Articles

Protonation of Platinated Adenine Nucleobases. Gas Phase vs Condensed Phase Picture

Judit E. Šponer,[†] Jerzy Leszczynski,[‡] Frank Glahé,[§] Bernhard Lippert,*[,§] and Jiří Šponer*[,†,∥,⊥]

J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, and Research Center for Molecular Clusters and Biomolecules, Dolejškova 3, 182 23 Prague, Czech Republic, Department of Chemistry, Computational Center for Molecular Structure and Interactions, Jackson State University, Jackson, Mississippi 39217, Fachbereich Chemie, Universität Dortmund, 44221 Dortmund, Germany, and Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

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Protonation of adenine carrying a Pt(II) moiety either at N7, N3, or N1 is possible in solution, but the site of protonation is influenced by the location of the Pt(II) electrophile and to some extent also by the overall charge of the metal entity (+2, +1, 0, −1), hence the other ligands (NH_3, Cl^-, OH^-) bound to Pt(II). Quantum chemical calculations based on density functional theory (DFT) have been carried out for intrinsic protonation energies of adenine complexes carrying the following Pt(II) species at either of the three ring N atoms: $[Pt(NH_3)_3]^{2+}$ (**1**), *trans*-$[Pt(NH_3)_2Cl]^+$ (**2a**), *cis*-$[Pt(NH_3)_2Cl]^+$ (**2b**), *trans*-$[Pt(NH_3)_2Cl_2]$ (**3a**), *cis*-$[Pt(NH_3)Cl_2]$ (**3b**), $[PtCl_3]^-$ (**4**), *trans*-$[Pt(NH_3)_2OH]^+$ (**5a**), *cis*-$[Pt(NH_3)_2(OH)]^+$ (**5b**), *trans*-$[Pt(NH_3)(OH)_2]$ (**6a**), *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**), and $[Pt(OH)_3]^-$ (**7**). The data have been compared with results derived from solution studies (water) and X-ray crystallography, whenever available. The electrostatic effects associated with the charge of the metal entity have the major influence on the calculated intrinsic (gas phase) proton affinities, unlike the condensed phase data. Nevertheless, the relative gas phase trends correlate surprisingly well with condensed phase data; i.e., variation of the pK_a values measured in solution is consistent with the calculated gas phase protonation energies. In addition to a systematic study of the ring proton affinities, proton transfer processes within the platinated adenine species were often observed when investigating Pt adducts with OH^- ligands, and they are discussed in more detail. To the best of our knowledge, this is the first study attempting to find a systematic correlation between gas phase and condensed phase data on protonation of metalated nucleobases. The gas phase data provide a very useful complement to the condensed phase and X-ray experiments, showing that the gas phase studies are capable of valuable predictions and contribute to our understanding of the solvent and counterion effects on metal-assisted proton shift processes.

Introduction

Protonated nucleobases occasionally occur in nucleic acids. For example, protonation stabilizes certain AC and GA mismatches, polyA forms a helical, double-stranded structure at low pH, and formation of N3 protonated cytosine is essential in stabilizing four-stranded intercalated i-DNA or achieving a third-strand binding of cytosine in pyrimidine−purine−pyrimidine triplexes.[1]

As revealed by condensed phase and X-ray experiments, protonation of nucleobases can be substantially affected if the base carries a metal moiety.[2,3]It has been suggested that proton shift processes are driven by specific changes in the electronic structures of bases upon metal binding. The experiments provide abundant data about changes of pK_a values of nucleobases. However, the experiments themselves do not allow quantification of the electronic effects exerted by metal ions on the

* Corresponding authors. E-mail: J.Š., sponer@indy.jh-inst.cas.cz; B.L., lippert@pop.uni-dortmund.de.

[†] J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, 182 23 Prague, Czech Republic.

[‡] Department of Chemistry, and Computational Center for Molecular Structure and Interactions, Jackson State University, Jackson, MS 39217.

[§] Fachbereich Chemie, Universität Dortmund, 44221 Dortmund, Germany.

[⊥] Research Center for Molecular Structure and Interactions.

[∥] Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic.

