Multiple metal binding to 6-oxopurine nucleobases as a source of deprotonation. The role of metal ions at N7 and N3†

Marta Morell Cerdà,a David Amantia,b Burkhard Costisella,a Andrew Houlton*b and Bernhard Lippert*a

Received 10th March 2006, Accepted 25th May 2006
First published as an Advance Article on the web 26th June 2006
DOI: 10.1039/b603650c

Simultaneous metal coordination to N7 (PtII) and N3 (PdII) of N9-blocked guanine leads to a 104 fold acidification of the guanine-N(1)H position and hence to a virtual complete deprotonation of the N(1)H position at neutral pH. The chelate-tethered nucleobase ethylenediamine-N9-ethylguanine was employed and relevant acid–base equilibria were studied by pD dependent 1H NMR spectroscopy. CH2 resonances of the tether were assigned on the basis of NOESY and COSY experiments. Our findings suggest a plausible method of formation of a previously reported trinuclear PtII complex of 9-ethylguanine with metals coordinated to N1, N3 and N7. According to this, a sequence with the first metal binding to N7, the second one binding to N3, and only the third one binding to N1 with deprotonation of this site is proposed.

Introduction

The N7 position of guanine is a kinetically preferred binding site for many metal ions M in nucleic acids at neutral and moderately acidic pH. At higher pH, the N1 position becomes the thermodynamically favoured metal binding site, causing a “crossover” of M to this site following its deprotonation (pKwa ≥ 9.5). Mechanistic details of this process are not very well understood. Both direct attack of a M–OH entity on N(1)H or indirect ways of N(1)H deprotonation under the influence of the acidifying effects of metal ions coordinated to other sites (e.g. N7, N3, O6) are feasible. Indeed, metal coordination to N1 of 6-oxopurine bases in conjunction with N7, N3,N7,4,5 and O6,N7 metalation6,7 are established, and none of these binding patterns requires excessively high pH. Here we present strong evidence that simultaneous metal binding to N7 and N3 of a guanine model nucleobase potentiates the well understood effect of a PtII at N7 on the acidity of the proton at N1 and in fact leads to an essentially complete deprotonation of this nucleobase at neutral pH. In principle, this then allows for metal binding at other sites, preferably at N1.

In order to direct a second metal ion selectively to the N3 position of a guanine nucleobase, advantage was taken of so-called chelate-tethered nucleobases, which have been demonstrated to achieve this goal in a number of cases. In these compounds, an ethylenediamine9 or a dithioether10 moiety is linked via an ethyl or propyl tether to the N9 position of the purine base, thus enabling facile coordination of a metal ion to N3.

This study adds to our understanding of processes leading to nucleobase deprotonation as a consequence of metal binding and their possible consequences for hydrogen bonding, base pairing, as well as nucleobase tautomerism. Moreover, it is relevant to the ongoing discussion on “shifted nucleobase pKw values”, according to which proton transfer processes are responsible for catalytic reactions occurring in ribozymes.11

Experimental

Syntheses

The ligand ethylenediamine-N9-ethylguanine was prepared as its hydrochloride salt (G-Et-en·HCl) as previously reported.10b [(dien)Pt(G-Et-en·H-)]1+ was prepared in D2O solution, yet not isolated, by mixing [(dien)PtI]I and G-Et-en·HCl in a 1 : 1 ratio in D2O, and by adding three equiv. of AgNO3. After stirring the mixture at room temperature with daylight excluded for 20 h, the precipitate of AgI and AgCl was centrifuged off, and the 1H NMR spectrum of the sample (pD 7.8) was recorded. The mixed Pt,Pd complex was obtained by adding one equiv. of K2PdCl4 to the solution of [(dien)Pt(G-Et-enH-N-)]1+.

NMR studies

1H NMR spectra were recorded on Varian Mercury 200 and Varian INOVA 600 instruments in D2O using sodium 3-(trimethylsilyl)propanesulfonate (TSP) as internal reference. 1D NOE, 1H,1H COSY and 13C,1H COSY spectra in D2O were recorded on a Varian INOVA 600 instrument.

