Synthesis and ^{1}H NMR Spectroscopic Characterization of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}]†

Christopher A. Lepre, Kenneth G. Strothkamp, and Stephen J. Lippard*
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Received February 17, 1987; Revised Manuscript Received April 17, 1987

ABSTRACT: The reaction of *trans*-[$Pt(NH_3)_2Cl_2$] with the sodium salt of [d(ApGpGpCpCpT)]$_2$ in aqueous solution at 37 °C was monitored by reversed-phase high-performance liquid chromatography and UV spectroscopy. Two intermediates, most likely monofunctional adducts, were observed, which subsequently formed one predominant single-stranded product, as well as several polymeric species proposed to be interstrand cross-linked products. The single-stranded adduct was structurally characterized by ^{1}H NMR spectroscopy. From the pH dependence of the chemical shifts, two-dimensional homonuclear chemical shift correlation (COSY) spectroscopy, and one- and two-dimensional nuclear Overhauser effect (NOESY) experiments, the platinum(II) moiety was found to be coordinated to the N7 positions of adenine(1) and guanine(3), with the intervening guanine(2) base destacked from its neighboring residues. This intrastrand 1,3 adduct induces changes in the backbone torsion angles and causes the deoxyribose ring of adenine(1) to switch from a C2′-endo to a predominantly C3′-endo conformation. The other deoxyribose rings retain B DNA type conformations. The structure of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}] differs from those previously reported for *cis*-DDP 1,2- and 1,3-intrastrand oligonucleotide adducts but is consistent with the structures of *trans*-DDP 1,3-intrastrand adducts of two previously reported trinucleotides.

The adducts formed between the antitumor agent *cis*-[$Pt(NH_3)_2Cl_2$] (*cis*-DDP)[1] and DNA, its putative cellular target, have been extensively studied for the purpose of elucidating the mechanism of action of the drug. Both single- and double-stranded *cis*-DDP/oligonucleotide complexes have been synthesized and studied by ^{1}H and ^{31}P NMR spectroscopies in order to provide structural information about *cis*-DDP/DNA adducts (Chottard et al., 1980; Caradonna et al., 1982; Girault et al., 1982; Marcelis et al., 1982; den Hartog et al., 1984, 1985; van Hemelryck et al., 1984; Caradonna & Lippard, 1987). Recently, the *cis*-DDP adducts of d(pGpG) (Sherman et al., 1985) and d(CpGpG) (Admiraal et al., 1987) have been characterized by X-ray crystallography.

The DNA binding properties of *trans*-DDP, which is clinically inactive and, at equivalent doses, less mutagenic and cytotoxic than the cis isomer (Roberts & Thomson, 1979), have been less thoroughly studied. For stereochemical reasons, *trans*-DDP is unlikely to form 1,2 adducts, cross-links between adjacent bases on the same strand (Stone et al., 1976; Cohen et al., 1980). In a system employing single-stranded phage M13 DNA, *trans*-DDP inhibited in vitro synthesis of the second strand by the large fragment of DNA polymerase I, binding with less selectivity than the cis isomer but exhibiting a preference for d(GpNpG), where N is any nucleotide, sequences (Pinto & Lippard, 1985b). In addition, the enzyme appeared to be inhibited by platinum binding near d(ApNpG) sites, suggesting that *trans*-[$Pt(NH_3)_2${d(ApNpG)}] adducts, like the analogous *cis*-[$Pt(NH_3)_2${d(ApG)}] adducts (Fichtinger-Schepman et al., 1985; van der Veer et al., 1986b), contribute to the pool of adducts formed by *trans*-DDP. Studies employing antinucleoside antibodies revealed that, at low binding levels, *trans*-DDP disrupts the DNA duplex to a greater extent than the cis analogue (Sundquist et al., 1986). Such differences in the character of cis and trans adducts may account for their different biological activities (Ciccarelli et al., 1985).

The only studies of *trans*-DDP/oligonucleotide complexes reported to date have been HPLC and ^{1}H NMR spectroscopic analyses of adducts formed with the trimers d(GpTpG) (van der Veer et al., 1986a) and d(GpCpG) (Gibson & Lippard, 1986). In this paper, the reaction of *trans*-DDP with a duplex deoxyribohexanucleoside pentaphosphate, [d(ApGpGpCpCpT)]$_2$, is described. This sequence was employed because the reaction of this hexamer with *cis*-DDP had already been studied in considerable detail (Caradonna et al., 1982; Caradonna & Lippard, 1987) and because use of a self-complementary sequence facilitated platination of duplex, rather than single-stranded, DNA.

As reported herein, the reaction yields the *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}] chelate as a principal single-stranded product. The solution structure of this product, which represents both the most complex *trans*-DDP/oligonucleotide model studied to date, as well as the first *trans*-[$Pt(NH_3)_2$[d(ApNpG)]] adduct to be structurally characterized, has been investigated by ^{1}H NMR spectroscopy. The results are compared with those for the previously noted platinum/oligonucleotide model complexes.

Materials and Methods

Synthesis of trans-[$Pt(NH_3)_2${*d(ApGpGpCpCpT)-N7-A(1),N7-G(3)*}]. The self-complementary deoxyribohexanucleoside pentaphosphate [d(ApGpGpCpCpT)]$_2$ was synthesized by the solid-phase phosphotriester method (Sproat & Gait, 1984) and purified by anion-exchange chromatography and reversed-phase HPLC. The purified hexamer was

† This work was supported by U.S. Public Health Service Grant CA-34992 from the National Cancer Institute. K.G.S. thanks Bryn Mawr College for granting him sabbatical leave time in order to pursue this project, which was supported under NSF Grant CHE85-42205 (to S.J.L.).

[1] Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); *trans*-DDP, *trans*-diamminedichloroplatinum(II); Na_2EDTA, disodium ethylenediaminetetraacetate; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, 2-D nuclear Overhauser effect spectroscopy; TMAC, tetramethylammonium chloride; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate; COSY, 2-D homonuclear chemical shift correlated spectroscopy.