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nucleobases and separation of them from two other major factors, viz., long-range electrostatic effects and the influence of the environment, mainly the solvent effects. All these contributions simultaneously affect the protonation and tautomeric equilibria of metalated nucleobases. The long-range electrostatic contribution associated with the charge residing on the metal entity dominates in the gas phase. However, the polar solvent and counterions in condensed phase and crystals largely modulate and compensate for the electrostatic contribution. It is well established that metalation of a neutral or monoanionic nucleobase by a positively charged metal entity does not prevent protonation of the nucleobase, even if it leads to a further increase of the total charge of the system. This is a process which is less likely to occur for a completely isolated system in the gas phase. It is important to underline that the magnitude of the solvent effects on the metalated nucleobases is expected to vary with experimental conditions. Thus, to make our understanding of metal-induced proton shifts in metalated bases more complete, it is quite tempting to supplement the existing condensed phase measurements with relevant data obtained in the absence of the environment. Such gas phase data can be presently obtained, with high accuracy, by using the ab initio quantum chemical approach. Computational quantum chemistry matured in recent years and modern ab initio quantum-chemical methods represent a respectable tool to study small and medium-sized systems in the gas phase with accuracy comparable to experiments. Ab initio methods were extensively used in studies of H-bonding and stacking of DNA bases,[4] their intrinsic tautomer equilibria[5] and protonation processes,[6−11] and various aspects of nucleobase metalation.[11−16] Recently, we have analyzed the effect of Pt(II) and Hg(II) binding to the amino groups of nucleobases and demonstrated that the metalation has a substantial nonelectrostatic effect on the basicity of the ring nitrogens of bases.[11] These results explained the experimentally observed proton shifts caused by the amino group metalation. In contrast, studies of N7 metalation of nucleobases rather suggest that metalation by Pt(II) influences the gas phase tautomeric and protonation equilibria of nucleobases primarily by the electrostatic effects,[15,16] except for cases with specific interactions between one of the ligands attached to the metal and the nucleobase.[15]

In the present paper we have carried out an extensive quantum-chemical analysis of the effects of metalation on protonation of adenine, complementing the available experimental data. We systematically study all possible combinations of the endocyclic ring nitrogenous sites of adenine involved in metalation and protonation, namely the N1, N3, and N7 positions. The calculations were carried out for Pt(II) adducts with charges varying from +2 to −1, and we considered several ligands. Computed protonation energies, defined as the difference of the total electronic energies between the protonated and nonprotonated forms, were compared in detail with available experimental pK_a values of free and metalated adenines in aqueous solution.

Method

The computations were done using two techniques differing in the treatment of relativistic effects.

First, we have utilized density functional theory (DFT) by using a combination of the nonlocal gradient corrected three parameter exchange functional developed by Becke[17] and the correlation functional in the formalism of Lee−Yang−Parr.[18] Nowadays this functional (abbreviated as Becke3LYP) is becoming standard for computation of transition metal complexes. Our previous contribution demonstrates its success in calculation of acid−base properties of platinated cytosine complexes.[11]

All first- and second-row elements have been described by the 6-31G* basis set. As an adequate description of highly polarizable atoms requires introduction of diffuse functions, on the chlorine atom we have used the 6-31+G* basis set.

On the platinum the lanl2dz relativistic pseudopotential has been imposed, which substitutes for the inner 60 electrons.[19] The remaining 18 electrons (corresponding to the 5s, 5p, and 5d shells) are explicitly considered throughout the calculations. The calculations have been carried out using the Gaussian 98 computer code.[19]

The second set of calculations has been carried out with the ADF (Amsterdam Density Functional) program package,[20,21] which offers a sophisticated approach to relativistic corrections.[22] The local-density approximation (LDA) in the Vosko−Wilk−Nusiar parametrization[23] was used together with Perdew's and Wang's nonlocal correction to the local expressions of the exchange and correlation energy.[24] The core orbitals were kept frozen up to 4f for Pt and to 1s for C, N, and

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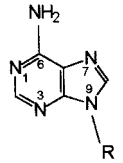
Table 1. Protonation Energies of Isolated Neutral Adenine in the Gas Phase (kcal/mol), Becke3LYP/6-31G* Method[a]

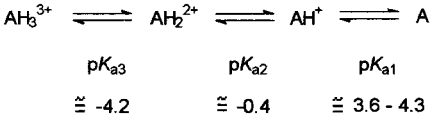
protonation site	absolute protonation energy (enthalpy)	relative protonation energy (enthalpy)
N7	−228.7 (−220.0)	+8.5 (+8.7)
N3	−235.8 (−227.3)	+1.4 (+1.4)
N1	−237.2 (−228.7)	0 (0)

[a] Values in parentheses show the 0 K protonation enthalpies after adding the zero point energy term. Note that relative protonation energies and enthalpies are identical.

Chart 1



Scheme 1



O. The valence orbitals were contracted into triple-ζ Slater orbitals. As polarization functions one 6p shell was used for Pt, one 3d for C and N, and one 2s for H (ADF basis set IV). All calculations were performed with relativistic corrections in the ZORA scheme.[25] All complexes in this study were optimized without applying any geometrical constraints.

Due to the large number of structures investigated, we did not verify the nature of optimized structures via harmonic vibration analysis; however, it is very unlikely that any of them represents a transition state. For the same reason we did not calculate the difference of zero point energy (ZPE). Thus all numbers provided in this study are the gas phase protonation energies and not enthalpies. Note, however, that despite a small difference in absolute values of protonation energies and enthalpies, the relative values of protonation enthalpies and energies for different nitrogenous sites should be identical. The difference between gas phase protonation energy and enthalpy is shown for a nonmetalated adenine in Table 1 below.

