy, R.; Marzilli, L. G. *Inorg. Chem.* **1982**, *21*, 2527.
(2) Marzilli, L. G.; Reily, M. D.; Heyl, B. L.; McMurray, C. T.; Wilson, W. D. *FEBS Lett.* **1984**, *176*, 389.
(3) den Hartog, J. H. J.; Altona, C.; Van Boom, J. H.; Reedijk, J. *FEBS Lett.* **1984**, *176*, 393.
(4) Fouts, C. S.; Reily, M. D.; Zon, G. F.; Marzilli, L. G., manuscript in preparation.
(5) Reedijk, J., personal communication.
(6) Gorenstein, D. G.; Luxton, B. A.; Goldfield, E. M.; Lai, K.; Vegeais, D. *Biochemistry* **1982**, *21*, 580.
(7) Reily, M. D.; Wilson, W. D.; Marzilli, L. G., unpublished results.
(8) Fazakerley, G. V.; Hermann, D.; Guschlbauer, W. *Biopolymers* **1980**, *19*, 1299.
(9) Hermann, D.; Guschlbauer, W. *Biochimie* **1978**, *60*, 1046.
(10) Hermann, D.; Houssier, C.; Guschlbauer, W. *Biochim. Biophys. Acta* **1979**, *564*, 456.
(11) Hermann, D.; Fazakerley, G. V.; Houssier, C.; Guschlbauer, W. *Biopolymers* **1984**, *23*, 945.
(12) Fazakerley, G. V.; Hermann, D.; Guschlbauer, W.; Hankes, G. E. *Biopolymers* **1984**, *23*, 961.

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hoped, then, to examine the ^{31}P NMR changes during reaction of poly(I)·poly(C) with Pt complexes and to obtain spectra on well-defined states.

In addition, the published studies[8] on poly(I)·poly(C) contained some observations contrary to general conclusions reached with ^1H NMR studies of oligonucleotides[14-23] and with studies on product distribution after enzymatic degradation of platinated DNA.[24-29] Namely, it was concluded[8] that *cis*-$Pt(NH_3)_2Cl_2$ formed mainly (~60%) mono-adducts (one Pt–N7 bond) with poly(I)·poly(C). Since degradation of DNA treated with *cis*-$Pt(NH_3)_2Cl_2$ has revealed mainly bis-adducts (two Pt–N7 bonds)[24,25,28] and other studies have suggested predominantly bis-adducts,[30] we wished to reinvestigate this problem, particularly since the published ^1H NMR studies[8] of poly(I)·poly(C) reacted with *cis*-$Pt(NH_3)_2Cl_2$ (abbreviated here as poly(I)·poly(C)·*cis*-Pt) are not in total agreement with oligonucleotide work.

Experimental Section

Materials. Poly(I)·poly(C) (in phosphate buffer from Miles Laboratories) was made suitable for NMR studies by using previously described methods except that phosphate buffer was used instead of PIPES buffer.[31] After 8–12 h of sonication, the mean length was from 170 to 200 base pairs as determined by polyacrylamide gel electrophoresis.[32] After filtration through 0.45-μm Nylon 66 filters, the phosphate buffer was exchanged for diluted PIPES 10 buffer (0.01 M PIPES, 0.001 M EDTA, 0.100 M $NaNO_3$, pH 7.0) by dialysis. The sample and buffer concentrations were then adjusted by lyophilization and the addition of appropriate volumes of H_2O, D_2O, or H_2O/D_2O. The final polynucleotide concentration was determined by UV spectroscopy (ϵ_{260} 5300 M^{-1} cm^{-1}) and the samples were characterized by melting (T_m = 67 °C and hypochromicity = 22% at 260 nm, PIPES 10) and ^{31}P and ^1H NMR spectroscopy.

Pt compounds, prepared by literature methods[33,34] or purchased from Aldrich, were added as solids to NMR tubes containing 0.01 or 0.02 M (bases) poly(I)·poly(C). Unless otherwise stated, reactions were carried out at ambient temperature, the mixtures were stirred gently in the dark until all the solid dissolved, typically 8–12 h for neutral Pt complexes, and then left in the dark for several days. For reactions at 12 °C, the ^{31}P NMR spectral changes were complete only after 2–4 weeks.

NMR Spectroscopy. ^1H NMR spectra were recorded on a Nicolet NB360 spectrometer equipped with a VT unit operating in the FT mode at 361.08 MHz. Normally, ^1H NMR samples in 5-mm tubes contained 10% D_2O and spectra were obtained using a Nicolet-supplied version of the Redfield 21412 pulse sequence. Irradiation was at 15 or 10 ppm (downfield from internal TSP) and pulse widths were 251 and 442 μs, respectively. The low-power transmitter was attenuated so that the pulse corresponded to a 90° tip angle. Spectra represent the average of 200–2000 16 K transients. In some cases, signal to noise was enhanced by exponential multiplication (LB = 0.5 Hz) of the FID prior to Fourier transformation.

NOE measurements: NOE experiments were carried out on samples lyophilized thrice from 99.8% D_2O and finally brought up in 99.96% D_2O just prior to spectral measurements. Concentrations of polymer–Pt complex, buffer, and salts were twice those described above, to improve signal to noise. The spectra were recorded at 60 °C in the alternate accumulation mode and represent the accumulation of 192 transients. Difference spectra were obtained by subtracting the off-resonance spectrum from the resonance-saturated spectrum.

$^{31}P\{^1H\}$ NMR spectra were recorded on an IBM WP200SY spectrometer equipped with a VT unit operating in the FT mode at 81.01 MHz. The accumulation parameters were as follows: 4K data points, sweep width ±2000 Hz, pulse width 14.8 μs (45° tip angle), accumulation time = 0.9 s, no relaxation delay, and continuous low-level ^1H decoupling. Generally, the samples were in D_2O containing 0.02% trimethylphosphate (TMP) as an internal standard, in 10-mm NMR tubes, and T was usually ~30 °C.