0006-2960/87/0426-5651$01.50/0 © 1987 American Chemical Society

passed through a cation-exchange resin to obtain the Na^+ salt, which was then allowed to react with an aqueous solution of *trans*-DDP at 37 °C in the dark at various ratios from 1.0 to 1.6 platinum atoms per strand. The hexamer concentration varied from 38 to 281 μM, the pH range was 5.7–6.5, and 0.1 mM Na_2EDTA was usually present. The progress of the reaction was monitored by reversed-phase HPLC, using a Perkin-Elmer Series 4 liquid chromatograph and a Waters μBondapak C_{18} column, eluting with a linear gradient of 6–7.5% acetonitrile in 0.1 M aqueous ammonium acetate at pH 6.5. The absorbance at 260 nm was recorded on an LCI-100 computing integrator. The total UV absorbance of the reaction mixture was followed independently with a Perkin-Elmer Lambda 7 UV–vis spectrophotometer. In order to estimate the extinction coefficient per platinum of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}], platinum atomic absorption spectroscopy was performed on a Varian AA-1475 instrument, with a GTA-95 graphite tube atomizer.

The principal products of the reaction were isolated by anion-exchange column chromatography, using Whatman DE-52 cellulose resin and a linear gradient of 0.06–0.6 M triethylammonium bicarbonate in 80% H_2O and 20% ethanol at pH 7.5–7.7. Reversed-phase HPLC was also employed in the purification as previously described. The *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] sample was prepared for ^1H NMR spectroscopic studies by lyophilizing it 3 times from 99.8% D_2O, in order to remove traces of H_2O and volatile salts, followed by dissolution in 0.4 mL of 99.995% D_2O to give a final concentration of ca. 1 mM. A trace of TMAC was added as an internal reference; the methyl singlet appears 3.180 ppm downfield of DSS.

NMR Spectroscopy. The pH titration experiment was carried out on a Bruker WM-250 spectrometer equipped with an Aspect 2000 data system, by using standard Fourier transform techniques. The pH was adjusted within the range from 2.5 to 10.1 with small aliquots of DCl and NaOD and was measured on an Orion Research digital pH meter, with a 5-mm electrode. The pH values were not corrected for the deuterium isotope effect.

The COSY (Aue et al., 1975), difference NOE, and NOESY (States et al., 1982) experiments were carried out at 500 MHz on a homebuilt spectrometer at the Francis Bitter National Magnet Laboratory, using a VAX 750 computer for data processing.

The COSY experiment was performed on a nonspinning sample at 25 °C and pH 7.69, using the pulse sequence $[\pi/2\text{–}t_1\text{–}\pi/2\text{–acq}]_n$. The data set consisted of 512 data points over a sweep width of 2650 Hz in each dimension, and 64 transients were accumulated per t_1 increment. Gaussian line broadening (Ernst, 1966) of 4 Hz was applied in both dimensions. Both the symmetrized and unsymmetrized contour maps were employed in making assignments.

The NOESY experiments were carried out without spinning at 30 and 35 °C, and at pH 6.80 and 8.00, respectively. The data sets consisted of 512 data points over a sweep width of 4550 Hz, and 64 transients were accumulated per t_1 increment. Mixing times of 400 and 600 ms were employed at 35 and 30 °C, respectively. The intensity of the residual HDO resonance was reduced by selective presaturation, and Gaussian window and base-line leveling functions were applied in both dimensions. Resonance assignments were made by using the symmetrized, unsymmetrized, and one-dimensional projections of the pure phase sensitive spectra.

Results and Discussion

Formation and Separation of Pt/DNA Adducts. During the course of the reaction, the position of the UV absorbance maximum of the reaction mixture shifts from 258 to 263 nm and is accompanied by an increase in the value of the maximum absorbance. Although the observed bathochromic shifts are comparable to those seen when *cis*-DDP coordinates to oligonucleotides, the hyperchromicity accompanying the *cis*-DDP reaction is less pronounced. During the reaction of *trans*-DDP with $[d(ApGpGpCpCpT)]_2$, the absorbance at 260 nm increases by greater than 12%. This value may be compared to an observed increase of only 3.5% when this hexamer reacts with *cis*-DDP to give the N7-G(2),N7-G(3) adduct (Caradonna & Lippard, 1987) and an increase of 2–6% during the analogous reaction with $[d(TpGpGpCpCpA)]_2$ (Girault et al., 1982).

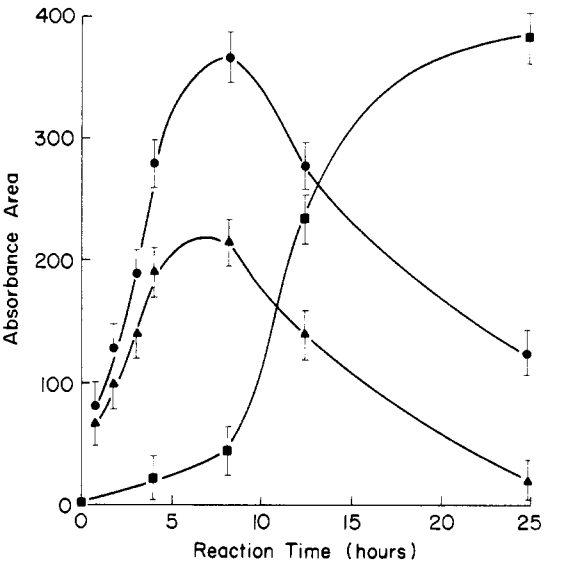

FIGURE 1: Concentration of principal single-stranded products in the reaction mixture as a function of reaction time. The integrated area of each major product peak in the reversed-phase HPLC elution profile (absorbance at 260 nm) is given in arbitrary units. (●,▲) Initial products; (■) *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}]. The reaction mixture contained 38 μM of both hexamer strand and *trans*-DDP at 37 °C. No Na_2EDTA was present.

Figure 1 illustrates the evolution of single-stranded products as a function of time, as monitored by measuring the integrated areas of each peak in the reversed-phase HPLC elution profile. Under the conditions employed, single-stranded adducts and unreacted hexamer are eluted while polymeric species, which comprise approximately 30% of the total optical density, are retained on the column.

An examination of Figure 1 reveals that two major products initially form in roughly a 3:2 ratio. Of the total amount of hexamer that reacts, approximately 70% does so within the 8-h time period before the two initial products reach their maximum concentrations. The time at which this maximum occurs varies with the initial concentrations of hexamer and *trans*-DDP in solution, occurring sooner at higher concentrations. The two initially formed products subsequently disappear from the reaction mixture with the simultaneous appearance of one principal single-stranded product, several minor products that each comprise less than 2% of the total absorbance area, and an unknown number of polymeric species.