Results and Discussion

Acid−Base Equilibria of Adenine Nucleobases. Let us first briefly summarize the condensed phase experimental data on adenine protonation. Adenine, A, (Chart 1; R = H) and its N9 substituted derivatives, 9-RA (R = alkyl group or sugar) can undergo protonation at the endocyclic nitrogen atoms N1, N3, and N7. Protonation of the exocyclic amino group N6 is not expected to take place as the lone pair of the amino nitrogen is largely delocalized into the heterocyclic ring. The existence of mono-, di-, and triprotonated adenine is established. pK_a[26] values are ca. −4.2, −0.4, and 4.0, respectively, for the three protonated forms (Scheme 1). It is widely accepted that the first proton added to A resides at N1, that the AH_2^{2+} species is protonated at N1 and N7, and that in AH_3^{3+} finally also N3 is protonated (see also below).

Deprotonation of A occurs in aqueous solution at N9 (pK_a ∼ 10),[27] followed by deprotonation of the exocyclic amino group (pK_{a2} ∼ 17).[28] With N9-substituted adenines only the second deprotonation process, viz., deprotonation of the NH_2 group, is relevant.

Table 2. Second Protonation Energies for Protonated Adenine (kcal/mol)[a]

adenine form	protonation site	absolute protonation energy	relative protonation energy
Ade(H7)+	N1	−140.1	0
	N3	−137.7	+2.4
Ade(H3)+	N1	−122.9	+17.2
	N7	−130.6	+9.5
Ade(H1)+	N3	−121.4	+18.7
	N7	−131.6	+8.5

[a] Energy for double protonation can be obtained by adding the first protonation energies listed in Table 1. Becke3LYP/6-31G* method.

In the following no explicit differentiation is made between nonsubstituted adenine (A) and N9 as well as N6′,N6′,N9-substituted adenines in that it is assumed that the overall picture is not changed dramatically by these modifications. For example, pK_{a1} values for adenosine are in the range 3.61[29]−3.78,[30] depending on temperature and ionic strength, for 9-methyladenine at ca. 4.3,[31] and for N6′,N6′,N9-trimethyladenine at ca. 4.15.[32] The pK_{a2} for the latter base is −0.75 ± 0.2. (However, it is obvious that the scenario of N9 deprotonation brought about by certain Pt hydroxo species is not relevant to 9-substituted adenine—see below).

Gas-Phase Protonation Energies of Nonmetalated Adenine. We have first investigated the protonation energies of neutral and singly protonated adenine to give species of +1 and +2 charge, respectively. Table 1 lists the protonation energies of neutral, nonmetalated adenine. These energies and their relative values represent the reference point with respect to which all our subsequent calculations are to be compared. Table 1 shows that the protonation energies are within the range of 230−240 kcal/mol and that protonation of N7 is markedly less favorable (−228.7 kcal/mol) compared to the N3 and N1 sites, which are almost isoenergetical (−235.8 and −237.2 kcal/mol, respectively). Our data are in agreement with protonation energies reported in other computational studies,[6−11] as protonation energies are not very sensitive to the level of calculations. For the sake of completeness, we have also calculated the protonation enthalpies of adenine (Table 1). The theoretical protonation energies are in excellent agreement with gas phase experimental value of adenine proton affinity (−224 kcal/mol, cf. the calculated enthalpy value in Table 1). We have carried out the calculations for nonmetalated adenine in order to have the protonation energies of adenine and metalated adenines evaluated exactly at the same level of computations.

Table 2 summarizes the protonation energies for a monoprotonated adenine, i.e., energy gains by adding a proton to the ring, which is already protonated. As expected, these protonation energies associated with the attachment of the second proton are in the absolute value much smaller compared to those in Table 1. This mainly reflects the electrostatic repulsion, which has to be overcome when increasing the charge of the system from a value of +1 to +2 in the gas phase. Besides this reduction (in absolute value) of the protonation energies, there are also considerable differences in the individual double protonation patterns. Clearly the most favorable situation for

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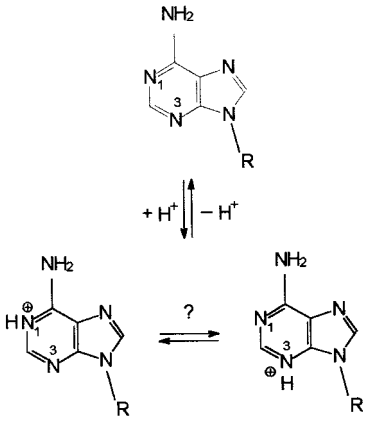
Scheme 2



the second protonation occurs when the first proton is attached to N7. Then protonation of N1 is weakly preferred over protonation of N3. Protonation of N7 after protonating N1 or N3 is apparently less favorable. The least favorable case is a simultaneous protonation of the closely spaced N1 and N3 positions. As far as total energies of the dicationic adenine species are concerned, the sequence is therefore N7, N1 > N7, N3 > N1, N3. The values in Table 2 represent a useful reference point for a direct comparison with protonation of adenine metalated by a metal adduct carrying a charge of +1 (see below).