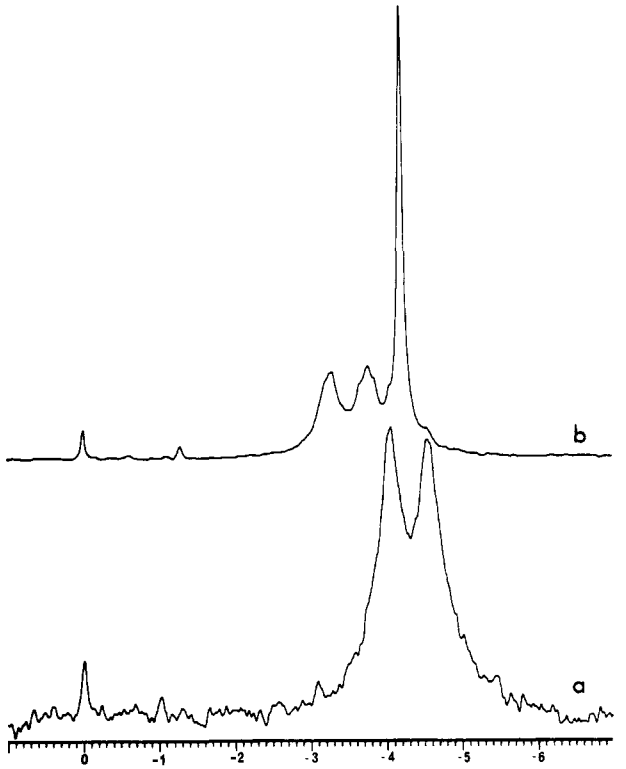


Figure 1. ^{31}P NMR spectra at 35 °C of 0.01 M poly(I)·poly(C) in PIPES 10 buffer, pH 7.0: (a) no Pt, (b) 0.2 Pt(en)Cl_2/base. Spectral conditions are listed in the Experimental Section. The small peak at −1 ppm is from residual inorganic phosphate.

Table I. ^{31}P NMR Spectral Data of Poly(I)·Poly(C) and the Effect of $Mg(NO_3)_2$[a]

$Mg(NO_3)_2$, mM	poly(I)	poly(C)	Δpoly(I)	Δpoly(C)
0	−4.03	−4.54		
2	−4.21	−4.59	−0.18	−0.05
4	−4.32	−4.62	−0.29	0.08
10	−4.52	−4.64	−0.49	−0.10

[a] See Experimental Section for solution conditions. Chemical shifts in ppm are relative to internal TMP.

Results

^{31}P NMR Spectral Studies. The $^{31}P\{^1H\}$ NMR spectrum of poly(I)·poly(C) in PIPES 10 buffer (Figure 1, Table II) shows two fairly well-resolved signals at −4.03 and −4.50 ppm upfield

(13) Hermann, D.; Fazakerley, G. V.; Guschlbauer, W. *Biopolymers* **1984**, *23*, 973.
(14) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *J. Am. Chem. Soc.* **1980**, *102*, 5565.
(15) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352.
(16) Marcelis, A. T. M.; Hartog, J. H. J.; van der Marel, G. A.; Wille, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *135*, 343.
(17) Neumann, J.-M.; Tran-Dinh, S.; Girault, J.-P.; Chottard, J.-C.; Huynh-Dinh, T.; Igolen, J. *Eur. J. Biochem.* **1984**, *141*, 465.
(18) den Hartog, J. H. J.; Altona, C.; Chottard, J. C.; Girault, J. P.; Lallemand, J.-Y.; deLeeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 4715.
(19) Van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J. P.; Huynh-Dinh, T.; Lallemand, J. Y.; Igolen, J.; Chottard, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 3037.
(20) Caradonna, J. P.; Lippard, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 5793.
(21) Girault, J. P.; Chottard, J. P.; Guittet, E. R.; Lallemand, J. Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157.
(22) Marcelis, A. T. M. Ph.D. Thesis, University of Leiden, The Netherlands, 1982.
(23) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528.
(24) Eastman, A. *Biochemistry* **1983**, *22*, 3927.
(25) Fichtinger-Schepman, A. M. J.; Lohman, P. H. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 5345.
(26) Tullius, T. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 4620.
(27) Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, M. H.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487.
(28) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry*, in press.
(29) Inagaki, K.; Kasuya, K.; Kidani, Y. *Inorg. Chim. Acta* **1984**, *91*, L13.
(30) Rahn, R. O. *J. Inorg. Biochem.* **1984**, *21*, 311.
(31) Wilson, W. D.; Jones, R. L. *Nucleic Acids Res.* **1982**, *10*, 1399.
(32) Maniatis, T.; Jeffrey, A.; van de Sande, H. *Biochemistry* **1975**, *14*, 3787.
(33) Dhara, S. C. *Indian J. Chem.* **1970**, *8*, 193.
(34) Watt, G. W.; Cude, W. A. *Inorg. Chem.* **1968**, *7*, 335.

Table II. Changes Induced in the ^{31}P NMR Spectrum of Some Polynucleotides by Various Pt Compounds[a]

nucleic acid	metal salt	description of ^{31}P spectrum[b] poly(I) signal	poly(C) signal
poly(I)·poly(C)	none	−3.9 (25)	−4.5 (25)
	cis-$Pt(NH_3)_2Cl_2$(0.2)	−3.4,[c] −3.8,[c] −3.9	−4.2 (8)
	$Pt(en)Cl_2$(0.2)	−3.3 (21), −3.7 (21)	−4.2 (8)
	$Pt(en)(H_2O)_2^{2+}$ (0.2)	−3.3 (21), −3.7 (21)	−4.2 (8)
	trans-$Pt(NH_3)_2Cl_2$(0.2)	−3.8,[c] −3.9, −4.2[c]	−4.5 (25)
	[Pt(dien)Cl]Cl(0.2)	−3.4 (~60), −3.9 (~60)	−4.9 (50)
poly(I)	none	−3.7 (12)	
	$Pt(en)(H_2O)_2^{2+}$ (0.2)	−3.2 (10),[d] −3.3 (10),[d] −3.6,[c] −3.7 (15)	
	trans-$Pt(NH_3)_2Cl_2$(0.2)	−3.4,[c] −3.8,[c] −3.9	
	[Pt(dien)Cl]Cl(0.2)	−2.8 (10),[d] −3.2 (20), −3.3 (8), −3.4,[c] −3.7, −4.0[c]	
poly(C)	none		−4.2 (8)
	$Pt(en)Cl_2$(0.2)		−3.9,[c] −4.2 (12)
	trans-$Pt(NH_3)_2Cl_2$(0.2)		−3.5,[c] −3.9,[c] −4.1,[c] −4.2
	[Pt(dien)Cl]Cl(0.2)		−4.2 (13), −4.5,[c] −4.7 (10)

[a] See Experimental Section for solution conditions. Chemical shift values relative to internal TMP. [b] The full width at half-height, in Hz, is indicated in parentheses. [c] Signals appear as upfield or downfield shoulders on main signal. [d] Signals are small (<5% of total area).

from internal trimethyl phosphate (TMP). The downfield and upfield poly(I)·poly(C) signals are assigned to the polyI strand and the poly(C) strand, respectively.[35] Poly(I)·poly(C) is reported to have an "A"-type helix;[10] the ^{31}P spectrum is consistent with a double-helical arrangement with phosphates on opposing strands in dissimilar environments.

