Synthesis and Structure of a Product, formed during DNA Nicking with a Cyclometallated Nuclease, consisting of an Adenine Bridging Two Palladium(II) Complexes

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Treatment of DNA with the cyclometallated nuclease obtained from Pd^{II} and derivatives of 2-phenyl-1,10-phenanthroline results in a bimetallic adenine complex, coordinated through N-3 and N-9.

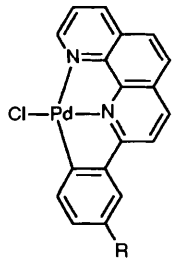
The cyclometallated product **1** obtained by reacting palladium salts with 2-phenyl-1,10-phenanthroline and its derivatives, acts as a nuclease toward DNA, generating nicks at dG, and to a lesser extent, dA sites.[1] While investigating the products formed in this reaction between **1a** and calf thymus DNA,† a major palladium-containing product was isolated as an orange precipitate. FAB mass spectra analysis (3-nitrobenzyl alcohol matrix) of the solid suggested it was an adduct of two ionized cyclometallated palladium complexes and one adenine (*m/z* 1059, $C_{51}H_{48}N_{11}O_2Pd_2^+$).

An authentic sample of this class of adduct was prepared by the reaction of the nitrate salt of **1a** or **b** (prepared by the reaction of **1** with silver nitrate) and adenine, in essentially quantitative yield.‡ Recrystallization from dichloromethane–methanol gave single crystals of the adenine–(**1b**)$_2$ complex **2** suitable for X-ray diffraction. The results of the structure determination are shown in Fig. 1.§ As seen in a few other bimetallic complexes bridging a free purine,[2] one of the palladium groups is coordinated to N-9, which has been deprotonated. The second palladium is coordinated to N-3. The two palladium atoms are separated by 3.162(3) Å, which

† Calf thymus DNA (38.9 mg) isolated from natural sources was heated with pale-yellow **1a** (26 mg) (2 : 1 nucleotide : complex ratio) in 0.1 nmol dm^{-3} HCl (5 ml) for 3 days at 95 °C. The cloudy orange solution was filtered through a 0.2 micron filter and the filtrate adjusted to pH 7 with NaOH. The resulting orange precipitate was recrystallized from methanol to yield 7.1 mg of **2**. Lower yields of **2**, characterized by FAB MS, were obtained when the reaction was run at 37 ° (and pH 7.4 in a TRIS buffer for 24 h. TRIS = tris(hydroxymethyl)methylamine.

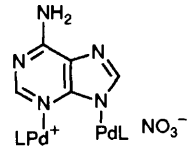
‡ The general procedure for all nucleobase adducts was to react the chloride salt (1 equiv.) in 50% aqueous MeCN with $AgNO_3$ (1 equiv.) under reflux for 2 h and separate the AgCl *via* centrifugation. The yellow filtrate containing the nitrate salt was mixed with a solution of adenine or other nucleobase at 25 °C overnight. Excess NH_4PF_6 precipated the complex, which was washed with H_2O and MeOH.

§ *Crystal data* for **2**·3H_2O: $C_{43}H_{36}N_{10}O_6Pd_2$, M = 1001.6, monoclinic, space group $P2_1/n$, a = 1377.1(4), b = 1773.0(8), c = 1672.8(6) pm, β = 104.32(2)°, U = 3.457(2) nm^3, Z = 4, D_c = 1.68 mg m^{-3} μ = 9.58 cm^{-1}, rectangular crystal 0.12 × 0.12 × 0.24 mm, 5649 measured reflections, 5183 non-equivalent reflections, 2533 reflections retained for refinement [$I > 3.5\sigma(I)$], scan range $3.5 < 2\theta < 45$, R (R_w) = 0.098 (0.091), number of parameters refined 518, final difference map Δ/σ (e $nm^{-3} \times 10^{-2}$), max; min 0.94; −0.31. Data were collected at 22 °C using a Nicolet $R3_m/E$ crystallographic system (Mo-Kα radiation λ = 71.073 pm). The structure was solved using automatic Patterson interpretation to locate the approximate Pd atom positions (SHELXTL/PC v.4.2). A difference map based on these Pd positions was used to locate 25 non-hydrogen astoms. The remaining 36 non-hydrogen positions were located by a series of difference Fourier syntheses. The relatively high values of R and R_w were due to a combination of the disordered nitrate and water molecules in the structure and the weak diffraction of the crystal. The nitrate (N–O bond distance 122 pm, O–N–O bond angle 120°) and water (O–H bond distance, 85 pm, H–O–H bond angle 106°) molecules were refined as rigid groups. Atomic coordinates, bond lengths and bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

