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Metal Coordination and Imine−Amine Hydrogen Bonding as the Source of Strongly Shifted Adenine pK_a Values

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Abstract: The X-ray crystal structure of a Pt^{II} complex of composition *trans*-[$(NH_3)_2$Pt(1,9-DimeA) (1,9-DimeAH)]$(ClO_4)_3$ (**2**) with 1,9-DimeA = 1,9-dimethyladenine and 1,9-DimeAH^+ = 1,9-dimethyladeninium) is presented. Complex **2** forms upon deprotonation of one of the exocyclic amino groups of the adeninium ligands in *trans*-[$(NH_3)_2$Pt(1,9-DimeAH)$_2$]$(ClO_4)_4$ (**1**), where the two nucleobases are in a head−tail arrangement. The low pK_{a1} of **1** (4.1 ± 0.2) is a consequence of a combination of the effects of metal coordination to N7 of the purine base and efficient stabilization of the deprotonated species. This feature is supported by the results of the structure determination of **2**, which displays a head−head orientation of the two bases and intramolecular H-bonding between the imine group of 1,9-DimeA and the amino group of 1,9-DimeAH. In the fully deprotonated species *trans*-[$(NH_3)_2$Pt(1,9-DimeA)$_2$]$(ClO_4)_2$ (**3**), the two nucleobases are again in a head−tail arrangement. The findings are of relevance with regard to the concept of "shifted pK_a values" of nucleobases. This concept is applied to rationalize acid−base catalysis reactions involving nucleobases of DNA and RNA which occur in the near-physiological pH range.

Introduction

In general, pK_a values of DNA nucleobases are in the range pK_a < 4 or pK_a > 9, rendering the heterocyclic entities neutral at physiological pH and permitting Watson−Crick pairing between the neutral forms of the complementary bases. However, special structural features such as the "i-motif" of hemiprotonated C,[1] CH^+GC triplet structures,[2] or pairs involving protonated bases in DNA duplexes[3] and RNA ribozymes[4] can lead to a situation where nucleobase pK_a values are shifted into the near-neutral pH range. Extensive hydrogen bonding can stabilize even deprotonated nucleobases, as is observed for the uracil anion during excision from DNA by a glycosylase,[5] for example. The topic of "shifted pK_a values" has lately attracted considerable attention in the context of general acid−base catalysis involving nucleic acids because it can rationalize the existence of nucleobases of differing protonation states at physiological pH. As a matter of fact, protein synthesis in the ribosomes is catalyzed by an adenine of rRNA having a p$K_a \approx$ 7.6,[6,7] and self-cleavage of the Hepatitis Delta Virus requires the presence of a protonated cytosine acting as an acid during catalysis.[8,9]

Here, we demonstrate a principle which leads to a large pK_a shift of a weakly acidic NH nucleobase proton, in this case of the exocyclic amino group of an adenine nucleobase, into the near-neutral range. The acidification is brought about by a synergy of metal coordination and efficient stabilization of the deprotonated species by hydrogen bonding which involves the NH_2 group of a second nucleobase as the H-donating group.

Results and Discussion

Our earlier observation[10] of an unusually low pK_a of 7.9 for the exocyclic amino proton of 9-methyladenine (9-MeA) in a triplatinum(II) tetrakis (nucleobase) complex and an extended study on related complexes[11] have led us to postulate that an extra stabilization of the resulting adenine anion by a suitably positioned amino group of a second nucleobase (adenine, cytosine) is required to account for the low pK_a (Scheme 1). The effect of the two Pt^{II} electrophiles as such causes an acidification of N(6)H_2 of ca. 6 log units at most and is therefore insufficient to rationalize the experimental data of a 10^9 acidification. By studying the acid−base properties of a mononuclear Pt^{II} complex of 1,9-dimethyladenine (1,9-DimeA), we have now been able to further confirm this view and at the same time to provide X-ray structural evidence in support of it. 1,9-DimeA is a model of the corresponding nucleoside, which occurs in its protonated form (1,9-DimeAH^+) as a rare base in tRNAs[12] and occasionally