The pD values were obtained by adding 0.4 to the pH meter reading.15 Changes in chemical shifts of all non-exchangeable protons in the complexes due to the change in pH were evaluated with a nonlinear least-squares fit after Newton–Gauss.16 The pKw values in H2O were calculated from the pKw* values in D2O according to pKw* = 1.015pKw + 0.45.17

See DOI: 10.1039/b603650c
Results and discussion

NMR characterization of G-Et-en

The $^1$H NMR spectrum of ethylenediamine-$N^\text{9}$-ethylguanine (G-Et-en) in D$_2$O consists of a singlet in the lowfield region due to H8 of the guanine as well as four triplets in the upfield region assigned to the four CH$_2$ groups of the tether as well as the ethylenediamine moiety. Fig. 1 gives a typical $^1$H NMR spectrum at pD = 9.2. Depending on the field strength applied and on the pD chosen, triplets of the CH$_2$ resonances can strongly overlap. The assignment of the four CH$_2$ triplets was achieved by a combination of different NMR experiments. Thus, assignment of the C(10)H$_2$ and C(11)H$_2$ resonances was based on a 1D NOE experiment following irradiation of the H8 resonance, which caused a strong positive signal for C(10)H$_2$ and a weak negative signal for C(11)H$_2$. As confirmed in a $^1$H,$^1$H COSY experiment (cf. ESI†), both resonances show cross-peaks, as is also the case with the remaining two signal sets of C(13)H$_2$ and C(14)H$_2$, which cannot be differentiated in this experiment, however. The $^{13}$C NMR resonances of the four methylene groups were determined in a $^{13}$C,$^1$H COSY experiment and assigned on the basis of the results obtained in a $^{13}$C,$^1$H Long Range Correlation Spectrum. According to it, the C(10)H$_2$ signal correlates with C11 (47.7 ppm) as well as the C8 (140.3 ppm) and C4 (152.0 ppm) resonances of the guanine ring, the chemical shifts of which were taken from the literature.$^{18}$ The C(11)H$_2$ resonance correlates with C10 (43.4 ppm) and C13 (45.7 ppm), C(13)H$_2$ with both C11 (47.7 ppm) and C14 (38.9 ppm), whereas C(14)H$_2$ correlates with C15 (45.7 ppm) only (cf. ESI†). The assignment of CH$_2$ resonances used in Fig. 1 and 2 was based on this analysis.

The G-Et-en molecule can be involved in a series of acid–base equilibria as outlined in Fig. 2. Consequently the chemical shifts of the proton resonances are pD dependent (Fig. 3).

The various protons display different pD sensitivities, depending on how close the site of protonation/deprotonation is. The H8 resonance of the guanine base (Fig. 3(a)) was used to determine equilibria involving protonation of G at N7 in acidic medium ($pK_a$ H$_2$O $\simeq$ 1.85(3)) and deprotonation of N(1)H in alkaline solution ($pK_a$ H$_2$O $\simeq$ 9.53(2)). The C(11)H$_2$ and C(10)H$_2$ triplets of the tether, on the other hand, undergo substantial shifts between pD 4 and 7, which amount to 0.6 and 0.3 ppm, respectively (Fig. 3(b)). These changes indicate protonation/deprotonation of the N12 amino group ($pK_a$ H$_2$O $\simeq$ 5.22(3)). Both methylene resonances reflect also guanine deprotonation in strongly acidic medium, but the sensitivity is reversed, as expected. In strongly basic solution these two triplets likewise are slightly pD dependent. It is not immediately clear, if this feature is due to guanine deprotonation ($pK_a$ H$_2$O $\simeq$ 9.53(2), see above) or due to the acid/base equilibrium involving the terminal amino group N15. Unfortunately the use of C(13)H$_2$ and C(14)H$_2$ is restricted due to severe overlap of resonances at neutral and acidic pH. However, in basic medium (pD > 7) the upfield shift of these resonances is pronounced ($\Delta$ $\simeq$ 0.2 ppm), suggesting indeed deprotonation of the terminal N(15)H$_3^+$ group with a $pK_a$, calculated for C(14)H$_2$ in H$_2$O, of ca. 9.60(7) and 9.4(2) for C(13)H$_2$. We therefore propose that deprotonation of the fully protonated cation of G-Et-en occurs in the sequence N7, N12, N1 $\simeq$ N15, with overlap between the last two steps.
Characterization of [(dien)Pt(G-Et-en)]<sup>+</sup> 1

Binding of (dien)Pt<sup>II</sup> to the N7 position of the guanine nucleobase in G-Et-en is evident from <sup>195</sup>Pt coupling with H8, which gives rise to typical <sup>195</sup>Pt satellites with \( \frac{\Delta J}{\Delta \nu} \approx 20 \) Hz. These satellites are, however, only seen if spectra are recorded at low magnetic field (e.g. 200 MHz). The assignment of the four CH<sub>2</sub> triplets of the Et-en moiety and the differentiation from the dien resonances is accomplished by <sup>1</sup>H NMR spectra recorded at 600 MHz.