After 125 h, the reaction is complete and no traces of the initially formed products remain. As shown in Figure 2, anion-exchange chromatography of the reaction mixture yields two overlapping bands, labeled 1 and 2, corresponding to single-stranded products with a charge of 3–. The predominant product, *trans*-[$Pt(NH_3)_2$[d(ApGpGpCpCpT)]], was subse-

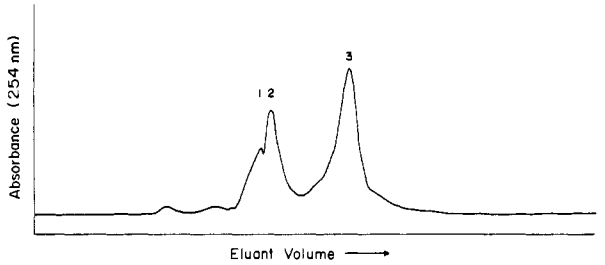

FIGURE 2: Elution profile for the purification of the reaction products on a (diethylaminoethyl)cellulose anion-exchange column (20 × 1.5 cm). The reaction mixture contained 1 equiv/strand of *trans*-DDP and $[d(ApGpGpCpCpT)]_2$ at 57 μM concentration, pH 5.5. The reaction proceeded for 2 days at 37 °C. Gradient, 0.06–0.60 M $(Et_3NH)(HCO_3)$, 20% EtOH, pH 7.53; flow rate, 1.2 mL/min; observation wavelength: 254 nm. Peaks 1 and 2 are single-stranded products, and peak 3 is unreacted hexamer. The high molecular weight species are eluted after the end of the gradient (not shown).

quently isolated from band 2 by reversed-phase HPLC, giving an overall yield of purified, desalted product of approximately 18%.

The elution of *trans*-$[Pt(NH_3)_2[d(ApGpGpCpCpT)]]$ as a trianion is consistent with the presence of only one bound platinum atom per strand. It eliminates the possibility that this product consists either of two *trans*-$[Pt(NH_3)_2]^{2+}$ moieties bound per strand or an interstrand cross-link formed by one or two platinum atoms, which would result in species of overall charge 1–, 8–, or 6–, respectively.

The extinction coefficient of *trans*-$[Pt(NH_3)_2[d(ApGpGpCpCpT)]]$ at 263 nm is approximately 56 000 cm^{-1} M^{-1} per platinum, consistent with one platinum adduct per strand (Caradonna, 1985; Caradonna & Lippard, 1987). Species with two platinum adducts per strand, or one platinum linking two strands, would have extinction coefficients per platinum that differ from the observed value by a factor of 2.

Up to 40% of the hexamer did not react with *trans*-DDP and served as a 5– charge marker in the anion-exchange elution profile (Figure 2, band 3). This material was recovered and shown by ^1H NMR spectroscopy to be unmodified starting material, indicating that the incomplete reaction is not due to inactivation of the oligonucleotide.

In addition to the single-stranded species, several strongly retained polyanionic species with charges in excess of 6– were eluted from the anion-exchange column by 0.6 M triethylammonium bicarbonate/ethanol buffer at pH 8.0. Since they elute under denaturing conditions, these highly anionic species are assigned as having two or more hexamer strands covalently linked by platinum. These interstrand cross-linked compounds are also strongly retained on the reversed-phase HPLC column, and their ^1H NMR spectrum exhibits very broad peaks in the nonexchangeable base proton region, with downfield shifts consistent with platinum bound at N7 of guanine or adenine (Chottard et al., 1980; Caradonna et al., 1984; den Hartog et al., 1984a; Chu et al., 1978). The specific sites of platinum binding cannot be assigned without further experimentation. It is interesting that no interstrand cross-linked products were observed during the reaction of *cis*-DDP with $[d(ApGpGpCpCpT)]_2$ (Caradonna & Lippard, 1987).

The HPLC results indicate that the formation of the final products proceeds through two intermediates. If additional intermediates exist, they are not resolved under the eluant conditions. The amount of hexamer that reacts after the initial products reach their maximum concentrations is insufficient to account for the total absorbance area of the products subsequently formed, even if hyperchromicity is taken into account, so the final products must arise by conversion of the initially appearing species. This explanation is also consistent with the eventual disappearance of the initial products from the reaction mixture.

We suggest that the observed intermediates correspond to monofunctional adducts of *trans*-DDP with the hexamer nucleobases, most likely at the N7 positions of G(2), G(3), or A(1), which subsequently react to form bifunctional intrastrand and interstrand adducts. This type of mechanism has already been proposed for the reaction of *cis*-DDP with DNA (Plooy et al., 1984; Reedijk et al., 1984), and monofunctional intermediates have reportedly been observed in a number of in vitro systems (Butour et al., 1986; Eastman, 1986; Dijt et al., 1984; Marcelis et al., 1980). Recently, monofunctional intermediates were seen during the reaction of *trans*-DDP with d(GpCpG) (Gibson & Lippard, 1987).

^1H NMR Characterization of trans-$[Pt(NH_3)_2\{d(ApGpGpCpCpT)$-N7-A(1),N7-G(3)$\}]$. The 500-MHz ^1H NMR spectrum of *trans*-$[Pt(NH_3)_2\{d(ApGpGpCpCpT)\}]$ is shown in Figure 3. This adduct, like the *cis*-$[Pt(NH_3)_2\{d(ApGpGpCpCpT)$-N7-G(2),N7-G(3)$\}]$ intrastrand cross-link (Caradonna & Lippard, 1987), is single-stranded, as deduced from the relatively slight temperature dependence, arising from base-stacking effects, of the chemical shifts (not shown). High-resolution spectra could not be obtained at temperatures below 25 °C, owing to severe broadening of the resonances, probably due to the effects of self-aggregation.

The nonexchangeable base proton resonances occur in the region from 9.3 to 7.6 ppm. The reduction in intensity of the purine H8 singlets, due to exchange with deuterium from the solvent, serves to identify these resonances (Schweizer et al., 1964), although the exchange rate was not measured. This exchange process caused a significant loss of H8 signal intensity during the course of the COSY and NOESY experiments, particularly at the elevated temperatures necessary to achieve optimal resolution.