The addition of Mg^{2+} shifts both resonances upfield, but to a different degree, Table I. At 10 mM added $Mg(NO_3)_2$, the poly(I) signal shifts more than 0.4 ppm and the poly(C) peak shifts less than 0.1 ppm. The upfield shifts are consistent with the effects of base-binding cations on the ^{31}P NMR spectrum of calf thymus DNA.[1]

The effect of *cis*-PtA_2Cl_2(A_2 = NH_3, $^1/_2$en) antitumor agents on the duplex (reaction T = 25 °C) is dramatic (Figures 1 and 2, Table II). A somewhat simpler spectrum is obtained with the en (ethylenediamine) compound and so the changes induced in the ^{31}P NMR spectrum of poly(I)·poly(C) by this compound will be described first, followed by a description of the less clear-cut case involving the NH_3 compound. At r = 0.05 (r = Pt/P ratio), the poly(I) resonance broadened and a new peak was observed at −3.3 ppm. Increasing the r to 0.1 revealed two new peaks at −3.7 and −4.2 ppm. In addition, we observed an increase in the size of the downfield peak at −3.3 ppm with a concomitant decrease in the original signals from duplexed poly(I)·poly(C). The resonance at −4.2 ppm is sharp and coincides with the signal from single-stranded poly(C) under identical conditions (vide infra). At r = 0.2, the three new signals contained almost all the intensity in the ^{31}P NMR spectrum (see Figure 1). The two downfield resonances are approximately equal in intensity and the fully developed signal from single-stranded poly(C) is evidence for essentially complete strand separation at this r value. The result is in agreement with UV and CD studies mentioned previously.[10] For r values above 0.2, the resolution of the spectra was decreased with many peaks evident and eventually (r ~ 0.3) the intensity of the unduplexed poly(C) peak decreased. New resonances attributable to platinated C were observed. Identical results were obtained when $Pt(en)(H_2O)_2^{2+}$ was used instead of the dichloride.

Reactions similar to those described above were carried out at 12 and 25 °C in the presence of 0.02 M Mg^{2+}. Under these conditions where the duplex should be more stable we nevertheless observed spectral changes consistent with identical product formation and essentially complete duplex loss, as was described above.

The products formed in an analogous reaction with *cis*-$Pt(NH_3)_2Cl_2$ did not have such well-resolved ^{31}P NMR spectra. The downfield peak at −3.3 ppm in the $Pt(en)Cl_2$ reaction appeared further upfield at ca. −3.5 ppm and was a poorly resolved shoulder of the −3.7 ppm signal (Figure 2). This difference is interesting in view of the ^1H NMR changes observed with the two antitumor agents (see below).

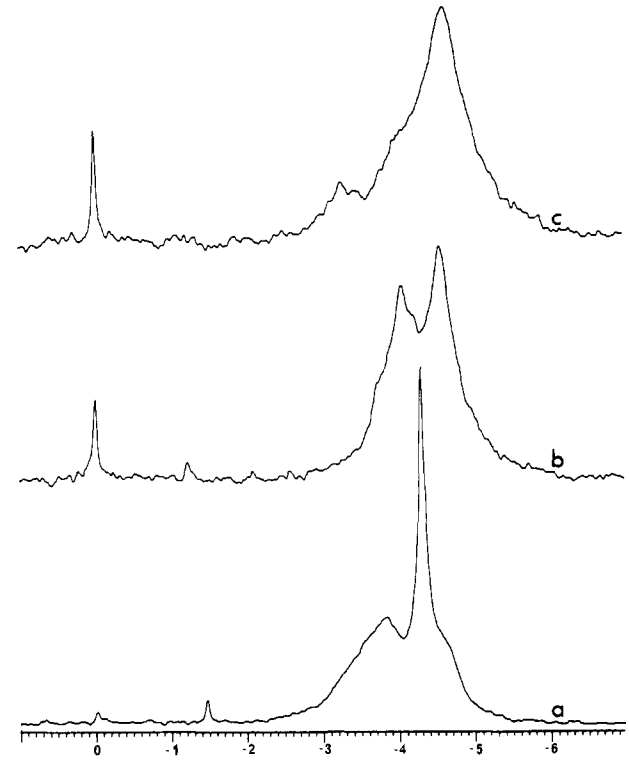


Figure 2. ^{31}P NMR spectra at 25 °C of various platinum adducts of poly(I)·poly(C) (0.01 M bases, 0.2 Pt/base): (a) *cis*-$Pt(NH_3)_2Cl_2$, (b) *trans*-$Pt(NH_3)_2Cl_2$, and (c) [Pt(dien)Cl]Cl. Spectral conditions are listed in the Experimental Section. The small peak at −1 ppm is from residual inorganic phosphate.

Heating a sample of platinated poly(I)·poly(C) (r = 0.2, $Pt(en)Cl_2$) caused sharpening of the three peaks with a more or less concerted small downfield shift (0.1–0.3 ppm at 65 °C) of all three signals. No new peaks were observed and the relative intensity of all the peaks remained constant over the temperature range studied. In contrast, addition of $Pt(en)Cl_2$ to poly(I)·poly(C) at 80 °C resulted in a ^{31}P NMR spectrum at 25 °C with a single narrow resonance at −4.2 ppm attributable to single-stranded poly(C) and a very broad resonance ($\nu_{1/2}$ ≃ 72 Hz, full width at half-height) at −3.7 ppm. This latter resonance is coincident with platinated single-stranded poly(I) (vide infra).

^{31}P NMR spectroscopy was used to follow several "stripping" reactions with ligands that are known to be strong Pt binders:

(35) Joseph, A. P.; Bolton, P. H. *J. Am. Chem. Soc.* **1984**, *106*, 437.