Comparison of Intrinsic Gas-Phase Trends with Solution Studies and X-ray Data. Protonation studies with adenine and its N9-substituted derivatives in water agree with the gas phase picture in that N7 is not the most basic site for proton binding to give the monocation AH^+. It is believed that in aqueous solution the first proton adds to N1, consistent with the large effect (downfield shift) of protonation on the H2 resonance of A seen in the ^{1}H NMR spectrum.[34] While N3 protonation might be expected to exercise a similar effect on H2, the relative insensitivity to the N9 substituent (e.g., CH_3 resonance in 9-methyladenine) does not strongly support the option of N3 being the preferred first site of protonation. On the other hand, a tautomeric equilibrium of both forms, with the N(1)H form dominating over the N(3)H form, cannot rigorously be excluded (Scheme 2). Clearly, the results of the gas phase calculations are qualitatively in agreement with the situation in water. In nonaqueous solution formation of AH_2^{2+} cations and in superacidic medium ($FSO_3H/SbF_5/SO_2$) and at low temperature (252 K) even formation of AH_3^{3+} cations, protonated at N1, N3, and N7, has been reported.[35]

X-ray crystallographic studies of adeninium compounds AH^+X^- are likewise consistent with N1 protonation.[36] The bonding patterns observed in several salts are unambiguous concerning the site of protonation. With AH_2^{2+} compounds, the sites of proton binding are N1 and N7.[37]

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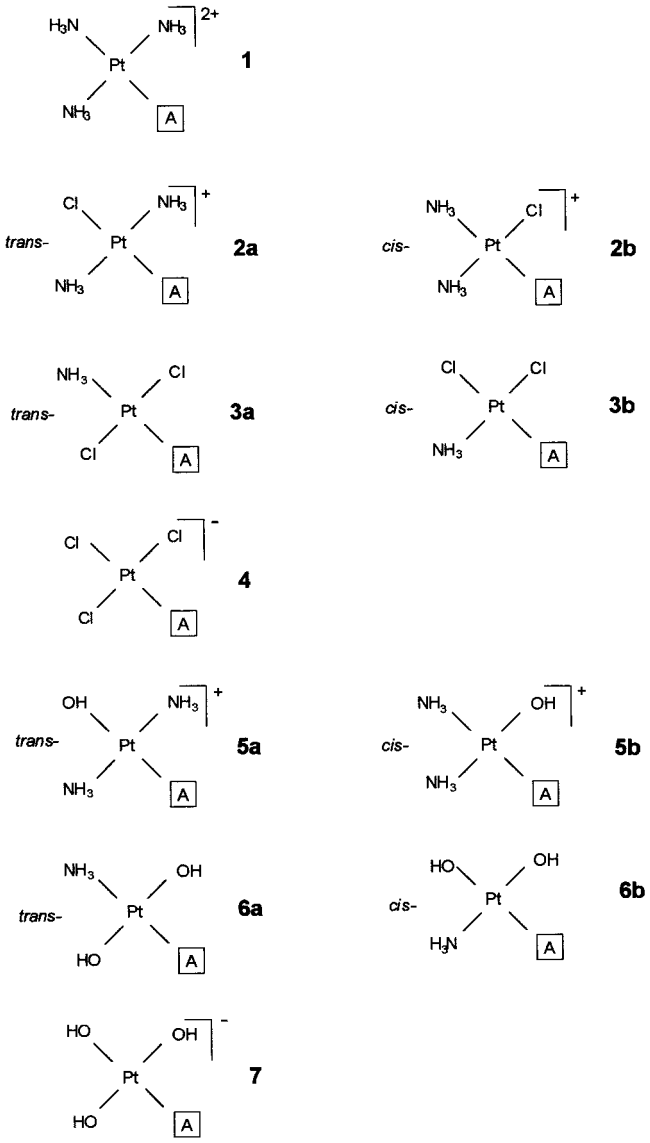

Figure 1. Schematic view of adenine (A) complexes **1-7** studied.

Gas Phase Protonation Energies of Adenine Carrying [Pt(NH$_3$)$_3$]$^{2+}$ (1), *trans-* and *cis*-[Pt(NH$_3$)$_2$Cl]$^+$ (2), *trans-* and *cis*-[Pt(NH$_3$)Cl$_2$] (3), and [PtCl$_3$]$^-$ (4) Units. In a subsequent set of calculations, we have investigated the effect of metalation of the adenine on its intrinsic protonation energies. Figure 1 lists the Pt(II) species used in the calculations and Table 3 summarizes the protonation energies of adenine carrying [Pt(NH$_3$)$_3$]$^{2+}$ (**1**), *trans*-[Pt(NH$_3$)$_2$Cl]$^+$ (**2a**), *cis*-[Pt(NH$_3$)$_2$Cl]$^+$ (**2b**), *trans*-[Pt(NH$_3$)Cl$_2$] (**3a**), *cis*-[Pt(NH$_3$)Cl$_2$] (**3b**), and [PtCl$_3$]$^-$ (**4**) moieties. The values listed are the absolute protonation energies. Relative values of protonation energies within the rows are given in parentheses, i.e., for structures with an equal charge and adduct.

