Insight into the Preferential Intrastrand Binding to Adjacent Guanine Bases in DNA by Platinum(II)

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Phosphorus-31 two dimensional exchange spectroscopy (^{31}P 2D-EXSY) and line width analysis reveal that *cis*-$[Pt(NH_3)_2(GMP)_2]$ in the head-to-head conformation lies about 50 kJ mol^{-1} below the energy of *cis*-$[Pt(NH_3)_2(AMP)_2]$ complex in the same conformation.

It is generally believed that the antitumour activity of *cis*-diamminedichloroplatinum(II) is due to an intrastrand binding to DNA predominantly through the two adjacent guanine bases.[1] An intrastrand binding by two adjacent purine rings gives rise to a head to head (HTH) configuration.[2,3] Although the preference for guanine over adenine is not clearly understood, much higher energy requirements for adenine in the HTH orientation as compared to the guanine adduct may be responsible for the binding of guanine bases.[4] The energy difference between the bis(GMP) and bis(AMP) complexes† in the HTH configuration might aid us to understand why *cis*-DDP preferentially binds to adjacent guanine bases. In this communication, we report rotational energy barriers in the Pt–N(7) bonds from 2D NMR spectroscopy and line shape analysis, and compare the energetics of GMP and AMP complexes in HTH conformations. To the best of our knowledge,‡ this is the first report that analyses the energetics of AMP and GMP complexes of *cis*-DDP and utilizes ^{31}P 2D-EXSY to evaluate quantitatively the activation energy associated with the rotations about Pt–N(7) purine bonds.

Slow rotations about platinum–N(7) purine bonds in bis(purine) platinum complexes give rise to two major conformations:[5–7] the two six-membered rings are on the same side (head to head, HTH) or on the opposite sides (head to tail, HTT) of the square plane. Rotamers in several platinum complexes containing bulky amine ligands are observed due to restricted rotations about the Pt–N(7) (oxopurine) bonds.[5–7] The two rotamers[6] in $[Pt(NH_3)_2(AMP)_2]$ and $[Pt(NH_3)_2(dAMP)_2]$ were observed owing to slow rotation about Pt–N(7) bonds and are diastereoisomers in HTT configuration owing to the lack of symmetry in these

† *Abbreviation used:* *cis*-DDP, *cis*-diamminedichloroplatinum(II); AMP, adenosine 5′-monophosphate; GMP, guanosine 5′-monophosphate.

‡ Earlier reports from various laboratories include molecular mechanics calculation[9] of purine nucleosides, characterization of rotamers utilizing bulky amine ligands,[5–7] and assessment of rotational energy for some of these complexes.

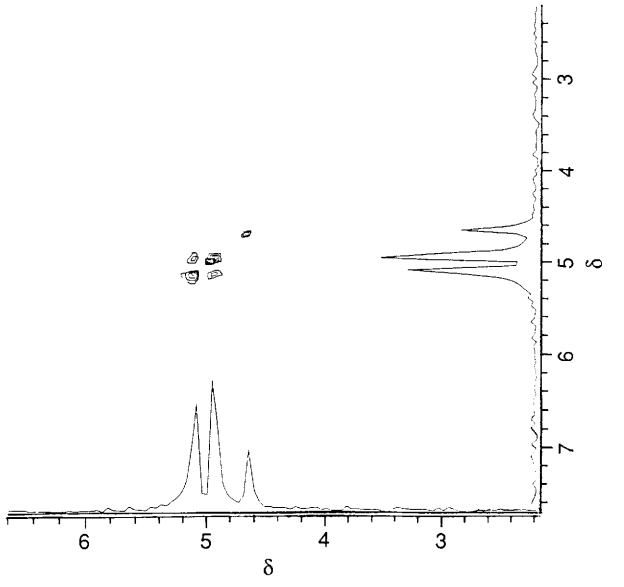

Fig. 1 126.5 MHz ^{31}P 2D-EXSY contours of *cis*-[Pt(NH$_3$)$_2$(AMP)$_2$] complexes in the presence of AMP. The signal at δ 4.65 is for the free AMP and the two other downfield resonances are for the two diastereoisomers. The presence of NOE connectivity between these two isomers is evident in the 2D-contour.