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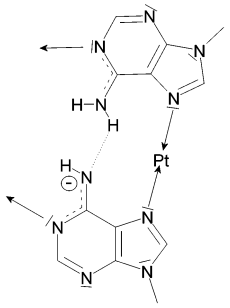
Scheme 1



in rRNAs.[13] The pK_a of the N(6)H_2 group of the cation is ca. 9.1[14] and is expected to decrease upon metal coordination at N7. We are aware that there is a difference between deprotonation of the cationic 1,9-dimethyladeninium cations in the here described complexes and deprotonation of the neutral ligand 9-methyladenine. However, this difference is a formal one only, as both the methyl group at N1 and the metal binding to this site are causing an acidification of the exocyclic NH_2 group. The effect is only larger in the case of methylation (ΔpK_a = 16.7 − 9.1 = 7.6) as compared to platination ($\Delta pK_a \approx 4$[11]), thus leading to a shift of qualitatively identical processes from strongly alkaline medium (Pt(9-MeA-*N1*), pK_a = 12−13) to less alkaline medium (1,9-DimeAH^+, pK_a = 9.1). In both instances, binding of (additional) Pt^{II} at N7 reduces these pK_a values further, by approximately identical 3 log units, and by ca. 5 log units in cases of favorable interbase hydrogen bonding such as that realized in **2**. Moreover, the steric situations (possibility of formation of intramolecular hydrogen bond) in both cases are closely similar.

Three forms of the bis(1,9-DimeA) complex of *trans*-$(NH_3)_2$-Pt^{II} containing Pt^{II} bonded to N7 with different protonation states of the adenine ligands have been prepared and characterized by X-ray analysis: *trans*-[$(NH_3)_2$Pt(1,9-DimeAH)$_2$]$(ClO_4)_4$·$4H_2O$ (**1**), *trans*-[$(NH_3)_2$Pt(1,9-DimeA)(1,9-DimeAH)]$(ClO_4)_3$·$1.5H_2O$ (**2**), and *trans*-[$(NH_3)_2$Pt(1,9-DimeA)$_2$]$(ClO_4)_2$·H_2O (**3**).[15] The pK_a values, as determined by pD-dependent ^{1}H NMR spectroscopy and converted to H_2O,[16] were found to be 4.1 ± 0.2 for **1** ⇄ **2** + H^+, hence for deprotonation of the first 1,9-dimethyladeninium ligand in **1**, and 6.4 ± 0.3 for **2** ⇄ **3** + H^+, hence for deprotonation of the remaining 1,9-dimethyladeninium ligand in **2** (Figure 1). While the latter value is in the expected range (cf. pK_a = 6.5 for *cis*-[Cl_2Pt(1,9-DimeAH)(NH_3)]$^+$),[17] the former reflects an additional increase in acidity which we attribute primarily to a favorable stabilization of 1,9-DimeA in **2**.[18] The two aromatic protons of the adenine ligands in **1**−**3** were differentiated and assigned by the observation of ^3J-coupling between the N7-bound Pt and H8. Both resonances undergo substantial broadening in the pD range in which the two deprotonation/protonation processes take place (Figure 2), suggesting a dynamic equilibrium of some kind, either a ligand

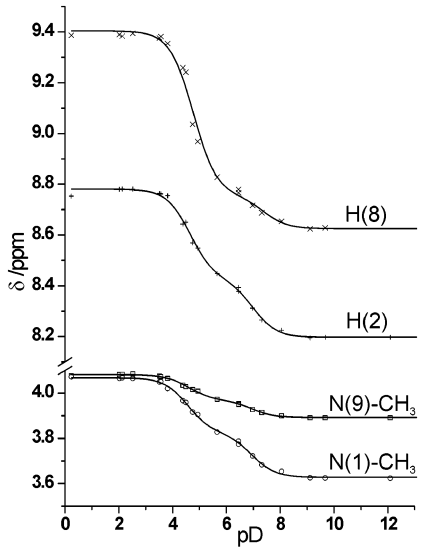


Figure 1. The pD dependence of the chemical shifts (δ) of the aromatic H2 and H8 as well as the methyl protons of the 1,9-dimethyladenine ligands in **1**, **2**, and **3**.

