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Study of the Binding of *cis*- and *trans*-Dichlorodiammineplatinum(II) to Calf Thymus DNA by Extended X-Ray Absorption Fine Structure Spectroscopy

Sir:

The complex *cis*-dichlorodiammineplatinum(II) (DDP) is an active antitumor drug while the *trans*-DDP isomer is ineffective, a result that may reflect differences in their ability to bind DNA.[1-5] While various chemical and physical techniques have been used to explore the interactions of these platinum compounds with DNA, there appears to be no direct structural information about the resulting platinum–DNA complex. Recent crystallographic study[5c] of the blue compound formed between *cis*-diammineplatinum and α-pyridone (**1**) suggested[5b] that thymine (**2**, R = CH₃) and guanine (**3**) bases in DNA
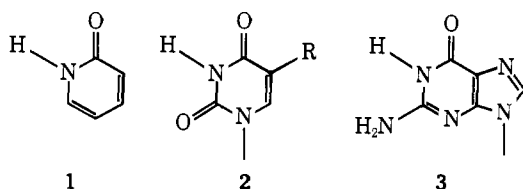
might bridge two *cis*-diammineplatinum units using the deprotonated amide nitrogen and the exocyclic oxygen as donor atoms. The resulting complex would have a short Pt–Pt distance, 2.5–3.2 Å, depending upon the platinum oxidation state. To test this possibility and to provide information about the binding sites of DDP, samples of *cis*- and *trans*-[Pt(NH₃)₂Cl₂] bound to calf thymus DNA, **4** and **5**, respectively, were prepared and studied by extended x-ray absorption fine structure (EXAFS) spectroscopy.

The platinum–DNA samples were prepared in the following manner. Solid DDP complexes were suspended in buffered solutions (10 mM NaNO₃, 5 mM Tris·NO₃, pH 8) of calf thymus DNA and left to stir in the dark at room temperature for 5 days. The formal ratio (r_f) of *cis*-DDP (concentration 5.1 mM in 15 mL) to that of DNA phosphate (5.3 mM) was 0.96, a value also used for the *trans*-DDP (5.3 mM in 75 mL) with DNA (5.5 mM) binding experiment. Following the incubation, the opalescent solutions were centrifuged at 190 000 × g for 16–18 h, pelleting the platinum–DNA complex.[6] The pellets were diluted with sucrose and a small amount of buffer to perform the measurements.

The raw data (Pt L$_1$ edge)[7a] in the form of $\mu x = \ln I_0/I$ vs. E (where μx is the absorption factor, I_0 and I are the incident and transmitted light intensities, and E is the x-ray photon energy) of **4** and **5** were recorded at ambient (cf. Figures 1a and 1b) and liquid nitrogen (cf. Figure 1c) temperatures with the synchrotron radiation of EXAFS I at SLAC-SSRL.[7b] After conversion of E into photoelectron wavevector **k** (where $\mathbf{k} = [2m/\hbar^2(E - E_0)]^{1/2}$ and E_0 is chosen as 13 900 eV and varied in the subsequent curve fittings) and μx into $\chi(\mathbf{k}) = (\mu - \mu_0)/\mu_0$,[8] the data were multiplied by $\mathbf{k}^3$ and the background was removed by a cubic spline technique (four sections with ~4 Å^{-1} each).[8] Fourier transforms of these data provide the radial distribution (RD) functions shown in Figure 1. To obtain interatomic distances from these Fourier transform functions, phase shift corrections must be acquired from other known systems and applied to the peak positions.[9] Knowing the average Pt–N distance of 2.043 Å in [Pt(en)₂]²⁺ (en = H₂NCH₂CH₂NH₂),[10] the phase shifts of 0.46 Å for Pt–N, at E_0 of 13 900 eV, were obtained. With the assumption that the phase shift for Pt–O can reasonably be assumed to be 0.46 Å, the average Pt–N (or –O) distance is 2.03 Å for both **4** and **5**.