The nonexchangeable base proton resonances and the sites of platinum binding were assigned primarily on the basis of the pH dependence of the nonexchangeable base proton chemical shifts, compared with results for the unplatinated hexamer reported previously (Caradonna & Lippard, 1987). The plots thus obtained (Figure 4) reflect the influence of protonation and deprotonation of the endocyclic nitrogen atoms upon the chemical shifts of the corresponding base protons and the stacking of the adjacent bases (Izatt et al., 1971; Saenger, 1984).

The A(1) H8 (9.268 ppm in Figure 3) and H2 (8.354 ppm) resonances are shifted downfield +1.30 and +0.36 ppm, respectively, from their positions in the unplatinated duplex. They may be differentiated on the basis of the fact that only the H8 resonance exchanges with deuterium. They exhibit no chemical shift changes near pH 3.7, where protonation of adenine N1 normally occurs (Izatt et al., 1971), but begin to shift near pH 2. This behavior parallels that previously reported for binding of *cis*-DDP at adenine N7 of the deoxyoligonucleotides d(ApG) (Fichtinger-Schepman et al., 1985) and d(GpApG) (van der Veer et al., 1986b), which lowers the pK_a of N1 from 3.7 to ~2. From these considerations, we therefore assign N7 of A(1) as a site of platinum binding, consistent with the known preference of *cis*-DDP for N7, rather than N1, of adenine (Inagaki et al., 1982; den Hartog et al., 1984a; Fichtinger-Schepman et al., 1985; van der Veer et al., 1986b; van Hemelryck et al., 1986; Bose et al., 1986).

The guanine H8 resonance at 8.684 ppm, which is shifted 1.0 ppm downfield from its position in the unplatinated duplex,

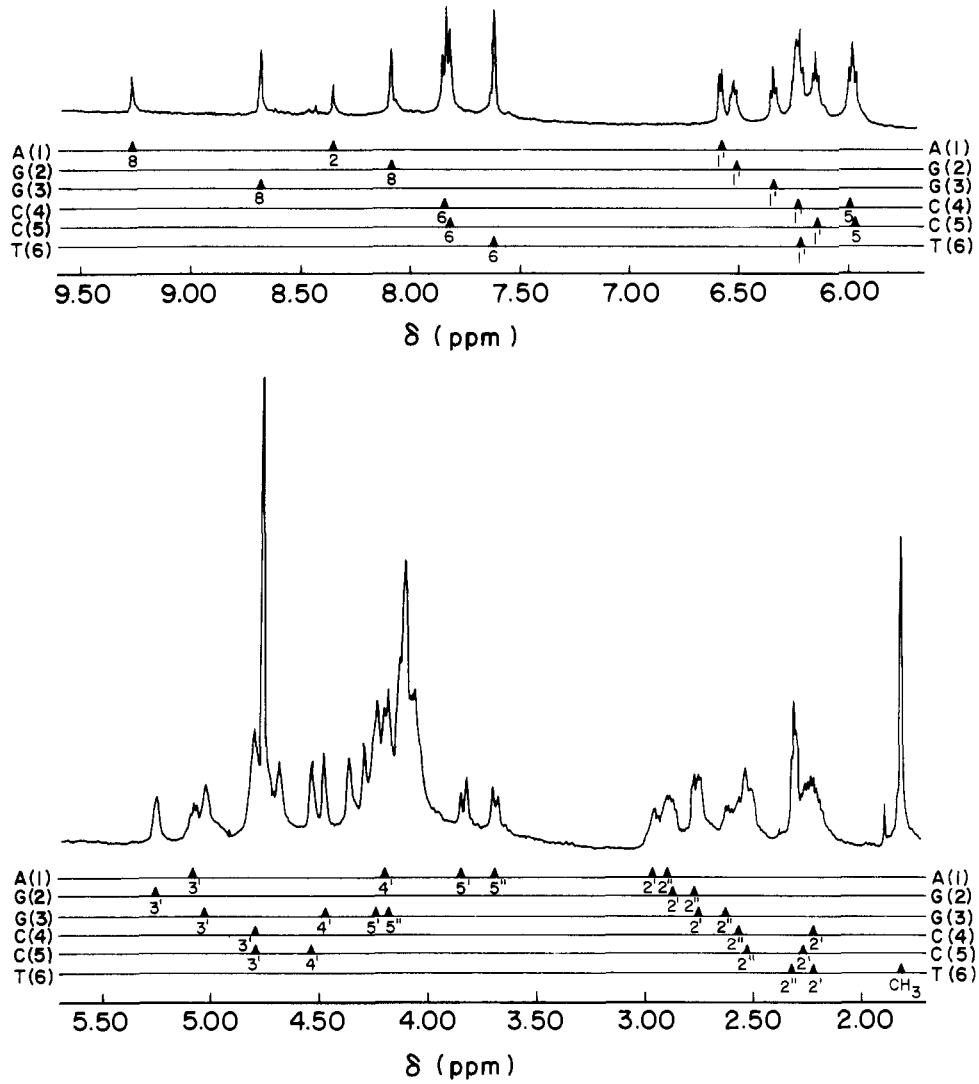

FIGURE 3: 500-MHz ^{1}H NMR spectrum of *trans*-[Pt(NH_3)$_2$[d(ApGpGpCpCpT)]], 1 mM in D_2O, pD 7.69, 25 °C. The scale below the spectrum denotes the resonance assignments, as described in the text. The peaks at 1.90 and 4.75 ppm correspond to traces of acetate and HDO, respectively.