Let us start the discussion with compounds **3a** and **3b**, i.e., the data for neutral adducts, and compare them with the data for free adenine in Table 1. Binding of *trans*-[Pt(NH$_3$)Cl$_2$] (**3a**) weakly reduces the ability of the adenine ring to become protonated, on average by about 5 kcal/mol. This indicates that there is a certain positive charge flux from the metal adduct to the ring. Despite the fact that the adduct is neutral, Pt binding introduces interesting differences in the relative protonation energies. Clearly, the highest probability of protonation should be expected when the Pt entity is attached to N7, and in this

Table 3. Protonation Energies (kcal/mol) of Adenine Metalated by $[Pt(NH_3)_3]^{2+}$ (**1**), *trans*- and *cis*-$[Pt(NH_3)_2Cl]^+$ (**2**), *trans*- and *cis*-$[Pt(NH_3)Cl_2]$ (**3**), and $[PtCl_3]^-$ (**4**) Entities[a]

	N1[b]		N3[b]		N7[b]	
	N3[c]	N7[c]	N1[c]	N7[c]	N1[c]	N3[c]
$[Pt(NH_3)_3]^{2+}$ (**1**)	−85.6 (+13.1)	−96.0 (+2.7)	−89.1 (+9.6)	−95.0 (+3.7)	−98.7	−98.1 (+0.6)
trans-$[Pt(NH_3)_2Cl]^+$ (**2a**)	−149.0 (+11.2)	−152.7 (+7.5)	−151.5 (+8.7)	−152.0 (+8.2)	−160.2	−158.35 (+1.85)
cis-$[Pt(NH_3)_2Cl]^+$ (**2b**)	−155.3 (+11.1)	−159.5 (+6.9)	−157.3 (+9.1)	−161.3 (+5.1)	−166.4	−163.6 (+3.1)
trans-$[Pt(NH_3)Cl_2]$ (**3a**)	−224.2 (+7.5)	−219.3 (+12.4)	−226.3 (+5.4)	−223.6 (+8.1)	−231.7	−228.1 (+3.6)
cis-$[Pt(NH_3)Cl_2]$ (**3b**)	−216.3 (+10.2)	−214.5 (+12.0)	−217.1 (+9.4)	−219.7 (+6.8)	−226.5	−222.4 (+4.1)
$[PtCl_3]^-$ (**4**)	−285.0 (+3.1)	−273.8 (+14.3)	−286.6 (+1.5)	−281.3 (+6.8)	−288.1	−283.5 (+4.6)

[a] Numbers in parentheses are the relative values of the protonation energies within the rows, with respect to N1 protonation of the N7 metalated adenine. Becke3LYP/6-31G* method. [b] Position of Pt. [c] Position of the proton.

case the protonation of N1 is moderately favored over protonation of N3. Metalation of N3 reduces the protonation energy of N1 by ca. 5.4 kcal/mol while protonation of N7 is even less likely. The protonation energies are further reduced when the platination occurs at N1, and the protonation of N3 is clearly favored over N7. The data for *cis*-$[Pt(NH_3)Cl_2]$ (**3b**) adduct are quite similar with a further reduction of the absolute values of protonation energies. This reduction of proton affinity with respect to **3a** can be easily explained by considering the position (separation) of the anionic Cl^- ligands with respect to the adenine moiety in both compounds.

With regard to binding of $[Pt(NH_3)_3]^{2+}$ (compound **1**, see Table 3), it is evident that the absolute gas phase protonation energies are sharply reduced compared with nonmetalated adenine (by ca. 130 kcal/mol) due to the long-range electrostatic effects. However, since protonation is known to occur for this adduct in aqueous solution rather readily, one can expect that these electrostatic effects are largely annihilated by the solvent (see below). Thus, we will primarily discuss the relative values of protonation energies. The most favorable situation is in **1**, when Pt binds to the N7 position. Then the protonation of both N1 and N3 appears to be almost isoenergetical. In contrast to the data for $[Pt(NH_3)Cl_2]$ (**3**) and nonmetalated adenine, however, protonation of N7 is relatively favorable for both N3 and N1 Pt binding. On the other hand, metalation of N1 disfavors protonation of N3 and vice versa. It should be noted that, besides the influence of proton affinities discussed above, the $[Pt(NH_3)_3]^{2+}$ attached to N7 can also induce formation of an adenine imino tautomer by shifting a hydrogen from the amino group to the N1 position (cf. Figure 1b in ref 15).