Table III. Selected ^{1}H NMR Spectral Data for Poly(I)·Poly(C) and Adducts Formed with Various Pt Species[a]

Pt compd	T, °C[b]	H8(I)	H2(I)	H6(C)[c]	H5(C)[c]	H1'(I)[d]	H1'(C)[d]
none	70	8.35 (1)	8.17 (1)	7.84 (1)	6.0[e]	6.0[e]	5.83 (1)
	25	7.90 (1)	7.55 (1)	7.19 (1)			
cis-$Pt(NH_3)_2Cl_2$, r = 0.1	70	9.06 (0.02)	8.35 (0.05)	7.84 (1.0)	5.96 (1.0)	6.18 (0.4)	5.79 (1.0)
		8.99 (0.14)	8.30 (0.03)				
		8.82 (0.14)	8.23 (0.14)				
		8.32 (0.10)	8.16 (0.18)				
		8.26 (0.23)	8.12 (0.14)				
		7.91 (0.20)	8.08 (0.26)				
			8.06 (0.20)				
	25	9.1	7.6	7.7			
		8.5		7.2			
$Pt(en)Cl_2$, r = 0.1	70	9.08	8.19	7.76	5.95	6.20	5.80
		9.08	8.16			6.13	
		8.54	8.12			5.98	
		8.39					
		8.35					
		8.32					
		8.29					
		7.92					

[a] 360 MHz data, δ values in ppm from internal TSP. Values in parentheses represent the number of protons relative to H6(C) = 1.0. See text for explanation of peak assignments. [b] Resonances at 25 °C are broad and poorly resolved. [c] Doublet; $^3J_{5,6}$ = 7.6 Hz. [d] Doublet; $^3J_{1',2'}$(C) = 2.5 Hz (80 °C); $^3J_{1',2'}$(I) = 6.0 Hz (80 °C). [e] These signals are coincident at this temperature.

CN^-, SCN^-, imidazole, and thiourea. The only effective reagent was CN^-. At a ratio of 20 CN^-/Pt, the downfield signals were reduced to ca. 10% of their original intensity after 5 days at ambient temperature at pH 7.

Addition of *trans*-$Pt(NH_3)_2Cl_2$ or [Pt(dien)Cl]Cl (dien = diethylenetriamine) did not cause such dramatic changes in the ^{31}P NMR spectrum of poly(I)·poly(C) (Table II, Figure 2). Although the latter Pt compound induced some significant downfield intensity, neither caused release of poly(C).

The ^{31}P NMR spectral changes observed on the reaction of poly(I) and poly(C) with various Pt compounds are summarized in Table II. At r = 0.1, $Pt(en)(H_2O)_2^{2+}$ did induce some downfield signals in the ^{31}P NMR spectrum of poly(I), but these were small and not well resolved. At r = 0.4 (equivalent to r = 0.2 with poly(I)·poly(C)) the resolution in the spectrum deteriorated and the spectrum consisted of a single peak at −3.7 ppm with a large tapering downfield shoulder. This signal is very similar to the poly(I) signal in the ^{31}P NMR spectrum of poly(I)·poly(C)·Pt(en) (r = 0.2) when the reaction was carried out at 80 °C (vide supra). [Pt(dien)Cl]Cl and *trans*-$Pt(NH_3)_2Cl_2$ affected the ^{31}P NMR spectrum of poly(I) in a fashion qualitatively similar to their effect on poly(I)·poly(C) (see Table II).

Clear changes were observed in the ^{31}P NMR spectrum of poly(C) on addition of each of the Pt compounds, verifying the lack of formation of poly(C)·Pt adducts when poly(I) is present in excess over Pt.

^{1}H NMR Spectral Studies. Base CH and H1' Region. The 360-MHz ^{1}H NMR spectrum of poly(I)·poly(C) at probe temperature (~23 °C) exhibits broad poorly resolved lines due to slow tumbling of the relatively rigid double helix. At temperatures above the melting temperature (T_m), however, the spectrum simplified and at 70 °C the only peaks observed can be attributed to unduplexed poly(I) and poly(C) (see Figure 3, bottom trace). At 70 °C three signals are observed in the 7.5–9.2 ppm region at 8.35 and 8.17 ppm, assigned to H8 and H2 of poly(I), and 7.91 ppm (H6 of poly(C), $^3J_{56}$ ~ 7 Hz).[8] Signals at 5.93 and 5.79 ppm can be identified as a combination of H5 of poly(C) and H1' of poly(I) and H1' of poly(C), respectively.[7]

Added $Pt(en)Cl_2$. After reaction of $Pt(en)Cl_2$ with poly(I)·poly(C) (r = 0.05), 11 signals were observed, at 70 °C, between 7.75 and 9.10 ppm. The data are summarized in Table III. The I residue signals that decreased markedly or disappeared altogether in various D_2O exchange experiments are assigned to H8 whereas those that remain are assigned to H2. The positions of the two most downfield signals were 9.07 and 8.54 ppm. When r = 0.1, compared to the other signals below 7.75 ppm, the relative intensity of these two most downfield signals was greater than in the r = 0.05 spectrum and, at both ratios, these signals appeared to have equal intensity. These two signals in a r = 0.1 spectrum were evaluated by a deconvolution program and appeared to have ~30–35% of the H8 signal intensity. For r = 0.1 or greater, a small downfield shoulder was observed on the 9.08 ppm H8 signal. Two downfield shifted H1' resonances of similar area were observed.

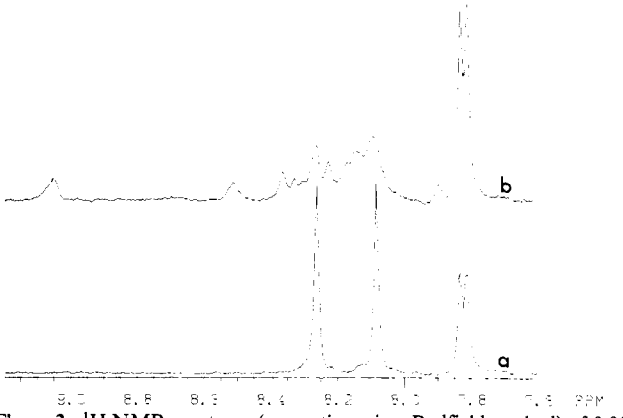


Figure 3. ^{1}H NMR spectrum (aromatic region, Redfield method) of 0.02 M poly(I)·poly(C) in PIPES 10 buffer, pH 7, 70 °C, 10% D_2O: (a) no Pt, (b) 0.2 $Pt(en)Cl_2$/base. Spectral conditions are listed in the Experimental Section.

Added *cis*-$Pt(NH_3)_2Cl_2$. Related experiments were performed with *cis*-$Pt(NH_3)_2Cl_2$. The major differences observed were in the shift of the upfield partner of the downfield H8 pair which was at lower field than in the $Pt(en)Cl_2$ experiment by ~0.3 ppm and in the resolution of the small peak in the ~9 ppm region which was clearly resolved at 9.06 ppm and for which the intensity could be estimated to be ~5% (of the total downfield H8 area) by deconvolution of the H8 signals at r = 0.1. This last resonance data does not appear to be paired with any other H8 resonances, although it would be difficult to detect such a small signal if it were in the region 8.2–7.8 ppm. Also, only one downfield-shifted H1' (I) is observed.