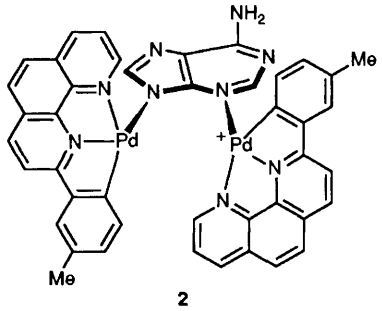


1 (PdLCl)

a; R = $OCH_2CH_2NMe_2$
b; R = Me
c; R = OMe
d; R = $CH(OMe)_2$



2



2

is within the range of metal–metal distances for other N(3)–N(9) bridging adenine complexes reported.[3] The tetracyclic ligands are stacked in a head-to-tail arrangement, with the ligand planes coplanar to within 2°. This coplanarity implies favourable stacking interactions between the cyclometallated ligands. The complex is quite stable to substitution. It can be recovered unchanged from dimethyl sulfoxide solution and an added excess of **1a** will not displace **1b** from **2**. In the crystal, adjacent adenines engage in normal symmetric hydrogen bonding betwen N(7)–N(6) (purine numbering). In addition, a linear spine of three water molecules are present per complex, one in a position to hydrogen bond to N-6, a second able to hydrogen bond to N-1 (purine numbering) and the third interacting with NO_3^-.

In addition to mass spectrometry, which provides a sensitive indication of complex formation, ^{1}H NMR spectroscopy suggested the stacking of two ligand planes from the pronounced upfield shifts of several protons. The chemical shifts (δ) of the protons in the chloride complex **1b** and (**2**) were (using the crystallographic numbering in Fig. 1): 15-H 7.45 (5.84, 5.76), 16-H 6.97 (6.02, 5.93), CH_3 2.30 (1.71), 18-H 7.62 (6.18, 6.20), 11-H 8.30 (7.09, 7.15), 10-H 8.73 (8.20, 8.23), 6-H, 7-H 8.11 (7.94, 7.86), 3-H 8.77 (8.53) 2-H 8.00 (7.58, 7.53), 1-H 8.82 (7.82, 7.67).¶ These assignments were confirmed by a series of 1D and 2D ^{1}H nuclear Overhauser effect experiments (in particular between H_{34} and H_1 and $H_{40adenine}$ and between H_{20} and H_{15} and $H_{40adenine}$), which showed that the structure in solution and in the solid state were similar, if not identical. The adenine protons H_{40ad} and H_{43ad} occur at δ 8.53 and 8.07, respectively in the complex. While H_{40ad} shows a significant downfield shift of 0.4 ppm relative to free adenine, the chemical shift of H_{43ad} is essentially unperturbed.

Complexes of the same stoichiometry (two palladium complexes to one purine) are formed *via* the reaction of **1c** with guanine, purine, xanthine and hypoxanthine, and also

¶ The ^{1}H resonances for **2** (which are in parenthesis) arise from the organometallic ligand complexed to either N-3 or N-9 of adenine.