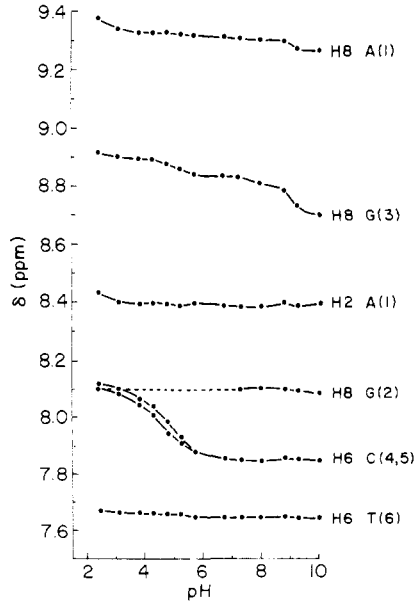

FIGURE 4: Chemical shifts of nonexchangeable base protons of *trans*-[Pt(NH_3)$_2$[d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*]] as a function of pH at 40 °C.

undergoes N1 deprotonation at pH 8.5 instead of the normal region of pH 9.8–10.0. Furthermore, it shows no sign of N7 protonation at the expected pH near 2.3 (Izatt et al., 1971; Saenger, 1984). This behavior is characteristic of guanine with a *cis*- or *trans*-[Pt(NH_3)$_2$]$^{2+}$ moiety bound at the N7 position (Inagaki et al., 1979; Chottard et al., 1980; Caradonna et al., 1982; van der Veer et al., 1986a). Since *trans*-[Pt(NH_3)$_2$]$^{2+}$ is stereochemically unlikely to form 1,2-intrastrand cross-links (Stone et al., 1976; Cohen et al., 1980) and since this adduct is single-stranded with one platinum bound per strand (vide infra), *trans*-[Pt(NH_3)$_2$[d(ApGpGpCpCpT)]] must contain a 1,3-intrastrand cross-link between A(1) N7 and G(3) N7.

The remaining purine base resonance (8.088 ppm) undergoes deuterium exchange and shows no sign of N1 deprotonation from pH 7 to 9, identifying it as the H8 resonance of unplatinated G(2). The expected protonation of its N7 site at pH 2.3 cannot be observed, owing to overlap with the cytosine H6 resonances and broadening.

Assignment of the pyrimidine nonexchangeable base protons is relatively simple. Although the cytosine resonances overlap considerably, two distinctive sets of H6 and H5 doublets are resolved at the appropriate conditions of pH and temperature. The pH dependence of these resonances is typical of unplatinated cytosine, with N3 protonation occurring at pH 4.5. This result is consistent with the known preference of platinum to bind to purine N7 rather than cytosine N3 sites (Inagaki & Kidani, 1979; van der Veer, 1986). The titration, however, does not permit assignment of the H6 and H5 resonances to

specific cytosines in the sequence.

The deprotonation of T(6) N3, pK_a 9.8, was not observed, but the T(6) H6 assignment (7.622 ppm) was readily confirmed by means of its NOE to the thymine methyl resonance at 1.820 ppm.

Almost all of the resonances in *cis*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] are deshielded relative to their chemical shifts in the unplatinated duplex. The A(1) H8 (9.268 ppm), A(1) H2 (8.354 ppm), and G(3) H8 (8.684 ppm) resonances exhibit the greatest shifts, with the H8 resonances shifting farther than the H2 resonance. This behavior is consistent with platinum coordination at the N7 positions of A(1) and G(3) (Chottard et al., 1980; den Hartog et al., 1984a; Chu et al., 1978).

A similar relative positioning of purine resonances is exhibited by the N7-A(2),N7-G(3) cross-linked complex *cis*-[$Pt(NH_3)_2${d(GpApG)}] (van der Veer et al., 1986b). These resonances appear farther upfield than those of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}], however, presumably due to the shielding effect of an uncoordinated 5′-deoxyguanosine unit in the former. A tendency for the *trans*-$[Pt(NH_3)_2]^{2+}$ unit to induce more pronounced downfield shifts than the *cis*-$[Pt(NH_3)_2]^{2+}$ moiety has been observed with the *cis*- and *trans*-$\{Pt(NH_3)_2\}^{2+}$ N7-G(1),N7-G(3) adducts of d(GpCpG) (den Hartog et al., 1983; Gibson & Lippard, 1987).

As was the case for the central deoxycytidine in *cis*-[$Pt(NH_3)_2${d(GpCpG)}] (den Hartog et al., 1983) and *trans*-[$Pt(NH_3)_2${d(GpCpG)}] (Gibson & Lippard, 1987), the central nucleoside of the present 1,3 adduct is less shielded than expected for a stacked single-stranded DNA, suggesting that it is destacked from its neighboring residues. The G(2) H8 resonance is also very readily broadened by paramagnetic trace metal ions. Under such circumstances, the signal can be restored by addition of Na_2EDTA to the solution. These results suggest that N7 of G(2) is exposed to metal ions in solution (Eichhorn et al., 1966) and, as already demonstrated, is not coordinated to the platinum atom.

The deoxyribose ring proton resonances were assigned by means of 1D and 2D nuclear Overhauser effect (NOESY) and 2D homonuclear chemical shift correlation (COSY) experiments in the following manner. Each set of sugar resonances was assigned to the corresponding base by means of the NOE between the base H8 or H6 and the deoxyribose 1′, 2′, 2″, and 3′ protons. Intrasugar NOE's and scalar couplings were then used to assign sequentially the remaining deoxyribose proton resonances. Redundancies in the base-to-sugar and intrasugar connectivities provided an internal consistency check on the assignments (Scheek et al., 1983; Hare et al., 1983; Gronenborn & Clore, 1985). Owing to the single-stranded nature of the product and rapid purine H8 exchange at the elevated temperatures necessary to obtain sufficient resolution, the weaker internucleotide NOE's could not be observed.

The deoxyribose H1′ resonances and the cytosine H5 doublets appear in the region from 6.0 to 6.6 ppm, with the H1′ multiplets partially overlapping one another at temperatures below 75 °C. The deoxyribose 2′, 2″, and 1′ protons comprise an ABX spin system, precluding direct measurement of $^3J_{1'2'}$ and $^3J_{1'2''}$. The coupling sum $^3J_{1'2'} + {}^3J_{1'2''}$ ($\sum^3J$), however, can be measured as the separation between the outermost peaks in each multiplet (Roberts, 1962), giving characteristic values indicative of the average conformation of the deoxyribose ring. The C2′-endo (B DNA, or S type) sugar pucker produces a pseudotriplet with $\sum^3J$ = 13.6 Hz, and the C3′-endo (A DNA, or N type) sugar pucker gives a doublet with $\sum^3J$ = 7.5 Hz (Altona, 1982).