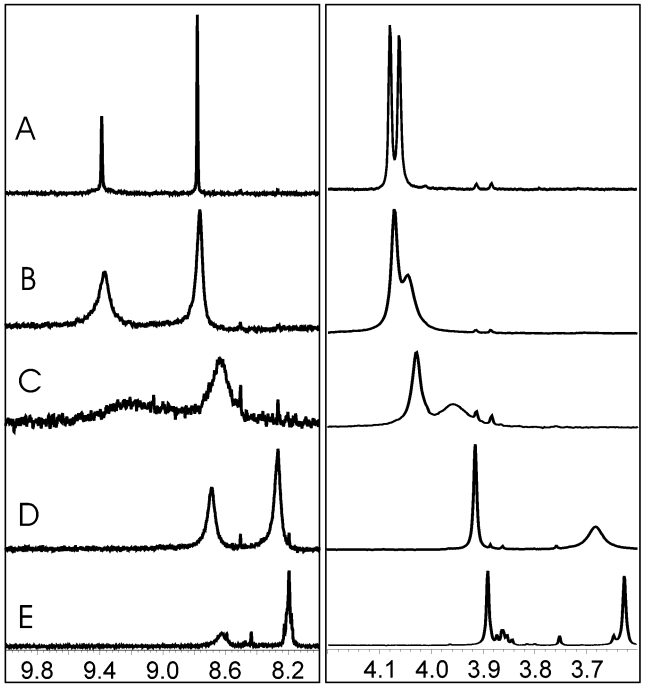


Figure 2. ^{1}H NMR resonances of H2 and H8 as well as the methyl protons of the 1,9-dimethyladenine ligands in their various protonated states (D_2O, 23 °C). pD values are A, 2.0; B, 3.5; C, 4.5; D, 7.3; E, 9.1. Minor peaks are due to an impurity and the formation of minor new species.

rotation (ht ⇄ hh) and/or proton exchange between the exocyclic amino group and its deprotonated imino form. Interestingly, it is the H8 resonance, on one hand, and the N(1)CH_3 resonance, on the other, which are broadened most. This fact makes it difficult to differentiate between the two scenarios.[19] However, the results of the X-ray structure determinations strongly suggest that nucleobase rotation contributes to signal broadening in the NMR spectra: Whereas in **1** and **3** the adenine bases are arranged head−tail (Figure 3), in **2** the bases adopt a head−head orientation which permits intramolecular H-bonding

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(18) We are aware that the charge of the compound might also have an influence on the pK_a value; see, however, ref 11.
(19) At elevated temperature, secondary reactions occur rapidly, which appear to involve, among others, Pt^{II} migration. Cf. also ref 11.