In one experiment, shown in Figure 4, we monitored the disappearance of the H8 signals on heating a sample in D_2O. The three downfield H8 signals had decreased to base line after heating for 1 h at 85 °C. The upfield H8 resonances also decreased but at a ~5 times slower rate. A broad signal at 7.95 ppm is clearly due to H8.

The ^{1}H NMR spectrum of a sample of poly(I)·poly(C) treated with *cis*-$Pt(NH_3)_2Cl_2$ at r = 0.1 was also studied as a function

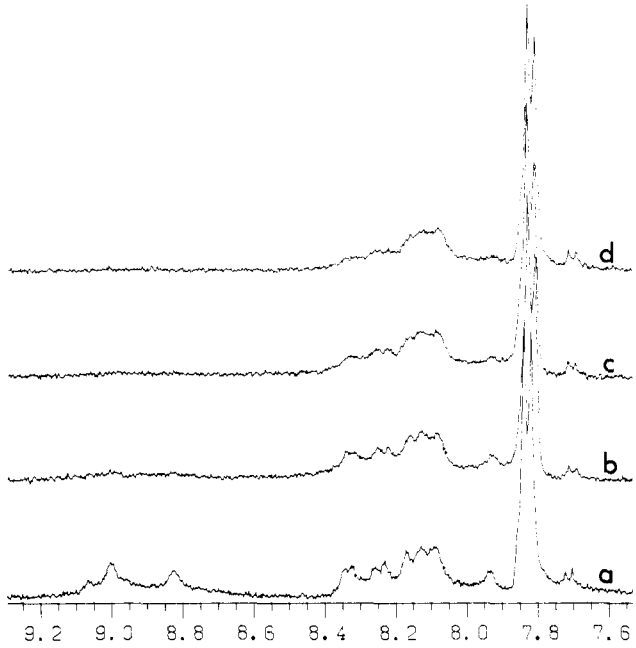


Figure 4. ^{1}H NMR spectrum (aromatic region, Redfield method, D_2O) of 0.02 M poly(I)·poly(C)·*cis*-Pt (r = 0.2). Spectra were recorded at 70 °C; otherwise, the sample was maintained at 85 °C. The spectra were recorded after the following times: (a) 0, (b) 1, (c) 3, and (d) 6 h. Spectral and solution conditions are listed in the Experimental Section.

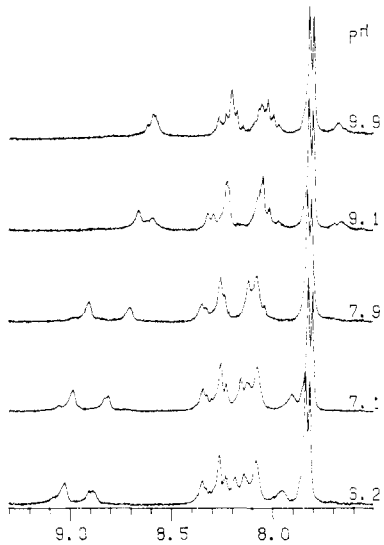


Figure 5. pH dependence of the ^{1}H NMR spectrum of poly(I)·poly(C)·*cis*-Pt (0.020 M bases, r = 0.1 Pt/base). Spectra were recorded at 60 °C but the pH values were recorded at 25 °C, with no correction for 10% D_2O. Spectral conditions are given in the Experimental Section.

of pH. In presenting these results, we will utilize assignments which are discussed in more detail in the Discussion. The largest effects were observed for the three downfield H8 signals and three downfield H2 signals. In Figures 5 and 6 are shown the changes induced in the base CH region of the ^{1}H NMR spectrum. pH measurements were made before and after recording each spectrum and after lowering the sample temperature to ~25 °C. The three downfield H8 signals and the H8 signal at 7.95 ppm display similar behavior and shift upfield ca. 0.3 ppm with an increase in pH from 6.2 to 9.9. A shift vs. pH plot yields sigmoid-type curves for the three downfield H8 protons with inflection between pH 8 and 9 (the upfield H8 is obscured by H6).

Of the next five clustered resonances to higher field (8.4–8.2 ppm), only the smallest resonance shifted significantly up to pH 8.0, whereupon addition of more base to pH 9.9 caused upfield shifts of the four other signals by 0.066, 0.095, 0.058, and 0.054 ppm, respectively. The smallest resonance probably is that of H2

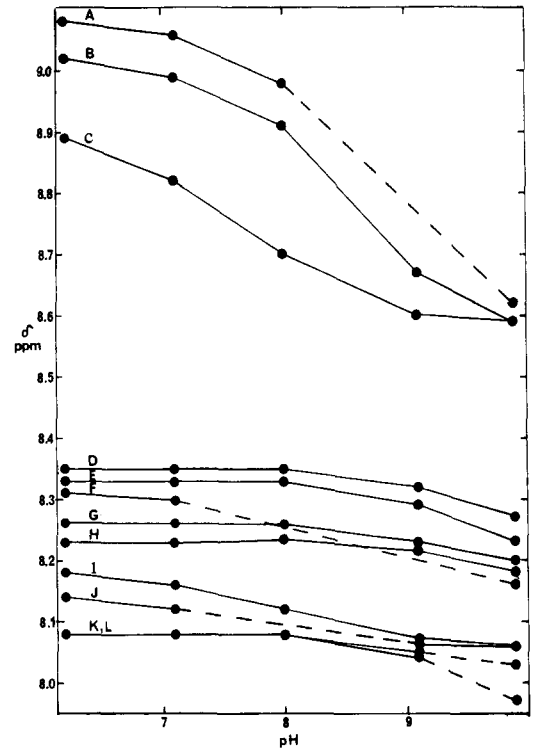


Figure 6. Chemical shift vs. pH for the 12 most downfield H8(I) and H2(I) signals in poly(I)·poly(C)·*cis*-Pt (0.20 M bases, 0.1 Pt/base). The signals correspond to those labeled A–L in Figure 8: see discussion for tentative assignments. Resonances which are obscured during the titration are indicated by dashed lines following the last pH where the resonance is observable. Signal M could not be observed at pH 8.0 due to H(6) of C (Figure 5). It is not included here to allow the pH dependence of the other signals to be displayed at greater amplification. M shift values are 7.95 and 7.90 ppm at pH 6.2 and 7.1, respectively. Solution and spectral conditions are listed in the Experimental Section; pH values are not corrected for 10% D_2O content.