In the unplatinated [d(ApGpGpCpCpT)]$_2$ duplex (Caradonna & Lippard, 1987), all of the H1′ resonances appear as pseudotriplets with $\sum^3J$ values of 14 ± 1.0 Hz, indicating a B DNA structure with all sugars in the S, or C2′-endo, conformation. In *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}], the A(1) H1′ resonance at 6.601 ppm appears as a doublet with a $\sum^3J$ value of 7.4 Hz, indicating that this sugar adopts a predominantly N, or C3′-endo, conformation. The remaining H1′ resonances have $\sum^3J$ values of 13.2 ± 0.8 Hz between 35 and 75 °C at pH 7.2, characteristic of the usual C2′-endo sugar geometry.

The deoxyribose H2′ and H2″ resonances appear in the region from 2.0 to 3.0 ppm. These resonances were assigned directly on the basis of the intranucleotide base H8/H6-to-deoxyribose H2′,2″ NOE's. The stronger of the two NOE's was assigned to H2′, which is approximately 1.3 Å closer to the base protons (Caradonna, 1985). Similarly, the H2′ and H2″ resonances could be distinguished because the H1′-to-H2″ NOE is stronger than the H1′-to-H2′ NOE and because in the COSY spectrum the different magnitudes of the H2′ and H2″ couplings to H3′ give rise to differences in the line shapes of the H1′-to-H2′,2″ cross-peaks (Hare et al., 1983).

The deoxyribose H3′ resonances appear in the region from 4.70 to 5.30 ppm. The three resonances belonging to nucleotides A(1) (5.076 ppm), G(2) (5.247 ppm), and G(3) (5.021 ppm), are well separated and strongly deshielded relative to their positions in the unplatinated duplex. The H3′ resonances of C(4), C(5), and T(6) are not resolved and represent a termination point in the sequential assignment of the deoxyribose proton resonances of these nucleotides.

The greatest deshielding is experienced by the G(2) H3′ proton (+0.257 ppm relative to the unplatinated hexamer), consistent with the earlier suggestion that this residue is destacked from its neighboring nucleotides. A(1) H3′ is the second most deshielded H3′ proton (+0.137 ppm) and exhibits a strong NOE to H8 of its base, indicative of the previously noted C3′-endo sugar pucker and a predominantly anti orientation of the base (Gronenborn & Clore, 1984). Furthermore, this resonance is only weakly coupled to the phosphorus nucleus of the 3′-phosphate group, as revealed by its narrow line width and well-defined multiplet structure. Since the magnitude of this coupling depends upon the value of the torsion angle ϵ, [C4′–C3′–O3′–P] (Altona, 1982), it appears that *trans*-$\{Pt(NH_3)_2\}^{2+}$ cross-linking induces significant changes in the geometry of the hexamer backbone.

The deoxyribose H4′, H5′, and H5″ resonances appear in the region from 3.675 to 4.700 ppm, and are difficult to assign, owing to extensive overlap. The exception is a well-resolved H5′, H5″ multiplet appearing between 3.677 and 3.846 ppm. The absence of ^{31}P coupling, with typical $^3J_{5'P}$ and $^3J_{5''P}$ values of 2–4 Hz (Altona, 1982; den Hartog et al., 1982, 1983), identifies this multiplet as arising from A(1) H5′ and H5″, since only the 5′ terminal residue of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] lacks an attached 5′-phosphate group. The downfield portion of the multiplet, at 3.834 ppm, is assigned to A(1) H5′ and the upfield portion, at 3.688 ppm, to H5″ in accord with the rule of Remin and Shugar (1972). The A(1) H5′, H5″, and H3′ resonances are all coupled to the same resonance at 4.198 ppm, identifying it as A(1) H4′ and providing two continuous and complementary sets of connectivities for the adenine deoxyribose protons.

Conclusions

The reaction of [d(ApGpGpCpCpT)]$_2$ with *trans*-DDP differs from the reaction with *cis*-DDP in both the number and character of products formed. Unlike the *cis*-DDP re-

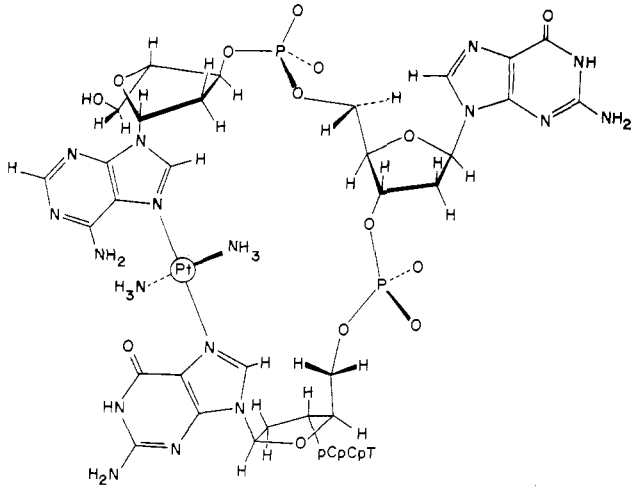

FIGURE 5: Schematic depiction of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}].

action, which yields exclusively the N7-G(2),N7-G(3) intrastrand cross-linked product (Caradonna et al., 1984; Caradonna & Lippard, 1987), the *trans*-DDP reaction produces several single-stranded species, of which *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] predominates. In addition, significant amounts of polyanionic, presumably interstrand cross-linked, high molecular weight products are formed.

As determined by ^1H NMR spectroscopy, *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] contains a 1,3-intrastrand cross-link in which platinum is coordinated to the N7 positions of A(1) and G(3). This structure is schematically depicted in Figure 5. The deoxyribose ring of A(1) adopts a C3′-endo conformation with its glycosyl torsion angle in the anti range. The other deoxyribose rings in the strand adopt primarily C2′-endo, or S-type conformations, including the central G(2) residue, which is apparently destacked from its neighbors.

The structure of *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] differs significantly from previously reported 1,2- and 1,3-intrastrand adducts of *cis*-DDP with oligonucleotides but is consistent with the structures of *trans*-[$Pt(NH_3)_2${d(GpCpG)}] and *trans*-[$Pt(NH_3)_2${d(GpTpG)}]. In these N7-G(1),N7-G(3) adducts, the 5′-guanosine deoxyribose ring also adopts a C3′-endo conformation and the other deoxyribose rings retain their B DNA type conformations, consistent with a model in which the central base is destacked (Gibson & Lippard, 1987; van der Veer et al., 1986a).