A closer scrutiny of the remaining cases shows, that for the $[Pt(NH_3)_2Cl]^+$ (**2**) the relative values of protonation energies are between the values obtained for $[Pt(NH_3)_3]^{2+}$ (**1**) and $[Pt(NH_3)Cl_2]$ (**3**) adducts. When comparing the absolute values of protonation energies with the data in Table 2 (double protonated adenine) one recognizes that protonation of adenine metalated by **2b** is considerably favored (by ca. 25−30 kcal/mol) over a second protonation of the nonmetalated adenine. This clearly shows that the effect exerted by the +1 metal adduct on adenine proton affinity is considerably smaller compared to the attachment of a proton. Thus, in the gas phase, binding of a metal adduct with a charge of +1 is considerably less destabilizing for a subsequent ring protonation than proton binding is. This point may be an important clue to rationalize the observation that metal adducts permit protonation in solution. The data for *trans*-$[Pt(NH_3)_2Cl]^+$ (**2a**) are very similar, though the absolute values of proton affinities are by about 5−10 kcal/mol lower compared to *cis*-$[Pt(NH_3)_2Cl]^+$ (**2b**). The last row of Table 3 shows protonation energies for the negatively charged $[PtCl_3]^-$ (**4**) entity. Also in this case the most favorable protonation is that of N1 after N7 metalation. However, the change in the charge of the adduct considerably enhances the ability of N1 to be protonated after N3 metalation, and vice versa.

Results Obtained in Condensed Phase. The number of Pt(II) adenine complexes studied in aqueous solution and by X-ray crystallography, respectively, is limited, and therefore a complete comparison with computations is not possible. As to platinated adeninium (AH^+) species, very few examples[38,39] are verified by X-ray crystallography and they have the Pt(II) bonded to N7. For example, in $[PtCl_3(9\text{-MeAH})]$[38] the N1 position is protonated and there is no ambiguity concerning the protonation site. The situation (N7 metal binding, N1 proton binding) is strictly analogous to that in $[ZnCl_3(AH)]$, which contains the parent adeninium ligand.[40] These findings agree with the gas phase trends.

Solution studies carried out by application of potentiometric titration, UV spectroscopy, or ^{1}H NMR spectroscopy, while providing a measure for the loss of overall adenine basicity as a consequence of Pt binding to N7, do not unambiguously establish the site of proton binding. It is generally *assumed* that the preferred protonation site is N1, but clearly a straightforward differentiation between N1 and N3 is not possible and neither is a tautomer equilibrium strongly favoring one species ruled out. UV spectroscopy might, in principle, give an answer provided one tautomer does not exceed the other by a factor of 10 or so.

As to the magnitude of the loss of adenine basicity (i.e., decrease in pK_{a1} as a consequence of the N7 platination), no strong influence of the charge of the Pt entity has been detected in aqueous solution. The evident lack of the charge dependence shows that the environment very efficiently compensates for the long-range electrostatic effects. In four complexes of 2′-deoxyadenosine with 2-fold positive *cis*- and *trans*-$[Pt(NH_3)_2(H_2O)]^{2+}$ moieties and the singly charged *cis*- and *trans*-$[Pt(NH_3)_2Cl]^+$ moieties at N7 each, for example, the drop in basicity amounts to ca. 1.7 log units each.[41] With 9-methyladeninium carrying $[Pt(dien)]^{2+}$ (dien = diethylenetriamine), *cis*-$[PtCl_2(NH_3)]$, and $[PtCl_3]^-$ at the N7 positions, pK_a values are 1.5 (UV)[42] and 1.9 (^{1}H NMR),[42] 1.9,[31] and 2.5,[31] respectively. Thus, although the loss in basicity ($4.3-pK_a$) is somewhat more spread (1.8−2.8 log units) in the case of 9-MeAH$^+$, more data are necessary to establish that this trend is general. The fact that $[PtCl_3]^-$ reduces the basicity of adenine to a smaller extent than the neutral and the cationic Pt entities is in agreement with the calculations.

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(39) Schreiber, A.; Lüth, M. S.; Erxleben, A.; Fusch, E. C.; Lippert, B. *J. Am. Chem. Soc.* **1999**, *121*, 3248 and references therein.
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(41) Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. *Chem. Eur. J.* **1999**, *5*, 2374 and references therein.
(42) (a) Schwarz, F.; Lippert, B.; Schöllhorn, H.; Thewalt, U. *Inorg. Chim. Acta* **1990**, *176*, 113. (b) den Hartog, J. H. J.; van den Elst, H.; Reedijk, J. *J. Inorg. Biochem.* **1984**, *21*, 83.