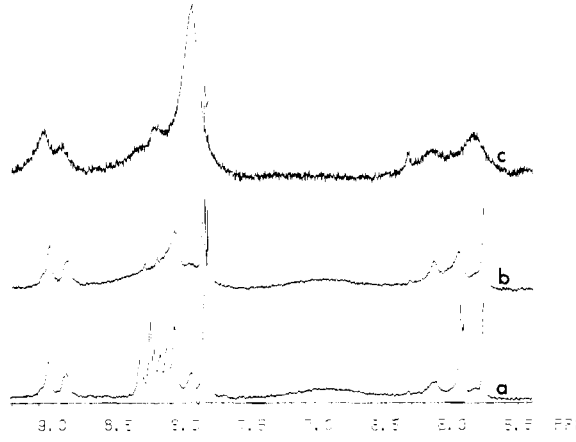
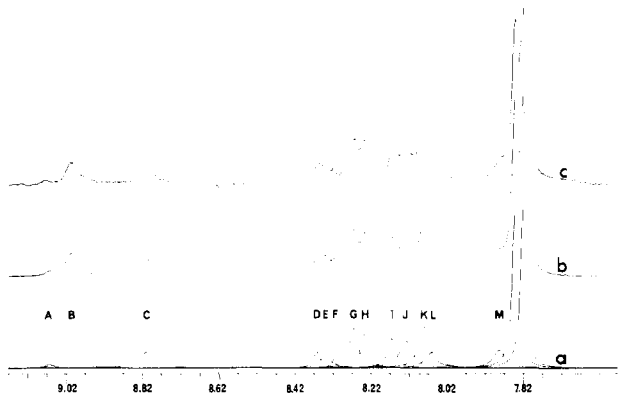


Figure 7. Effect of $Cu(NO_3)_2$ on the ^{1}H NMR spectrum of poly(I)·poly(C)·*cis*-Pt (0.020 M bases, 0.1 Pt/base, pH 6.2, 70 °C, 10% D_2O). The Cu^{2+} concentrations are as follows: (a) 0, (b) 2.0×10^{-5}, and (c) 1.0×10^{-4} M. Solution and spectral conditions are given in the Experimental Section.

on platinated I (see Discussion).

At higher field (8.2–8.0 ppm), there is a second cluster of CH resonances. The effect of pH on the two most downfield (H2) signals (total shifts of ca. 0.1 ppm) somewhat paralleled, but was less dramatic than, the effect on the three most downfield H8 resonances. The other signals in this cluster did not shift until pH >8.0 and then shifted upfield ca. 0.05 ppm by pH 9.9.

The spectrum of poly(I)·poly(C)·*cis*-Pt (r = 0.1) in the presence of Cu^{2+} (2×10^{-5} M) exhibited almost total loss of signal in the 8.2–8.4 ppm (H8) region with only general broadening of all other signals (Figure 7). The downfield H8 signals were still observed



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The ^{1}H NMR spectral changes as a function of pH were determined for a sample of poly(I)·poly(C)·*cis*-Pt identical with that used in the Cu^{2+} study (Figures 5 and 6). Seven of the 13 CH resonances shift upfield with a change in pH from 6.2 to 9.1 (apparent pK_a ~ 8). The two signals that we assign to H8 of the bis-adduct shifted upfield by 0.4 and 0.3 ppm for the downfield and upfield partners, respectively. This behavior can be compared, for example, with that of the product of platination d(TCTCGGTCTC) by *cis*-$Pt(NH_3)_2Cl_2$.[23] Over a similar pH range the two H8 resonances move upfield by ~0.3 and ~0.2 ppm for the downfield and upfield partners, respectively, again with a similar pK_a ~ 8.

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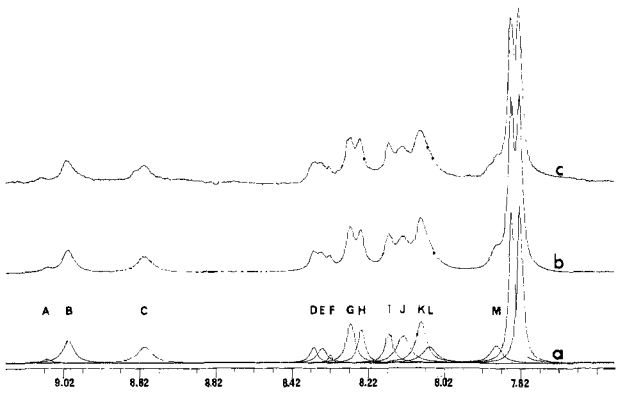

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H8, unplatinated.). The H8 signal at ca. 7.9 ppm shifted upfield (when the pH was raised from 6.2 to 7.1) and thus displayed behavior characteristic of an N7-platinated species. We do not believe this signal is for an N7-platinated I for the following reasons: First, the signal disappeared in the D_2O exchange experiment, but at a rate ca. 5 times slower than the downfield H8 signals of platinated I. Second, the total area of M, A, B, and C equals ~0.5 protons. This is not possible at $r = 0.10$. Third, the shift of the resonance is probably too far upfield for an H8 on I platinated at N7; an unusually large ring-current shift of ca. 1 ppm would be required to cause such an effect. Thus, all the techniques provide a consistent assignment, although some caution must be exercised due to the inherent complexity of polymers.

Finally, the H1′ resonances are consistent with our conclusion based on the downfield base ^{1}H NMR signals. About 40% of the H1′ signal for I is shifted downfield in poly(I)·poly(C)·*cis*-Pt ($r = 0.1$) (see bottom trace, Figure 9). For a bis-adduct, 40% of the I bases will be platinated at N7.

Pt(en)Cl_2. This compound, although studied in less detail, changed the ^{1}H NMR spectrum of poly(I)·poly(C) in a manner very similar to *cis*-Pt$(NH_3)_2Cl_2$. Analogous assignments of resonances are possible. Thus, there are three downfield H8 signals (0.3 protons at $r = 0.1$) with two of similar area and the one most downfield relatively small. However, the most upfield H8 signal, attributable to the bis-adduct, appears at 8.54 ppm, ~0.3 ppm upfield from the corresponding signal for poly(I)·poly(C)·*cis*-Pt. This difference can be attributed to a larger ring current experienced by this H8 in the Pt(en) bis-adduct.[14] This ring current probably arises from the other base on Pt since such effects are found with dinucleoside monophosphates.[14] The 8.0–8.4 ppm region is obviously complex and a peak-to-peak correspondence between the two Pt species is not expected, although the total area in this region does correspond to ~1.2 protons, as found for poly(I)·poly(C)·*cis*-Pt. The anomalous upfield H8 at 7.9 ppm is also observed with poly(I)·poly(C)·Pt(en) and has the same relative area.