The formation of a *trans*-$\{Pt(NH_3)_2\}^{2+}$ 1,3-intrastrand adduct forces the deoxyribose ring of the 5′ residue in the resulting 23-membered ring to switch from a C2′-endo to a C3′-endo conformation. This type of induced conformational change is also seen in the 5′ residue of *cis*-$\{Pt(NH_3)_2\}^{2+}$ 1,2-intrastrand cross-links (den Hartog et al., 1982, 1984b; Marcelis et al., 1983; Neumann et al., 1984; Caradonna & Lippard, 1987) but has not been reported in any model system containing the *cis*-[$Pt(NH_3)_2${d(GpNpG)}] (N = A, C, or T) 1,3 adduct (den Hartog et al., 1983, 1985; Marcelis et al., 1983; van der Veer et al., 1986b). In the 1,3 adduct formed by *cis*-$\{Pt(NH_3)_2\}^{2+}$, the sugar rings of both the 5′ and 3′ residues adopt predominantly C2′-endo conformations and their bases either undergo rapid syn/anti equilibria or else are locked into some intermediate conformation. The central residue appears to be "bulged out" with the deoxyribose ring predominantly C2′-endo and the base in the anti conformation (den Hartog et al., 1983).

The formation of the *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)}] 1,3 adduct is consistent with the results of the M13 replication mapping experiment (Pinto & Lippard, 1985b), and the proposed destacking of the central guanosine residue agrees with the interpretation of studies employing antinucleoside antibodies (Sundquist & Lippard, 1986). The d(ANG) cross-link characterized in this study may comprise a significant fraction of the adducts formed by *trans*-DDP on DNA and is likely to exhibit structural features representative of other *trans*-DDP 1,3-intrastrand cross-links involving purine residues.

ACKNOWLEDGMENTS

500-MHz proton NMR experiments were performed at the NMR Facility for Biomolecular Research located at the Francis Bitter National Magnet Laboratory at MIT. The NMR Facility is supported by Grant RR00995 from the Division of Research Resources of the National Institutes of Health and by the National Science Foundation under Contract C-670. We are also grateful to Engelhard Corp. for a loan of K_2PtCl_4 from which *trans*-DDP was prepared.

Registry No. *trans*-DDP, 14913-33-8; d(ApGpGpCpCpT)·*x*Na, 109284-33-5; *trans*-[$Pt(NH_3)_2${d(ApGpGpCpCpT)-*N7-A(1),N7-G(3)*}], 109284-34-6.

REFERENCES

Admiraal, G., van der Veer, J., de Graaf, R., den Hartog, J., & Reedijk, J. (1987) *J. Am. Chem. Soc. 109*, 592–594.

Altona, C. (1982) *Recl.: J. R. Neth. Chem. Soc. 101*, 413–433.

Aue, W. P., Bartholdi, E., & Ernst, R. R. (1975) *J. Chem. Phys. 64*, 2229–2246.

Bose, R., Cornelius, R., & Viola, R. (1986) *J. Am. Chem. Soc. 108*, 4403–4408.

Butour, J. L., & Johnson, N. P. (1986) *Biochemistry 25*, 4534–4539.

Caradonna, J. C. (1985) Ph.D. Dissertation, Columbia University.

Caradonna, J. C., & Lippard, S. J. (1987) (submitted for publication).

Caradonna, J. C., Lippard, S. J., Gait, M. J., & Singh, M. (1982) *J. Am. Chem. Soc. 104*, 5793–5795.

Chottard, J.-C., Girault, J.-P., Chottard, G., Lallemand, J.-Y., & Mansuy, D. (1980) *J. Am. Chem. Soc. 102*, 5565–5572.

Chu, G. Y. H., Mansy, S., Duncan, R. E., & Tobias, R. S. (1978) *J. Am. Chem. Soc. 100*, 593–596.

Ciccarelli, R. B., Solomon, M. J., Varshavsky, A., & Lippard, S. J. (1985) *Biochemistry 24*, 7533–7540.

Cohen, G. L., Ledner, J. A., Bauer, W. R., Ushay, H. M., Caravana, C., & Lippard, S. J. (1980) *J. Am. Chem. Soc. 102*, 2487–2488.

den Hartog, J. H. J., Altona, C., Chottard, J.-C., Girault, J.-P., Lallemand, J.-Y., de Leeuw, F., Marcelis, A., & Reedijk, J. (1982) *Nucleic Acids Res. 10*, 4715–4731.

den Hartog, J. H. J., Altona, C., van Boom, J. H., Marcelis, A. T. M., van der Marel, G. A., Rinkel, L. J., Wille-Hazeleger, G., & Reedijk, J. (1983) *Eur. J. Biochem. 134*, 485–495.

den Hartog, J. H. J., van den Elst, H., & Reedijk, J. (1984a) *J. Inorg. Biochem. 21*, 83–92.

den Hartog, J. H. J., Altona, C., van der Marel, G., & Reedijk, J. (1984b) *Eur. J. Biochem. 147*, 371–379.

den Hartog, J. H. J., Altona, C., van den Elst, H., van der Marel, G., & Reedijk, J. (1985) *Inorg. Chem. 24*, 983–986.

Dijt, F. J., Canters, G. W., den Hartog, J. H. J., Marcelis, A. T. M., & Reedijk, J. (1984) *J. Am. Chem. Soc. 106*, 3644–3647.