Table 4. Protonation Energies (kcal/mol) of Adenine Metalated by $[Pt(NH_3)_3]^{2+}$ (**1**), *trans*- and *cis*-$[Pt(NH_3)_2OH]^+$ (**5**), *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**), and $[Pt(OH)_3]^-$ (**7**)[a]

	N1[b]		N3[b]		N7[b]	
	N3[c]	N7[c]	N1[c]	N7[c]	N1[c]	N3[c]
$[Pt(NH_3)_3]^{2+}$ (**1**)	−85.6 (+13.1)	−96.0 (+2.7)	−89.1 (+9.6)	−95.0 (+3.7)	−98.7	−98.1 (+0.6)
trans-$[Pt(NH_3)_2OH]^+$ (**5a**)	−154.7 (+9.8)	−157.7 (+6.8)	−157.2 (+7.3)	−156.6 (+7.9)	−164.5	−163.0 (+1.5)
cis-$[Pt(NH_3)_2OH]^+$ (**5b**)	−157.9 (+17.9)	−163.7 (+12.1)	−153.6 (+22.2)	[−172.4][d]	−175.8	−161.7 (+14.1)
cis-$[Pt(NH_3)(OH)_2]$ (**6b**)[d]	[−232.3]	[−231.3]	−217.8[e]	[−250.4]	[−248.0]	[−238.1]
$[Pt(OH)_3]^-$ (**7**)[d]	[−318.2]	[−303.3]	[−313.7]	[−320.7]	[−324.6]	[−313.6]

[a] Numbers in parentheses are the relative values of the protonation energies within the rows, with respect to N1 protonation of the N7 metalated adenine. Becke3LYP/6-31G* method. [b] Position of Pt. [c] Position of the proton. [d] For this metal adduct, N→O proton shift in the protonated form occurred, leading to an aqua ligand and often associated with rotation of the platinated adduct. Therefore, the individual protonation energies are given in brackets and relative protonation energies are not listed. [e] This number corresponds to the protonation energy of the isomeric form without proton shift. This protonation energy, therefore, can be compared (and in fact is identical) with the corresponding number in Table 3 for **3b**. For details see the text.

It is interesting to notice that the loss of adenine basicity due to a binding of a *neutral* Pt adduct in solution correlates well with the reduction of protonation energy caused by the same adduct in the gas phase (cf. Table 1 with data for compounds **3a** and **3b** in Table 3). At the same time, solution protonation of adenine metalated by +1 adduct is facilitated over a second (double) protonation of a nonmetalated adenine. This again clearly correlates with the gas phase trends (cf. Table 2 vs data for **2a** and **2b** in Table 3). The gas phase protonation energies of adenine and singly protonated adenine envelope the values of protonation energies of adenine metalated with Pt adducts with charges 0 and +1, and this relation remains qualitatively unchanged when considering solution pK_a values. Thus, the dependence of both gas phase protonation energies and solution pK_a values on the total charge has a reduced slope for the platinated adenine compared with the multiple-protonation process. It leads to the apparent independence of the pK_a from the charge carried by the metal adduct in aqueous solution.

Very few adenine complexes carrying Pt(II) at the N1 position have been studied.[42,43] For the $[Pt(dien)]^{2+}$ complex pK_a values of ca. 0.3 (UV) and ca. 0.7 (^{1}H NMR) have been reported.[42] The large effect of the protonation process on the H8 resonance of 9-MeA strongly suggests that N7 is the site of protonation, again in agreement with the gas phase calculations. A few examples of Pt(II) complexes exist, in which the metal is bonded to N3 of the adenine base (N6′,N6′,N9-trimethyladenine).[32] pK_a values of ca. 0.2 have been estimated for the bound adeninium from ^{1}H NMR and UV data with $[Pt(dien)]^{2+}$, *trans*-$[Pt(NH_3)_2(9\text{-EtGH-}N7)]^{2+}$ (9-EtGH = 9-ethylguanine), and *trans*-$[Pt(NH_3)_2Cl]^+$ species. From the ^{1}H NMR spectra it has been concluded that protonation occurs at N7, again consistent with the gas phase data (Table 3). Most importantly, both N3 and N1 metal binding reduce the pK_a value compared with the N7 metalation, an observation entirely consistent with the gas phase trend. In general, the comparison of solution and gas phase data gives a clear impression that while solvent efficiently eliminates the major part of the pure electrostatic effects, the relative trends seen in the gas phase remain expressed under the solution experimental condition. This is especially evident when comparing condensed phase and solution data for systems with equal charges.

Gas Phase Protonation Energies of Adenine Carrying *trans*- and *cis*-$[Pt(NH_3)_2OH]^+$ (5), *cis*-$[Pt(NH_3)(OH)_2]$ (6b), and $[Pt(OH)_3]^-$ (7) Entities. In a subsequent set of calculations, we repeated the above calculations with OH^- substituting for Cl^-. The reason for this substitution was to see to what extent the results depend on the ligands attached to the platinum. The data are summarized in Table 4.