A further indication that the orientations of the I units in the bis-adduct are less equivalent than in poly(I)·poly(C)·*cis*-Pt is the presence of two H1 signals for platinated I. These signals are of similar intensity, as expected.

^{1}H NMR Summary. The ^{1}H NMR studies reported here and our reinterpretation of the earlier study provide very strong evidence that the major product formed at low temperature is a bis-adduct and that it has structural characteristics similar to those of oligonucleotides.

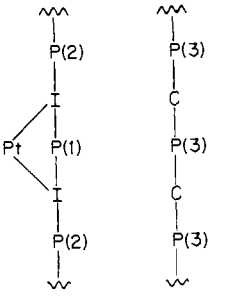
At $T = 25$ °C, the N1H resonance of poly(I)·poly(C) decreases by half at $r = 0.1$ and disappears at $r = 0.2$ on platination by Pt(en)Cl_2 or Pt(en)$(H_2O)_2^{2+}$. This result is consistent with either a greatly weakened duplex, where bases can be readily exposed to H_2O and undergo NH–H_2O exchange, or essentially total disruption of the duplex so that the I NH's are nearly always exposed to exchange with H_2O.[43] Previous CD, UV, electric birefringence, density equilibrium centrifugation studies, etc. also indicate disruption of the helix and release of poly(C).[9,10]

Finally, although the NOE results are necessarily less clear than we would like, all the data are consistent with an adduct very similar to that found with oligonucleotides. The I bases are probably in a "head to head" arrangement. This arrangement can explain the differences in shift of the two H8 resonances since one signal appears more downfield and one more upfield. Thus, if one H8 is in the upfield shielding region of the other bound hypoxanthine ring whereas the second H8 is either in the downfield shifting region or near the crossover between regions, these resonances will occur at different shifts.[14] It is interesting that the en compound induces a greater difference between shifts, although this same effect is also observed in complexes formed by d(TGGT) with Pt(en)Cl_2 and *cis*-Pt$(NH_3)_2Cl_2$.[4] The origin of this difference is unclear but may arise from subtle differences in structure. In this regard, the shifts of the downfield H8 resonances are relatively unaffected by temperature increases from 23 to 80 °C and, therefore, the conformations may be constrained.

^{31}P NMR of Platinated Poly(I)·Poly(C). At 25–35 °C, there is a close correspondence between the general trends observed for the spectroscopic changes in the ^{31}P NMR spectrum of DNA and of poly(I)·poly(C) in comparative studies of Pt(en)Cl_2, *cis*-Pt$(NH_3)_2Cl_2$, *trans*-Pt$(NH_3)_2Cl_2$, and [Pt(dien)Cl]Cl. The DNA spectrum is hardly influenced by the agents lacking cis leaving groups, except for general broadening of the main signal.[2,44] Similarly, the major changes in the poly(I)·poly(C) spectrum are caused by Pt(en)Cl_2. In addition, the ^{31}P NMR spectrum of poly(A)·poly(U) is not greatly influenced by Pt(en)Cl_2.[45] Likewise, the poly(dA)·poly(dT) ^{31}P NMR spectrum was not influenced much by this bifunctional agent.[2] Thus, polyribonucleotides appear to reflect, in a broad sense, the response of polydeoxyribonucleotides. In the same way, IpI and GpG form similar complexes as dIpI[4] and dGpG.[14]

Although undoubtedly several products are formed, especially at $r = 0.2$, the main product of the reaction of Pt(en)Cl_2 with poly(I)·poly(C) is a bis-adduct. The sharp signal at −4.2 ppm for the spectrum at $r = 0.2$ corresponds exactly with poly(C), unplatinated. Since only two H bonds are present between base pairs, the poly(I)·poly(C) duplex is weak in comparison to DNA or poly(dG)·poly(dC). After platination by *cis*-Pt$(NH_3)_2Cl_2$ or Pt(en)Cl_2 the poly(C) is either free or at best loosely associated with the platinated poly(I) strand. The absence of platination in poly(C) is clearly evident both in comparison to our studies with poly(C), itself, and by the changes in spectra observed at high ratios. This finding is also another piece of evidence that poly(I)·poly(C) responds similarly to DNA where C residues are not attacked at low r values.[24,25,28,30]

We feel that the much weaker duplex and the nearly 100% reactive sites on the poly(I) strand allow for the formation of essentially one type of product mixture illustrated schematically below:



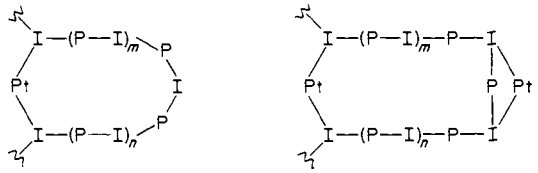
We suggest, in analogy with studies on oligonucleotides,[3,4,5] that the signal of P(1) is the most downfield poly(I) signal. This resonance corresponds to the downfield resonance observed on platination of DNA, poly(dG)·poly(dC), and nucleosomes by antitumor drugs.[2] The upfield poly(I) signal is assigned to P(2) which is in a relatively unperturbed PO_4 group. This signal has the same shift as free poly(I). The area of the P(1) signal is directly proportional to r for $0 < r < 0.25$. At $r = 0.2$ the I residues are nearly all coordinated and the signals from P(1) and P(2) are equal (Figure 2). The poly(C) signal, P(3), is clearly identified from our studies of poly(C) alone and has an area equal to the total for the P(1) and P(2) signals. The ^{31}P NMR results with *cis*-Pt$(NH_3)_2Cl_2$ are similar to those with Pt(en)Cl_2 but two downfield ^{31}P NMR signals are not as clearly resolved. This difference may arise from subtle conformational differences.

(43) Reid, B. R. *Annu. Rev. Biochem.* **1981**, *50*, 969.

(44) This comparison also holds for poly(dG)·poly(dC) with Pt(en)Cl_2 and *trans*-Pt$(NH_3)_2Cl_2$.

(45) Poly(A)·poly(U) was obtained from Miles and prepared as above. The ^{31}P NMR spectrum contained three signals. Two signals at −3.99 and −4.36 ppm were large and $\nu_{1/2} \simeq 25$ Hz. One sharp signal (ca. 5% of total intensity) occurred at −3.76 ppm and may be due to excess poly(U). At $r = 0.2$ (for Pt(en)Cl_2), the major downfield signal broadened and shifted to ca. −4.15 ppm. The upfield major signal broadened slightly and shifted slightly to −4.40 ppm. The small sharp resonance remained sharp and appeared to shift to −3.83 ppm but this shift could be a consequence of the proximity to the broad major signals. We tentatively suggest that the downfield major signal is due to the poly(A) strand.

