EXAFS Studies of a Pt-dimer–DNA Complex

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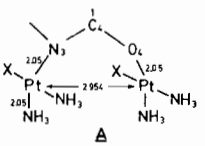
cis-$Pt(NH_3)_2Cl_2$ (**1**) is a useful anticancer agent which is thought to operate through specific Pt–DNA interactions which inhibit DNA replication. The mechanism of this interaction is not understood [1]. A possible mechanism, which is supported both by kinetic studies [1] and the structures of model compounds, involves conversion of **1** to a dimer aquation product [2], $[(NH_3)_2Pt(OH)_2Pt(NH_3)_2]^{2+}$ (**2**) before interaction with DNA. The reactions of **2** and DNA bases yield two types of complexes; dimeric species containing two Pt atoms separated by about 2.95 Å [3] and 'platinum blues' which, on the basis of Lippard's model compound [4] probably contain four platinum atoms in a chain with a shortest Pt–Pt distance of *ca.* 2.8 Å. A typical Pt environment, as shown in the Pt-1-methyluracil complex [3c] is shown in A.



A

The interaction product (**3**) of **2** with DNA may contain two platinum atoms bound to a single base as found in the dimeric model complexes. Alternatively bonding of the two platinum atoms to two adjacent bases may occur, either instrastrand, as in the Eichhorn co-stacking model [5, 6] or interstand resulting in longer Pt–Pt distances (>3.4 Å). A third possibility is the dissociation of the dimer species before or during interaction which could result in a complex without a well-defined Pt–Pt distance. We have used extended X-ray absorption fine structure (EXAFS) spectroscopy [7] above the Pt L_3 edge to study the Pt environment in the complex formed by the reaction of the hydroxo-bridged Pt dimer cation, **2**, with calf thymus DNA.

The platinum DNA sample was prepared as follows. The solid nitrate salt of the Pt dimer species **2** (0.0409 g), was suspended in a buffered solution (10 m*M* $NaNO_3$, 5 m*M* tris-NO_3, pH 8) of calf thymus DNA (0.4130 g/100 ml) and left to stir in the dark for 24 hours. This ratio of **2** to DNA provided a ratio of 1 platinum atom to every 10 nucleotide bases. Following incubation, the opalescent solution was centrifuged at 190.000 g for 16 hours. The supernatant was decanted off and more buffer was added (12 ml). The mixture was stirred thoroughly and then centrifuged at 190,000 ,g for 16 hours. Again the supernatant was removed and the semi-dry material (**3**) was used for the EXAFS experiments. The spectra of the solid Pt-dimer (**2**) and solid $[Pt(NH_3)_2(C)_2](NO_3)_2(C)$ (C = 1-methylcytosine) (**4**) were recorded as standards as their structures are known from single crystal X-ray studies [2a, 8].

The X-ray absorption spectra (PtL_3) in the form $\mu x = \ln (I_o/I)$ *versus* E (where μx is the absorption factor, I_o and I are signals proportional to the incident and transmitted light intensities and E is the X-ray photon energy) of **2**, **3**, and **4** were recorded at ambient temperatures with synchrotron radiation at the C2 station of the Cornell High Energy Synchrotron radiation Source (CHESS) at Cornell. The EXAFS signal $[\chi(k) = (\mu - \mu_o)/\mu_o]$ was isolated by subtraction of a background generated by multiple (150–200) three-point smooths of the data. The energy above the PtL_3 edge was then converted into photoelectron wavenumber ($k = (0.261 (E - E_o))^{1/2}$, k in $Å^{-1}$, E in eV]. (In this work, E_o was fixed at the midpoint of the absorption jump.) The $\chi(k)$ data over the range **4** to 14 $Å^{-1}$ (60 to 750 eV above the edge) was weighted by k^n (n = 3, 4, 5) and Fourier transformed to obtain the spectra shown in Fig. 1. An alternate cubic spline background subtraction procedure yielded similar results although each background subtraction technique introduced characteristic artifacts in the Fourier transform (FT) below 1.5 Å. To obtain interatomic distances from the Fourier transform functions (Fig. 1) phase shift corrections must be applied [7]. Approximate phase shift corrections of 0.31 Å for Pt–(O,N) distances and 0.04 Å for Pt–Pt distances* are derived from the EXAFS spectrum of the Pt-dimer **2** [R(Pt–N) = 2.01 Å R(Pt–O) = 2.03 Å R(Pt–Pt) = 3.09 Å] [3a] and the assumption of phase shift transferability [9]. The purpose of the work reported in this note is a qualitative study of the Pt environment in Pt–DNA. Detailed analysis of the EXAFS spectra to obtain accurate interatomic distances will be the subject of a further paper.