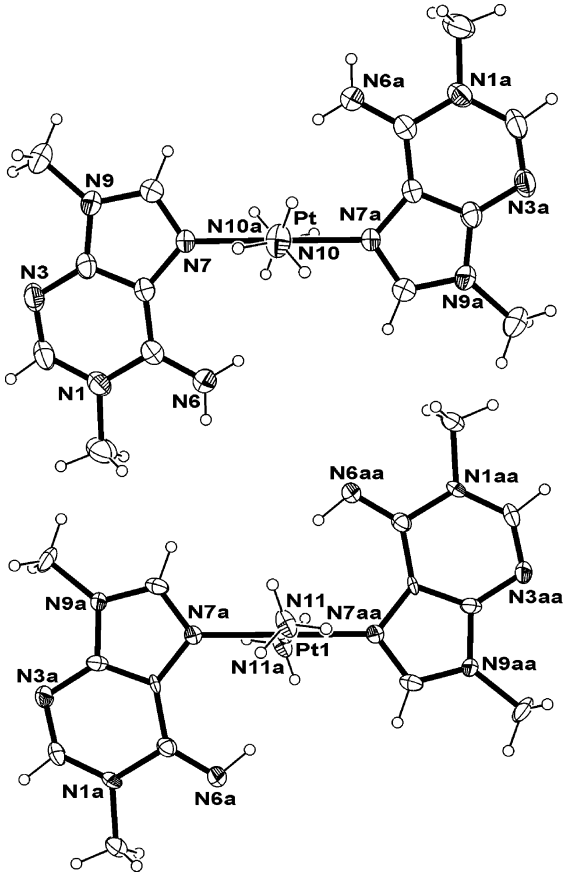

Figure 3. Cations of *trans*-$[(NH_3)_2Pt(1,9\text{-DimeAH})_2](ClO_4)_4\cdot 4H_2O$ (**1**) (top) and of *trans*-$[(NH_3)_2Pt(1,9\text{-DimeA})_2](ClO_4)_2\cdot H_2O$ (**3**) (bottom). In both cases, the nucleobases are arranged head−tail.

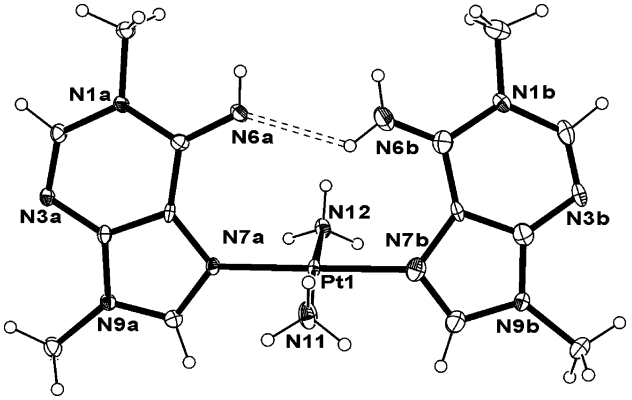

Figure 4. View of one (I) of the two crystallographically independent cations of **2**. The nucleobases are oriented head−head to permit intrabase H-bond formation.

between N(6)H of 1,9-DimeA and N(6)H_2 of 1,9-DimeAH$^+$ (Figure 4). The separations between exocyclic N6 sites are 2.856(8) and 2.966(7) Å for two crystallographically independent cations I and II of **2**. Angles between the adenine planes are 7.3(1)° and 5.2(1)°, respectively. Differences in C6−N6 distances of the neutral and cationic adenine ligands (1.253(9) vs 1.324(8) Å in cation I, 1.284(7) vs 1.326(8) Å in cation II) suggest that there is no major disorder of the proton shared between the two N6 sites. For comparison, in 1,9-DimeAH$^+$-Cl^-, C6−N6 distances are 1.313(3) and 1.311(3) Å for two crystallographically independent cations[20] and 1.301(7) Å in the 1,9-DimeAH$^+$ ligands in **1**, while for the (neutral) 1,9-DimeA ligands in **3** distances are 1.26(1) and 1.29(1) Å. Although there are reported examples of H-bonding between R_2N^- and HNR_2 entities,[21] imine−amine interactions between nucleobases as seen in **2** are scarce.[22] An interesting detail of **3** is the relatively short separation between Pt and N(6)H (2.65(7) and 2.75(6) Å for two crystallographically independent cations), hence the fact that the protons of the imino groups rather than the lone electron pairs are pointing toward the metal.[23]

Conclusion

In summary, it is shown that the combined action of metal coordination and favorable stabilization of the deprotonated nucleobase can lead to a substantial drop in pK_a values of even weakly acidic nucleobase protons such as adenine-NH_2 protons. The X-ray crystal structure of **2** confirms the H-bonding pattern previously postulated by us.[10] Our findings furthermore suggest that there may exist a scenario in which metal ions play an indirect role in general acid−base catalysis involving nucleic acids, simply by acidifying nucleobases upon coordination.[24] In fact, such a possibility could further add to the diversity of RNA cleavage reactions.[25] An involvement of the exocyclic adenine amino group is attractive in this respect because it could accomplish the transfer of protons in different directions, very similar to the function of the histidine side chain in proteins. The main question at this point is whether physiologically relevant metal ions can utilize such an indirect way of acid−base catalysis. For DNA- and RNAzymes containing nonphysiological metal ions,[26] this possibility should not be ruled out.