The most definitive result that can be deduced from the radial distribution functions shown in Figure 1 is the lack of distinct Pt–Pt bonding[11] in the DNA complexes of both the *cis*- and *trans*-DDP. This conclusion is further supported by a detailed comparison of Figure 1 with the Fourier transforms of the EXAFS spectra of the structurally characterized[12] [(Pt(en)(Guo)₂]²⁺, Guo = guanosine, which does not contain any Pt–Pt bond, and of a series of *cis*-diammine- or *cis*-di-(alkylamine)platinum uridine blues which have been shown to contain Pt–Pt bonds of ~2.9 Å.[13] Specifically, the RD curves of **4** (Figures 1a and 1c; except for the shoulder at 2.01 Å in the latter) and **5** (Figure 1b) are quite similar to that of [Pt(en)-(Guo)₂]²⁺ (cf. Figure 1d of ref 13) but grossly different from that of the platinum blues (cf Figures 1a and 1b of ref 13).[13] This conclusion is reinforced by the observation that the amplitude of the Pt··X (X = C, N, O) peaks decreases with increasing distances and that for similar distances the Pt–Pt peaks are two to three times higher than the Pt··X peaks.[13,14]

The number of nearest neighboring atoms and the corresponding Pt–N (or –O) distances can be obtained by Fourier filtering[8a] the largest peak in Figure 1 with a smooth window of 1.0–2.4 Å (cutoff limits) and transforming it back into **k** space. The resulting EXAFS spectra can then be fitted (as shown in Figure 2)[8b] with a "single-distance" model based on the amplitude and phase functions (chemical transferabilities
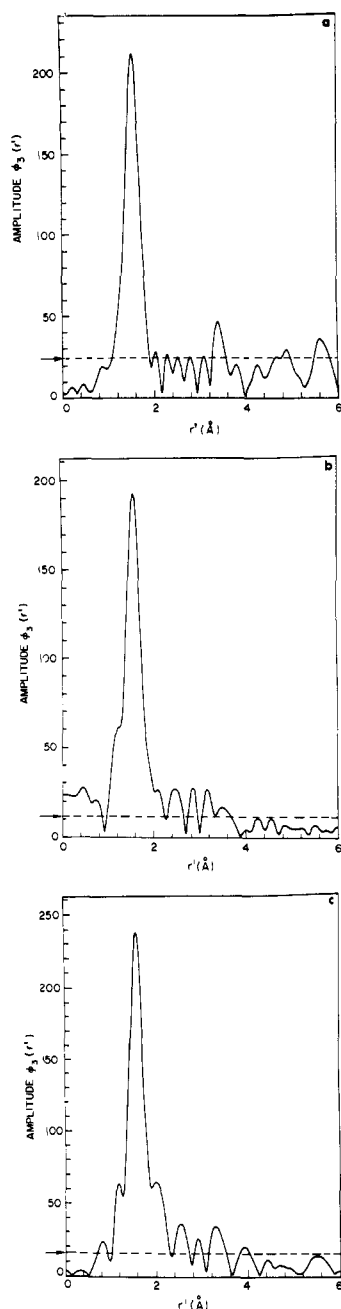
Figure 1. Fourier transforms $\phi_3(r')$ (radial distribution curves) of the $k^3\chi(k)$ EXAFS spectra, corresponding to data recorded at ambient (a and b) and liquid nitrogen (c) temperatures, of complexes formed in the reaction of *cis*-DDP (a and c) and *trans*-DDP (b) with calf thymus DNA. The dashed lines indicate the noise levels. $r'(\text{Å})$ is related to the interatomic distance $r(\text{Å})$ by a phase shift (see text).
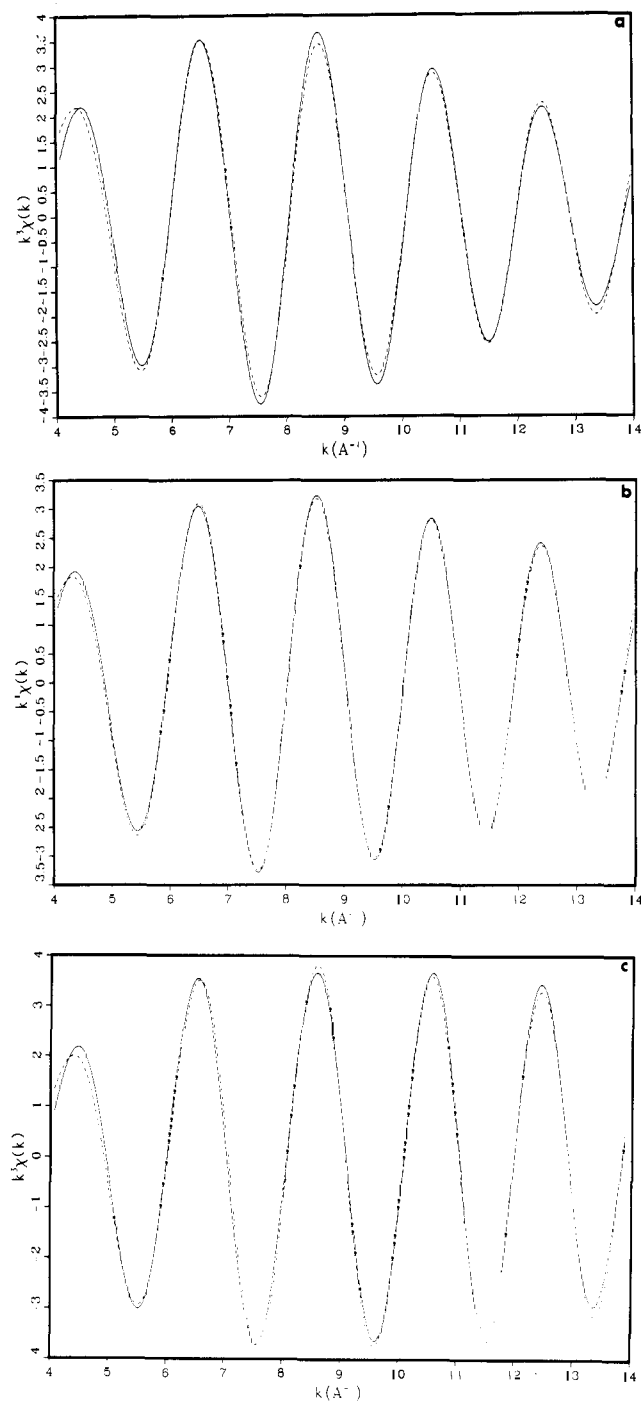