*Note that the phase shift correction for Pt–Pt distances is 0.18 Å when derived from the EXAFS spectrum of Pt metal. The differences in the phase shift correction derived from **2** and Pt metal indicates some of the uncertainties in obtaining quantitative interatomic distances from EXAFS.

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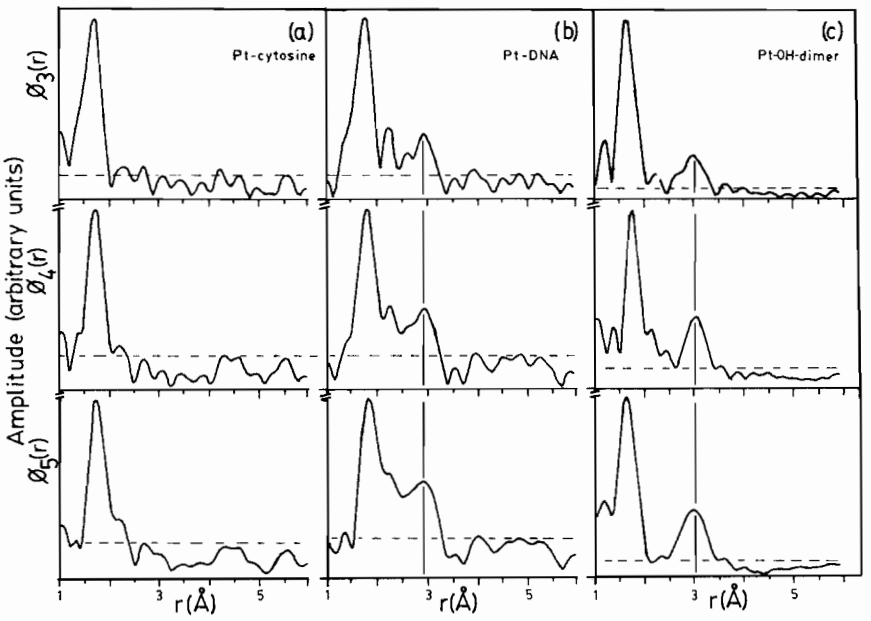

Fig. 1. Fourier transforms $\phi_3(r)$, $\phi_4(r)$, $\phi_5(r)$ of the $k^n \chi(K)$ data [$4 < k < 14$ Å^{-1}] above the PtL_3 edge of (a) $[Pt(NH_3)_2(C)_2]\cdot(NO_3)_2$[C] (C = 1-methylcytosine) (**4**) (b) the Pt–DNA complex (**3**) resulting from the reaction of **2** with calf thymus DNA in a 1:50 Pt:base pair ratio; and (c) $[(NH_3)_2Pt(OH)_2Pt(NH_3)_2](NO_3)_2$ (**2**). The actual interatomic distances (R) are related to the 'radial distribution' peaks (r) by phase shifts. The dashed lines indicate the effects in the Fourier transforms of noise in the original absorption data.

The most definitive result that we deduce from the Fourier transforms (approximate radial distribution functions) is that a short Pt–Pt distance exists in the Pt–DNA complex (**3**). This is indicated by the 2.87(8) Å peak in the FT of **3** which is at about the same position as the Pt–Pt peak in the FT of the Pt dimer species (3.05(10) Å). (Distances indicated are the average (standard deviation) of several determinations of peak positions in $\phi_3(r)$ spectra.) The FT of the PtL_3 EXAFS of **4** does not contain a peak around 3 Å as expected since the shortest Pt–Pt distance in **4** is greater than 4.5 Å [6]. We observe for both **2** and **3** (see Fig. 1) that the 3 Å peak height increases by approximately a factor of 1.5 relative to the nearest neighbour peak height (1.70(5) Å–Pt(N,O)) when the k weighting of the $\chi(K)$ data is increased from 3 to 5. This strongly supports identification of the 3 Å peak with backscattering from a Pt atom since the backscattering amplitude for a high Z atom is larger at large k than that for a low Z atom and increasing the k weighting of the data emphasizes the larger k regions of the EXAFS data.