Experimental Section

Starting Materials. *trans*-$[(NH_3)_2PtCl_2]$ was prepared from K_2PtCl_4 according to the method of Kauffmann and Cowan.[27] 1,9-DimeAH$^+$-ClO_4^- was prepared according to the published method[28] from 9-MeA, which was prepared according to Krüger.[29]

Synthesis of *trans*-$[(NH_3)_2Pt(1,9\text{-DimeAH-}N7)_2](ClO_4)_4\cdot 4H_2O$ (1). First, 120.0 mg (0.4 mmol) of *trans*-$[(NH_3)_2PtCl_2]$ was suspended in 5 mL of H_2O and then, after addition of 165.9 mg (0.8 mmol) of $AgClO_4$, was stirred for 4 h at 60 °C in the dark. Precipitated AgCl was removed, and to the remaining solution was added 210.9 mg (0.8 mmol) of 1,9-DimeAH$^+ClO_4^-$, the solution was diluted to 20 mL, and acidity was adjusted to pH 1 with $HClO_4$. After 10 min, the mixture was filtrated, and the remaining solution was stirred 1 week at room temperature in the dark. The solution was cooled in a refrigerator, where, within 1 week, colorless crystals suitable for X-ray analysis were obtained. The crystals were isolated and washed with 1 mL of H_2O. Yield was 189.5

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(26) See, for example: Lu, Y. *Chem.-Eur. J.* **2002**, *8*, 4589−4596.
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mg (46%). Anal. Calcd for $C_{14}H_{28}N_{12}O_{16}Cl_4Pt \cdot H_2O$: C, 17.2; H, 3.1; N, 17.2. Found: C, 17.2; H, 3.0; N, 17.8.

Synthesis of *trans*-[$(NH_3)_2$Pt(1,9-DimeAH-*N7*)(1,9-DimeA-*N7*)]-$(ClO_4)_3 \cdot 1.5H_2O$ (2). Several crystals of **1** were dissolved in 1 mL of D_2O, and the pD was adjusted to 5.7 with NaOD. The solution was cooled to 4 °C in a refrigerator and yielded, within several days, a mixture of different, colorless crystals, among which crystals of **2** (in our experiments: the larger spindle-shaped crystals) were found.

Synthesis of *trans*-[$(NH_3)_2$Pt(1,9-DimeA-*N7*)]$(ClO_4)_2 \cdot H_2O$ (3). Several crystals of **1** were dissolved in 1 mL of D_2O, and the pD was adjusted to 9.1 with NaOD. The solution was cooled to 4 °C in a refrigerator and yielded, within several days, very small crystals of **3**.

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Varian Mercury 200 FT NMR spectrometer. Sodium 3-(trimethylsilyl)propane sulfonate (TSP; $\delta = 0.00$ ppm) was used as an internal standard. Assignment of the methyl groups in **1**−**3** was achieved by selective 1D ^{1}H NOE spectroscopy, performed on a Varian Inova AS600 FT-NMR spectrometer. pD values were determined by means of a glass electrode and addition of 0.4 units to the meter reading.[30]

pK_a values. pK_a values were determined by means of pD-dependent ^{1}H NMR spectroscopy. The resulting curves in a plot of δ versus pD were fitted by equations published in the literature.[31] The pK_a values for D_2O, obtained by this method, were then calculated for H_2O.[32]

X-ray Crystallography. Crystal data for compounds **1**−**3** were collected at 100 K on an Enraf-Nonius-KappaCCD diffractometer using graphite-monochromated Mo Kα radiation ($\lambda = 0.71069$ Å). For data reduction and cell refinement, the Bruker-Nonius HKL 2000 Suite was used. The structures were solved by direct methods and subsequent Fourier syntheses and refined by full-matrix least squares on F^2 using the SHELXTL PLUS and SHELXL-97 programs.[33] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were generated geometrically and given fixed isotropic thermal parameters or refined isotropically.

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Supporting Information Available: X-ray data of **1**−**3**, and pD-dependent ^{1}H NMR shifts (PDF, CIF, and TXT). This material is available free of charge via the Internet at http://pubs.acs.org.

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