The assignment of the C(10)H<sub>2</sub> and C(11)H<sub>2</sub> resonances follows the same pattern as in G-Et-en, as confirmed by a 1D NOE experiment. Thus, irradiation of the H8 resonance causes a strong positive signal for C(10)H<sub>2</sub> and a weak positive signal for C(11)H<sub>2</sub>. As confirmed in a <sup>1</sup>H<sup>1</sup>H COSY experiment, both resonances show cross-peaks. C(13)H<sub>2</sub>, C(14)H<sub>2</sub>, and the <sup>13</sup>C resonances were assigned, as in the case of G-Et-en, with help of a <sup>13</sup>C<sup>1</sup>H COSY and a <sup>13</sup>C<sup>1</sup>H Long Range Correlation Spectrum. All the resonances follow the same pattern as in the G-Et-en, except that C(13)H<sub>2</sub> and C(14)H<sub>2</sub> change position (ESI†).

The upfield region of the <sup>1</sup>H NMR spectrum of 1 (D<sub>2</sub>O, pD 5) is shown in Fig. 4.

The pD dependence of the <sup>1</sup>H NMR spectra of the Pt compound 1 in the pD range 3–11 (Fig. 5) reveals at least two acid–base equilibria with pK<sub>a</sub> values of 5.41(8) (D<sub>2</sub>O, average from C(11)H<sub>2</sub> and C(13)H<sub>2</sub>), corresponding to 4.9(1) in H<sub>2</sub>O, as well as 8.45(9) (D<sub>2</sub>O), corresponding to 7.9(1) in H<sub>2</sub>O. Since the C(11)H<sub>2</sub> triplet is most sensitive to pD in the range 4–7 (Δδ 0.42 ppm), we assign this pK<sub>a</sub> to the preferential protonation/deprotonation of the amine group N(12).

On the other hand, H8 of the guanine nucleobase is most sensitive in the pD range 7–9, consistent with base deprotonation at the N1 site. The pK<sub>a</sub> value of the platinated guanine base is well within the range generally found, and the magnitude of chemical shift difference of H8 between neutral and deprotonated state (Δδ ca. 0.23 ppm) is likewise as expected.

Binding of a second metal (Pd<sup>II</sup>) to N3

Addition of one equivalent of K<sub>2</sub>PdCl<sub>4</sub> to an aqueous solution of 1 produces the dinuclear complex [(dien)Pt(N<sup>7</sup>-G-Et-en-N<sup>3</sup>)PdCl]<sup>3+</sup> or the corresponding aqua species, respectively. This process is accompanied by a drop in pD (from 7.8 to 2). Pd<sup>II</sup> binding to N3 of the guanine base is inferred from the highly diagnostic downfield shift of the C(10)H<sub>2</sub> resonance of 1 and the separation of the original triplet into two triplets of 1 : 1 intensity, now around 6.1 and 4.9 ppm (pD 2.1), with the latter superimposed with residual water. The H8 resonance of the guanine base gives rise to a sharp singlet at around 8.32 ppm (Fig. 6).

Upon addition of NaOD the H8 resonance undergoes an upfield shift, consistent with guanine deprotonation (Fig. 7). The pK<sub>a</sub> value can only be estimated because the deprotonation process is accompanied by a loss of Pd<sup>II</sup> from the N3 position. We conclude this from the gradual broadening and eventual disappearance of the strongly shifted C(10)H<sub>2</sub> signal at 6.1 ppm (pD 2.1) and the concomitant appearance of new guanine H8 resonances as the pD of the solution approaches 7. From the δ/pD plot a pK<sub>a</sub> of ca. 6 can be estimated for D<sub>2</sub>O solution, corresponding to ca. 5.5 in H<sub>2</sub>O. The upfield shift of the H8 resonance of 2 between
Fig. 6 1H NMR spectrum (D₂O, pD 2.1) of 2. The second half of the C(10)H₂ signal is partly overlapping with the solvent signal at 4.9 ppm.

Fig. 7 pD dependence of H8 resonance of guanine of [(dien)Pt(N⁷-G-Et-en-N³)PdCl]⁺⁺ 2 or of the corresponding aqua species. Above pD 6 multiple new H8 resonances are formed and the C(10)H₂ signal is lost.

pD ~ 2 ~ 6 (which corresponds to 50% deprotonation) is ca. 0.1 ppm. If extrapolated to full deprotonation, a total upfield shift of ca. 0.2 ppm for this resonance is to be expected. The magnitude of this shift lends strong support to the view that it is indeed guanine deprotonation which is detected by 1H NMR spectroscopy (Fig. 8). Although deprotonation of a hydrolysed Pd–Cl group, hence of Pd(OH₂) occurs in a similar range, such a process is unlikely to have such a profound effect on guanine H8. Our attempt to also apply pH dependent UV spectroscopy to answer this question was ambiguous in that isosbestic behaviour was not observed. This finding is consistent with the formation of multiple new H8 resonances in the 1H NMR spectra. The fate of the Pd¹І ion is unclear. Migration to another site, e.g. N1 or even to the exocyclic amino group N2 (with loss a proton) seems possible.