Eastman, A. (1986) *Biochemistry 25*, 3912–3915.
Eichhorn, G. L., Clark, P., & Becker, E. D. (1966) *Biochemistry 5*, 245–253.
Ernst, R. R. (1966) *Adv. Magn. Reson. 2*, 1–135.
Fichtinger-Schepman, A. M. J., van der Veer, J. L., den Hartog, J. H. J., Lohman, P., & Reedijk, J. (1985) *Biochemistry 24*, 707–713.
Gibson, D., & Lippard, S. J. (1987) *Inorg. Chem.* (in press).
Girault, J.-P., Chottard, J.-C., Guittet, E. R., Lallemand, J.-Y., Huynh-Dinh, T., & Igolen, J. (1982) *Biochem. Biophys. Res. Commun. 109*, 1157–1163.
Gronenborn, A. M., & Clore, G. M. (1985) *Prog. Nucl. Magn. Reson. Spectrosc. 17*, 1–32.
Hare, D. R., Wemmer, D. E., Chou, S. H., Drobny, G., & Reid, B. R. (1983) *J. Mol. Biol. 171*, 319–336.
Inagaki, K., & Kidani, Y. (1979) *J. Inorg. Biochem. 11*, 39–47.
Inagaki, K., Kuwayama, M., & Kidani, Y. (1982) *J. Inorg. Biochem. 16*, 59–60.
Izatt, R. M., Christensen, J. J., & Rytting, J. H. (1971) *Chem. Rev. 71*, 439–481.
Marcelis, M., van Kralingen, C. G., & Reedijk, J. (1980) *J. Inorg. Biochem. 13*, 213–222.
Marcelis, A., den Hartog, J. H. J., van der Marel, G. A., Wille, G., & Reedijk, J. (1983) *Eur. J. Biochem. 135*, 343–349.
Neumann, J. M., Tran-Dinh, S., Girault, J.-P., Chottard, J.-C., Huynh-Dinh, T., & Igolen, J. (1984) *Eur. J. Biochem. 141*, 465–472.
Pinto, A. L., & Lippard, S. J. (1985a) *Biochim. Biophys. Acta 780*, 167–180.
Pinto, A. L., & Lippard, S. J. (1985b) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4616–4619.
Plooy, A., van Dijk, M., & Lohman, P. (1984) *Cancer Res. 44*, 2043–2051.
Reedijk, J., den Hartog, J. H. J., Fichtinger-Schepman, A., & Marcelis, A. (1984) *Platinum Coordination Complexes in Cancer Chemotherapy* (Hacker, M., Douple, E., & Krakoff, I., Eds.) p 39, Martinus-Nijhoff, Boston.
Remin, M., & Shugar, D. (1972) *Biochem. Biophys. Res. Commun. 48*, 636–642.
Roberts, J. D. (1962) *An Introduction to the Analysis of Spin–Spin Splitting in High Resolution NMR Spectra*, Benjamin, New York.
Roberts, J. J., & Thomson, A. J. (1979) *Prog. Nucleic Acid Res. Mol. Biol. 22*, 71–133.
Saenger, W. (1984) *Principles of Nucleic Acid Structure*, Chapter 5, pp 105–115, Springer-Verlag, New York.
Scheek, R. M., Russo, N., Boelens, R., Kaptein, R., & van Boom, J. H. (1983) *J. Am. Chem. Soc. 105*, 2914–2916.
Schweizer, M. P., Chan, S. I., Helmkamp, G. K., & Ts'o, P. O. P. (1964) *J. Am. Chem. Soc. 86*, 696–700.
Sherman, S., Gibson, D., Wang, A., & Lippard, S. J. (1985) *Science (Washington, D.C.) 230*, 412–417.
Sproat, B. S., & Gait, M. J. (1984) *Oligonucleotide Synthesis. A Practical Approach* (Gait, M. J., Ed.) pp 83–114, IRL, Washington, DC.
States, D. J., Haberkorn, R. A., & Ruben, D. J. (1982) *J. Magn. Reson. 48*, 286–292.
Stone, P. J., Kelman, A. D., & Sinex, F. M. (1976) *J. Mol. Biol. 104*, 793–801.
Sundquist, W. I., Lippard, S. J., & Stollar, B. D. (1986) *Biochemistry 25*, 1520–1524.
van der Veer, J. L. (1986) Ph.D. Thesis, State University Leiden.
van der Veer, J. L., Ligtvoet, G. J., van den Elst, H., & Reedijk, J. (1986a) *J. Am. Chem. Soc. 108*, 3860–3862.
van der Veer, J. L., van den Elst, H., den Hartog, J. H. J., Fichtinger-Schepman, M. J., & Reedijk, J. (1986b) *Inorg. Chem. 25*, 4657–4663.
van Hemelryck, B., Girault, J.-P., Chottard, G., Valadon, P., Laoui, A., & Chottard, J.-C. (1987) *Inorg. Chem. 26*, 787–795.

Effects of Mutation on the Downfield Proton Nuclear Magnetic Resonance Spectrum of the 5S RNA of *Escherichia coli*†

Daniel T. Gewirth‡ and Peter B. Moore*,‡,§
Departments of Molecular Biophysics and Biochemistry and Chemistry, Yale University, New Haven, Connecticut 06511
Received February 5, 1987; Revised Manuscript Received April 28, 1987

ABSTRACT: The imino proton spectra of several mutants of the 5S RNA of *Escherichia coli* are compared with that of the wild type. Three of the variants discussed are point mutations, and the fourth is a deletion mutant lacking bases 11–69 of the parent sequence, all obtained by site-directed mutagenesis techniques. The spectroscopic effects of mutation are limited in all cases, and the differences between normal and mutant spectra can be used to make or confirm the assignments of resonances. Several new assignments in the 5S spectrum are reported. Spectroscopic differences due to sequence differences permit the products of single genes within the 5S gene family to be distinguished and their fates followed by NMR.

Site-directed mutagenesis is proving to be a powerful tool for investigating the relationship of primary structure to function in ribosomal RNAs (Goringer et al., 1984; Gregory et al., 1984; Stark et al., 1984; Jemiolo et al., 1985; Christiansen et al., 1985; Goringer & Wagner, 1986; Meier et al., 1986; Gregory & Zimmermann, 1986). The application of site-directed mutagenesis methods to the solution of a specific problem having to do with the physical properties of the 5S RNA of *Escherichia coli* is discussed below.

The downfield resonances in the proton spectra of nucleic acids dissolved in water, between 10 and 15 ppm, represent

† This work was supported by National Institutes of Health Grants GM 32206 and GM 22778. The Yale 490-MHz NMR spectrometer is supported in part by NIGMS Shared Instrumentation Grant GM 32243-02S1.
* Author to whom correspondence should be addressed.
‡ Department of Molecular Biophysics and Biochemistry.
§ Department of Chemistry.

0006-2960/87/0426-5657$01.50/0 © 1987 American Chemical Society