Figure 2. Nonlinear least-squares fits (dashed curves) of the Fourier-filtered (smooth window of 1.0–2.4 Å) coordination sphere $k^3\chi(k)$ EXAFS spectra (solid curves) for complexes formed in the reaction of *cis*-DDP (a and c) and *trans*-DDP (b) with calf thymus DNA (cf. Figure 1 caption).

assumed[9]) of Pt–N deduced from the structurally known $[Pt(en)_2]^{2+}$ complex.[10] Four parameters are least-squares refined: the overall magnitude, the Debye–Waller factor σ, the distance r, and the energy threshold E_0.[9e,f] With this approach, the numbers of nearest neighbors were found to be 4.3 (5) and 3.6 (3), and the corresponding interatomic distances 2.025 (9) and 2.033 (6) Å, for **4** and **5**, respectively.[15] The corresponding values for **4** at liquid nitrogen temperature are 3.7 (5) and 2.027 (9) Å.[15] These reasonable values of coordination numbers and bond lengths provide strong evidence that the platinum atoms in both **4** and **5** are coordinated to four nitrogen and/or oxygen atoms as normal single bonds. The Debye–Waller factors[15] of 0.056 (8) and 0.049 (7) Å for **4** and **5**, respectively, at room temperature are comparable with those of 0.050 (12) and 0.052 (10) Å found in $[Pt(en)_2]^{2+}$ and