Possible relevance to the formation of threefold platinated guanine

The present study was motivated by our wish to better understand the formation of a trinuclear Pt¹І complex, {[(NH₃)₃Pt]₂(G-N⁷,N³,N¹)PdCl]⁺⁺ (G = 9-ethylguanine anion), which we had prepared before and which displays Pt¹І binding to three different ring nitrogen atom of the nucleobase. This compound had been synthesized by reacting the mononuclear complex [(NH₃)₂Pt(H₂O)]⁺⁺ with an excess of [(NH₃)₃Pt(HG-N⁷)]⁺⁺ and was isolated from slightly acidic aqueous solution. Since a N1,N7 bridged species was obtained as well, this suggested a sequence of formation N7 → N7,N1 → N7,N1,N3 (Fig. 9, route I). As binding of a single Pt¹І to N7 of guanine causes only a moderate acidification of the N(1)H (ca. 1.5–2 log units; pKₐ ≃ 8,19), formation of the dinuclear species would have required essentially a direct substitution of the proton by a Pt¹І moiety.

Our findings reported here and previously¹⁰ suggest that there may be alternative pathways leading to the trinuclear complex (routes II and III in Fig. 9), which involve the sequence of metal coordination N7 → N7,N3 → N7,N3,N1. Indeed, a cyclic Pd¹І-based guanine quartet with Pd¹І coordination via N7 and N3 has been obtained with a related ethylenediamine-N⁷-propylguanine (G-Prop-en) ligand and structurally characterized by some of us.¹⁰ In this complex, which was isolated from aqueous acetonitrile solution, the N1 position of the guanine is still protonated. It is

Fig. 8 Proposed acid–base equilibrium of mixed Pt¹І,Pd¹І complex 2.
obvious that removal of this proton will generate an attractive site for additional metal binding (route II). The extent of acidification, ca. 4 log units in \( K_{a} \) is sufficiently high to permit metal coordination even in weakly acidic solution.

Isolation of N7,N1 dimetalated guanine species\(^1\) does not necessarily rule against route II. It appears possible that following N1 deprotonation, a linkage isomerization of the initial N7,N3 product to a thermodynamically more stable N7,N1 product could take place. With an excess of metal, a third metal might add to position N3. The complexity of the \( ^{1}H \) NMR spectra of compound 2 at \( pD \geq 6 \) does not permit an unambiguous answer to such a possibility, however.

**Potential relevance of shifted \( pK_{a} \)**

There is yet another aspect of this work worth while discussing, viz. the extent of nucleobase acidification under the influence of two coordinated metal ions. While the normal \( pK_{a} \) values of guanine are well outside the physiological pH range (N7 protonated guanine has \( pK_{a} \approx 3.1 \); neutral guanine deprotonates at N1 with \( pK_{a} \approx 9.6 \), here the second \( pK_{a} \) has shifted to \( \approx 5.5 \). Reasons of shifted nucleobase \( pK_{a} \) values are manifold (chemical modification; microenvironment; specific factors stabilizing protonated or deprotonated forms of nucleobases\(^{12,21} \)), as are potential biological consequences (changes in hydrogen bonding patterns; acid–base catalysis\(^{15,23} \)). As we have discussed in more detail elsewhere,\(^24 \) metal ions coordinated to nucleobases can dramatically shift \( pK_{a} \) values, even from ca. 17 (NH\(_2\) groups of cytosine and adenine) down to 7 and beyond, depending on the site of metal binding and the number of metals attached to the nucleobases. What it implies is that metal ions, once coordinated to a nucleobase, can provide H\(^+\) from this nucleobase even at physiological pH. At least with RNA, this opens the possibility for acid–base catalysis, with the role of the metal ion being then to generate protons, rather than being directly involved in the catalytic process.

**Summary**

With the present study we continue our work on model nucleobase displaying multiple metal binding patterns, and reasons for and consequences of their formation. The here described system, one metal at N7 of guanine, and one at N3, can be considered a model for a nucleobase in a nucleic acid duplex, which carries simultaneous metal ions at a site located in the major groove (N7) and the minor groove (N3). The idea that metal binding to a site carrying a proton, e.g. N1H of guanine, is facilitated by initial binding of this metal to a less basic and consequently unprotonated site, is attractive in that it could rationalize a number of experimental findings on multinuclear metal–nucleobase complexes, containing anionic nucleobases, both purines and pyrimidines.\(^25 \) Apart from unprotonated ring nitrogen sites, also exocyclic oxygen atoms could function as anchors for metal ions, which through their polarizing effects, could facilitate nucleobase deprotonation.

**Acknowledgements**

Support by the Deutsche Forschungsgemeinschaft and the EPSRC for an Advanced Research Fellowship (A. H.) and research funding. COST D20 is thanked for a Short Term Scientific Mission to D. A.

**References**