One could suggest that the Pt–Pt signal in the EXAFS of the Pt–DNA complex (**3**) arises from unreacted Pt dimer (**2**). However this is definitely not the case since (1) the shift of 0.18 ± 0.13 Å ($\phi_3(r)$) between the peak position in **2** and **3** is real and reproducible; (2) there is a 4 eV increase in the PtL_3 edge position [from 11.562 eV in **2** to 11.566 eV in **3** – energy calibration was achieved and frequently checked by setting the midpoint of the PtL_3 edge in Pt metal to 11.564 eV] and there is no indication of a pre-edge rise in the absorption spectrum of **3** which would be observed if unreacted **2** was present; and (3) the sample preparation procedure included a washing step which was expected to remove unreacted Pt dimer.

The FT spectra of the Pt–DNA complex **3** appear to contain one or two additional peaks at *ca.* 2.2(1) and 2.5(1) Å in between the nearest neighbour [Pt–(N,O)] and Pt–Pt peaks. These may be associated with Pt–C distances (see A) in which case approximate phase shift corrections would yield distances of 2.5 Å and 2.8 Å. One complication with this interpretation is that the phase shift and amplitude functions of heavy atom backscatters such as Pt are known to distort the peak shape in the FT spectra and introduce side lobes at low R [10]. Thus at least part of the signal in between the 1.7 and 3.0 Å peaks is likely associated with the low R side lobes of the 3 Å peak. A definitive interpretation of these features, along with a rigorous determination of the Pt–Pt distance, awaits further analysis.

The result of this work, namely that a short Pt–Pt distance exists in the complex formed between Pt hydroxide dimer (**2**) and DNA, is in apparent contradiction to the results of Teo *et al.* [11]. They have used Pt L_1 EXAFS to show that short Pt–Pt

distances do not occur in the complexes formed in aqueous media [10 m*M* $NaNO_3$, 5 m*M* tri-NO_3, pH 8] between calf thymus DNA and either *cis* or *trans*-$Pt(NH_3)_2Cl_2$. Since **1** is known to form **2** in aqueous media [2a] one might argue that the reaction carried out by Teo *et al.* [11] between *cis*-$Pt(NH_3)_2Cl_2$ and DNA should have produced a complex similar to that which we have studied (**3**). However, our previous work [3] has shown that reaction of *cis*-$Pt(NH_3)_2Cl_2$ or freshly prepared *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$ with DNA bases yields primarily monomeric species, and only small fractions of Pt dimeric compounds (*i.e.* species containing two Pt atoms bound to one base and separated by ~3.0 Å [4]. Thus the two sets of results are not contradictory.

Although biological tests show that *cis* and *trans*-$Pt(NH_3)_2Cl_2$ react differently *in vivo*, model studies do not show any great difference in the ability of DNA bases to bind both the *cis* and *trans* complexes. The cation **2** can only be formed from *cis*-$Pt(NH_3)_2Cl_2$. The present results show that **2** reacts with DNA in the manner suggested by model compounds [4] and differently to *cis* and *trans*-$Pt(NH_3)_2Cl_2$. Further, the likely binding sites of a dimer with the preferred base, guanine, are N1–O6 [4a] or N7–O6 [12]. Binding to these sites will interfere with the hydrogen bonding between DNA strands and thus the replication process. Speranzini [13] has calculated, on the basis of kinetic [14] and thermodynamic results [15] that the concentration of **2** in the cell after 24 hours is at least as high as 10^{-12} *M*. Thus we feel that a mechanism for the anti-cancer activity of *cis*-$Pt(NH_3)_2Cl_2$ based on the interaction of *2* with DNA cannot be ruled out.

More detailed analyses of the PtL_3 and L_1 EXAFS of the Pt–DNA complex **3** and Pt-base compounds are currently underway. This will allow a more detailed and quantitative determination of the Pt environment in the complex formed between **2** and DNA.

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