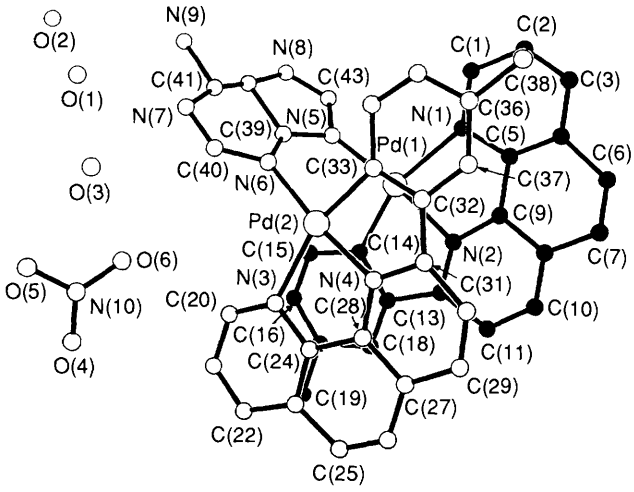


Fig. 1 Atom numbering scheme for the {[(2C′,*N*,*N*)-2-(5′-methylphenyl)-1,10-phenanthroline Pd^{II}]$_2$(μ-N-3, N-9-adenine)} ·[NO_3]·3H_2O complex **2**. Hydrogen atoms have been omitted for clarity.

imidazole, as established by FAB MS. These complexes (except for the imidazole adduct) have analogous ^{1}H NMR spectra with pronounced upfield shifts for several protons. As yet, however, none of these have produced diffraction quality crystals. Purines in which either N-3 or N-9 are alkylated, such as caffeine and 9-ethylguanine, give no evidence by FAB MS or NMR spectroscopy for the formation of analogous bimetallic complexes. Recently, guanidinium complexes coordinated to two $Pt(terpy)^{+2}$ units have been reported;[4] terpy = 2,2′:6′,2″-terpyridyl. In the case of palladium complexes, purines are able to form analogous complexes *via* coordination through N-3 and N-9.

Unexpectedly, the [$Pt(terpy)^+$]$_2$–guanidinium complexes reported by Kostic *et al.*[4] have been reported to bind to DNA, possibly acting as intercalators, even though the aromatic ligands are held 3 Å apart.[5] Because of this result, we looked for evidence of DNA binding by **2**. Viscometric titration[6] of sonicated calf thymus DNA indicated that **2** bound to and lengthened or stiffened DNA since increasing amounts of **2** caused a linear increase in flow times. Either intercalation or groove binding could give rise to this result. Modelling an intercalation event[7] indicates that it is physically possible for a species such as **2**, with heteroaromatic ligands separated by approximately 3 Å to intercalate between a DNA base pair, as long as each of the backbone torsion angles in the polymer exist in their most extended range.

Complexes **1a–d** produce nicks and abasic lesions in plasmid DNA at pH 7.4 without the need for additional reagents.[1] The structure of **2** suggests a mechanism by which this can occur. First, alkylation at N-3 of purine nucleosides strongly destabilizes the glycosidic bond. 3-Methyl-2′-deoxyguanosine, for example,[8] undergoes spontaneous hydrolysis of the glycosidic bond at pH 7, 37 °C. Second, coordination complexes with aromatic ligands [such as $Ru(phen)_3^{2+}$ (phen = 1,10-phenanthroline)] bind in the DNA minor groove.[9] The width of the DNA minor groove provides a cavity which accommodates such hydrophobic residues. Thus, even though N-7 is the favoured site for coordination of simple late transition metal ions, the phen ligand would direct the complex to the minor groove, where coordination to N-3 could take place. Since palladium is a kinetically labile metal, it may also reversibly bind to N-7, but need not remain bound to that site. Subsequent depurination of the N-3 complex, by analogy with the effect of alkylation at N-3 and addition of a second complex to N-9 (guided by stacking interactions) would produce **2**.

We thank the NIH for support *via* grant GM-39614.

Received, 7th September 1992; Com. 2/04805A

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