molecules. Fig. 1 shows the ^{31}P 2D-EXSY contours§ of *cis*-DDP (3.0 mmol dm^{-3}) and AMP (8.0 mmol dm^{-3}) mixed in D_2O at 25 °C and pH 5.3. The two downfield ^{31}P signals at δ 5.08 and 4.94 are for the *cis*-[Pt(NH$_3$)$_2$(AMP)$_2$] products and the peak at δ 4.65 for the unreacted nucleotide. The existent of cross peaks in these contours establishes the exchange processes between the two rotamers. In these 2D-EXSY experiments, free AMP was kept intentionally in order to demonstrate that there are no cross peaks between the unreacted AMP and the products. Both ^{1}H and ^{31}P signals broaden significantly with increasing temperature initially and then coalesce at 80 °C. The rates of exchange of these rotamers obtained from the line shape analysis are in excellent agreement with those obtained from 2D-EXSY experiments. The rate data at various temperatures afforded an activation energy of 69 ± 3 kJ mol^{-1}.

Only one H-8 resonance was observed for *cis*-[Pt(NH$_3$)$_2$(GMP)$_2$] complex in the temperature¶ range −15 to 40 °C. Bis-GMP complexes with small amine ligands do not exhibit such rotamers at room temperature owing to rapid rotations about Pt–N(7) bonds.[5,7,8] However, the line widths at the lower temperatures were significantly broadened. Based on line shape analysis, the activation energy can be estimated to be 25 ± 5 kJ mol^{-1}. This activation energy lies within the range of values predicted from the molecular mechanics calculation for *cis*-[Pt(NH$_3$)$_2$(guanosine)$_2$] and other guanine complexes.[9]

The NOE enhancements in the ^{31}P 2D spectra and the line broadening in the 1D spectra are attributed to the conversion of one HTT isomer into the other. To convert one HTT rotamer into the other HTT through the rotation about the Pt–N(7) bonds, a HTH configuration must be encounted. The latter configuration must lie at the highest potential energy surface along the reaction coordinate. Since these activation energies represent the highest potential energy surface along the reaction coordinate during the exchange process, these energies then represent the difference in ground state energies between HTH and HTT rotamers.

Reasons for an increased rotational energy barrier, about 50 kJ mol^{-1}, for the AMP complexes compared to that of GMP complexes should be addressed. This additional rotational energy barrier in the aminopurine system cannot be of purely steric origin since both the oxopurine and aminopurine nucleotides are of about the same size. Further, the hydrogen bonding between the phosphate group and hydrogen atoms of coordinated amine must be present in both cases. This additional energy barrier in the AMP-complexes must originate from the specific interactions between the platinum and exocyclic amine group. Hambley[9] in his molecular mechanics calculation stressed the importance of hydrogen bonding between the exocyclic amine and coordinated amine ligand. In addition, a 'pseudo-octahedral' environment positioning two NH_2 groups for two AMP molecules above and below the platinum plane may establish a weak interaction with the platinum d_{z^2} orbital. Although a weak interaction may also exist in the oxopurine complexes with two exocyclic oxygen atoms, the magnitude of such interaction must be substantially less than in the aminopurine complexes.

An intrastrand binding by two adjacent guanine bases in the DNA to *cis*-DDP is expected to result in a HTH conformation. Indeed, X-ray crystallographic data from Sherman *et al.*[2] and Admiraal *et al.*[3] for short oligonucleotide complexes of *cis*-DDP establish such an HTH configuration. Results presented here support the view that intrastrand binding through two adjacent adenine bases would be unfavourable by about 50 kJ mol^{-1}. Finally, the 2D ^{31}P EXSY can be used to evaluate rotational energy barriers for multisite exchanges in GMP complexes with bulky amine ligands.[5–7]

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§ Phase sensitive 2D-EXSY NMR experiments were carried out on a 300 MHz instrument (GN 300) utilizing the conventional pulse sequence: $\pi/2 - t_1 - \pi/2 - \tau_m - \pi/2 - A_t$, where t_1, τ_m and A_t are the evolution, mixing and acquisition times, respectively. The selected mixing times 700–900 ms, lie in the range 0.5 T_1 to 1.5 T_1 and the rate constants were independent of mixing times. The longitudinal relaxation times of the two diastereoisomers are evaluated to be 1.13 and 1.17 s. The rate constants at 15, 23, 30, 37 and 47 °C are 0.15, 0.30, 0.46, 0.72 and 2.66 s^{-1}, respectively.

¶ Temperatures below 0 °C in aqueous solutions were achieved by adding $NaNO_3$.

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