Several additional types of bis-adducts could possibly be formed with poly(I) either single stranded or duplexed with poly(C). These are illustrated below:



I $n \geq 0$ $m \geq 0$ II

These adducts are considered in the next section.

Poly(I) and Poly(C). We did not carry out extensive studies with these single-stranded polymers. Our interest in studying them was primarily based on a need for comparison with the poly(I)·poly(C) duplex. The perturbations induced in the poly(I) (and poly(C)) ^{31}P NMR spectrum by $Pt(en)(H_2O)_2^{2+}$ are much smaller than the effects on poly(I)·poly(C). Therefore, we are, in fact, observing reaction products formed from *duplexed* poly(I)·poly(C). For example, addition of $Pt(en)(H_2O)_2^{2+}$ to poly(I) leads to relatively smaller downfield intensity (~50% of that found for poly(I)·poly(C)) at a given ratio of Pt to I when r is small (<0.2). The downfield intensity in the poly(I) experiment should be half that in the poly(I)·poly(C) experiment since examination of published ^{1}H NMR studies[8] of poly(I) treated with *cis*-$Pt(NH_3)_2Cl_2$ suggests to us that about half the Pt forms an adjacent intrastrand cross-link. At higher r values, the ^{31}P NMR spectrum broadened and the downfield intensity increased with total loss of resolution. These effects may be caused by the formation of adducts such as II.[46]

The ^{31}P NMR results with [Pt(dien)Cl]Cl and poly(I) support the suggestion[13] that some unusual structure is formed with this monofunctional species, since a sharp downfield signal was observed. In our studies of deoxyribopolymers, this monofunctional agent did not cause any appreciable downfield intensity. With poly(I)·poly(C), some downfield intensity was also observed but much less than in the poly(I) case. It is worth noting that poly[d(GC)] does give a downfield ^{31}P NMR signal on treatment with [Pt(dien)Cl]Cl under conditions where Z DNA would be favored.[47] In the present case, without further study, we are unable to interpret the significance of the downfield signal.

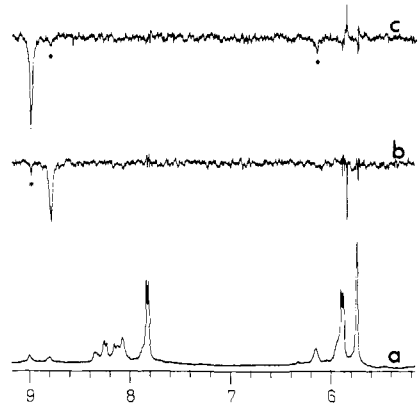


Figure 9. ^{1}H NMR NOE experiments on poly(I)·poly(C)·*cis*-Pt (r = 0.1) in D_2O at 60 °C, (a) recorded with 0.90-s preirradiation at 9.2 ppm; (b and c) difference spectra obtained by subtracting the spectrum in (a) from spectra that were recorded with preirradiation at 8.82 and 8.99 ppm, respectively. Solution and spectral conditions are listed in the Experimental Section.

Conclusion

The evidence for a relationship between the downfield ^{31}P NMR signal of platinated deoxyribonucleotides, either free in solution or in nucleosomes, and adjacent intrastrand cross-linked 6-oxopurine residues is clear. The 2-amino group in guanosine, which is lacking in inosine, is not an important determinant of the bis-adduct. Additionally, the bis-adduct in a poly*ribo*nucleotide also can induce this characteristic downfield ^{31}P NMR signal. A clear parallel exists between the nature of platinated polymers and platinated oligonucleotides (both ribo and deoxyribo) as judged not only by ^{31}P NMR spectroscopy but also with ^{1}H NMR spectroscopy.

Finally, it is clear that integrated intensities of H8 signals can be useful in assessing adducts formed in polymers when detected with high-field NMR instruments, given the caveat that we must allow for intensity loss resulting from ^{195}Pt coupling and CSA. The ^{1}H NMR experiments for the aromatic CH signals required the use of elevated temperatures. However, the platination reactions were carried out at low temperature (12 or 25 °C) and ^{31}P NMR spectra as a function of temperature revealed no major changes in the spectrum of poly(I)·poly(C)·Pten or poly(I)·poly(C)·*cis*-Pt. Thus, ^{31}P NMR spectroscopy has advantages in the examination of duplexes, particularly in larger molecules.

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Registry No. *cis*-$Pt(NH_3)_2Cl_2$, 15663-27-1; $Pt(en)Cl_2$, 14096-51-6; $Pt(en)(H_2O)_2^{2+}$, 50475-23-5; *trans*-$Pt(NH_3)_2Cl_2$, 14913-33-8; [Pt(dien)Cl]Cl, 14215-58-8.

(46) Adducts of poly(I) similar to I but with n = 1 or greater with *trans*-$Pt(NH_3)_2Cl_2$ are possible. It is not clear whether one or two H8 signals could be expected at each value of n. Intermolecular cross-links are also possible. Six closely spaced H8 resonances at ca. 9.05 to 9.25 ppm were reported for this reaction.[12] Again, analysis[12] of the data did not allow for ^{195}Pt CSA. The 1.3 ± 0.1 protons per Pt reported[12] corresponds to a corrected value of 1.9 ± 0.15 protons per Pt. The major resonance, which accounted for ~0.7 ± 0.05 protons (uncorrected) decreased by 40% on addition of imidazole. If this resonance arises from 60% bis-adduct and 40% mono-adduct (it was assigned as 100% mono-adduct in ref 12) a corrected ca. 0.6 protons/Pt corresponds to the mono-adduct and the remainder of the intensity corresponds to ca. 1.5 protons/Pt. Thus, the numbers can be rationalized as well within the reported error of the measurements to be ~75% bis-adduct and ~25% mono-adduct. (For poly(I)·poly(C) + *trans*-$Pt(NH_3)_2Cl_2$, we can reinterpret the published data to be consistent with 1.8 ± 0.2 protons/Pt. Again, the amount of bis-adduct is underestimated in ref 12.)

(47) Malfoy, B.; Hartman, B.; Leng, M. *Nucleic Acids Res.* **1981**, *9*, 5659. Ushay, H. M.; Santella, R. M.; Caradonna, J. P.; Grunberger, D.; Lippard, S. J. *Nucleic Acids Res.* **1982**, *10*, 3573.