$[Pt(en)(Guo)_2]^{2+}$, respectively, indicating that the spreads of the Pt–X (X = N or O) bond distances are probably on the order of 0.06–0.08 Å (assuming a 2:2 distance model and $\sigma_{vib} \approx 0.038$ Å). At liquid nitrogen temperature, the Debye–Waller factor[15] of **4** decreases to 0.038 (14) Å. Furthermore, the L_I absorption edges of **4** and **5** occur at energies similar (within 2 eV) to those of other platinum(II) complexes such as $[Pt(en)_2]^{2+}$, $[Pt(NH_3)_4]^{2+}$, and $[Pt(NH_3)_2Cl_2]$, thereby suggesting that the platinum atoms adopt a Pt(II) square-planar configuration. The present results rule out the possibility that three Pt–N(O) and one Pt–Cl bonds comprise the coordination sphere in either **5** (as has been proposed[4b]) or **4**. Chloride ions are clearly lost upon DNA binding.[16] The

EXAFS results also demonstrate that oxidative addition to form a six-coordinate Pt(IV)–DNA adduct with the retention of both ammines and chlorides does not occur. From the EXAFS spectra, no appreciable differences were detected at this stage *in the platinum coordination spheres* of the complexes formed by *cis*- and *trans*-DDP with DNA. This result, however, should not be taken to indicate that subtle differences do not exist.

In conclusion, this work provides, for the first time, strong structural evidence against the possibility of distinct metal–metal bonding in the complexes of both *cis*- and *trans*-DDP with calf thymus DNA. The data are consistent with the presence of four Pt–N (or –O) bonds in a presumably square-planar Pt(II) coordination sphere. Further stereochemical details from EXAFS study must await determination of interatomic distances other than those in the coordination shell using spectra of higher signal-to-noise ratio (in progress).

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(15) The fitting error quoted here for each parameter is calculated by changing that particular parameter (while least-squares refining the others) until the χ^2 doubled. The systematic errors (not included) due to background removal, Fourier filtering, and chemical bonding effects may amount to $\lesssim$0.5, 10, and 20% in distance, Debye–Waller factor, and coordination number, respectively.
(16) The Pt–Cl bonds differ drastically from the Pt–X (X = N or O) bonds in phase shift and backscattering amplitude^{9e,f} which is easily discernible in EXAFS spectroscopy. The EXAFS spectra of *cis*- and *trans*-Pt(NH$_3$)$_2$Cl$_2$, for example, exhibit an interference pattern characteristic of two types of distances (Pt–N and Pt–Cl) with a "beat mode" at k $\approx$ 10.4 Å^{-1}. Fourier transforms of these spectra result in two peaks at $r' = 1.48$ and 1.86 Å with an intensity ratio of ~0.8:1.0. Nonlinear least-squares curve fitting of the Fourier filtered k$^3\chi$(k) data with a two-distance model gave Pt–N and Pt–Cl distances at 2.05 and 2.33 Å, respectively, which compare favorably well with the observed values of 2.01 (4) and 2.33 (1) Å (G. H. W. Milburn and M. R. Truter, *J. Chem. Soc.* A, 1609 (1966)).

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β-Cyclodextrinylbisimidazole, a Model for Ribonuclease

Sir:

Tabushi has reported[1] the preparation of **1**, in which β-cyclodextrin (cycloheptaamylose) is capped as a disulfonate derivative. We had been interested[2] in such selectively difunctionalized cyclodextrins for the synthesis of bifunctional catalysts. As an example, we find that on heating with imidazole in DMF at 85–90 °C for 96 h **1** is converted to the bisimidazole derivative **2**.[3] Tabushi has recently described[4] several other displacement reactions of **1**. We now wish to report that **2** has the ability to catalyze the hydrolysis of a cyclic phosphate

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