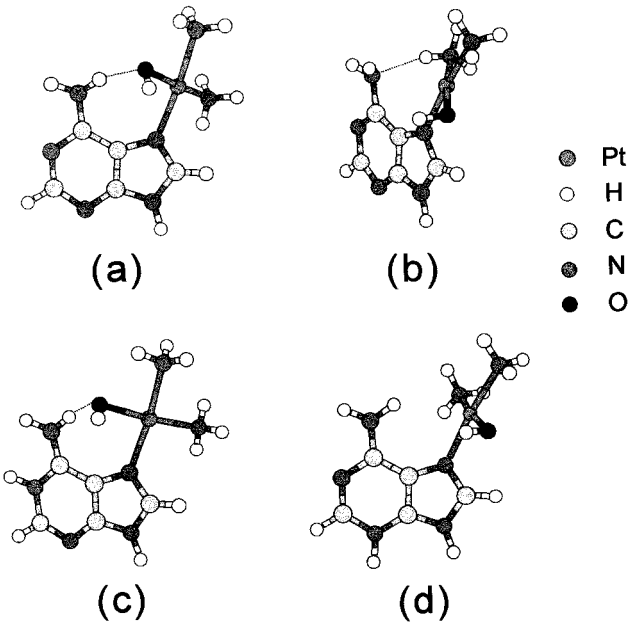


Figure 2. *cis*-$[Pt(NH_3)_2OH]^+$ binding to N7. (a) Global minimum for neutral adenine with a hydrogen bond between adenine amino group and the OH^- ligand; (b) local minimum with amino-acceptor interaction between the pyramidal amino group nitrogen of adenine and the NH_3 ligand; (c) protonation of N1 of adenine reinforcing the hydrogen bond between the amino group of adenine and the OH^- ligand; (d) N3 protonated structure with metal adduct perpendicular to the nucleobase.

Relative protonation energies for the *trans*-$[Pt(NH_3)_2OH]^+$ (**5a**) adduct, having the OH^- ligand trans to the adenine ring N atom, are close to those obtained for *trans*-$[Pt(NH_3)_2Cl]^+$ (**2a**). The absolute proton affinities are slightly higher with OH^- than with Cl^-.

On the other hand, the data for the *cis*-$[Pt(NH_3)_2OH]^+$ (**5b**) adduct are quite complicated. This is caused by a close contact between the OH^- ligand and the adenine moiety allowing specific H-bonding interactions with the adenine. These interactions significantly influence the relative protonation energies. Figure 2 shows the case of the N7 metalation by **5b**. For neutral adenine, the N7-bound **5b** adopts preferably such an orientation, that the OH^- group serves as an H-bond acceptor for the adenine amino group (Figure 2a). However, further calculations reveal a second local minimum which is basically isoenergetical (being less stable by just 1.2 kcal/mol). In this geometry (Figure 2b) the Pt entity is rotated by 180° so that the NH_3 ligand contacts the adenine amino group. The amino group is pyramidal and its nitrogen serves as a H-bond acceptor[16] with respect to the

(43) Beyerle-Pfnür, R.; Brown, B.; Faggiani, R.; Lippert, B.; Lock, C. J. L. *Inorg. Chem.* **1985**, *24*, 4001.

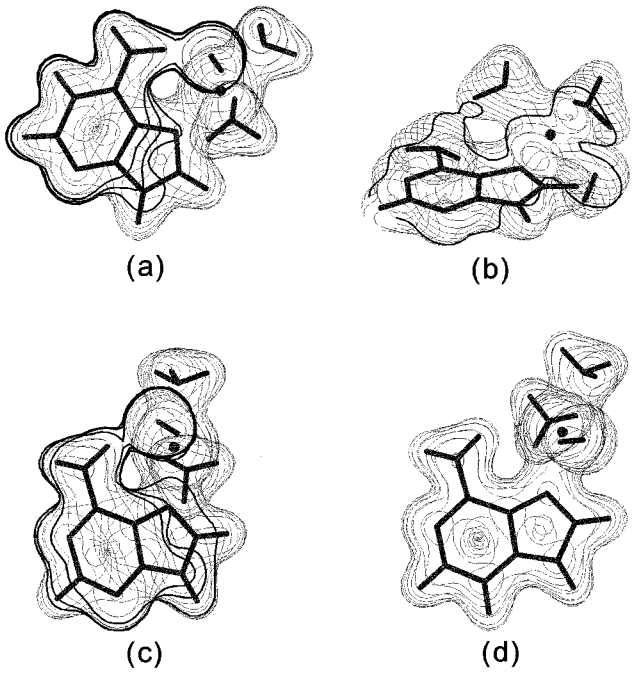


Figure 3. Isodensity surfaces for the models shown in Figure 2. Contours plotted at 0.025 au.

NH_3 ligand, with a distance between the two nitrogen atoms of 2.99 Å. The degree of the adenine amino group pyramidalization can be illustrated by the sum of the amino group valence angles, which amounts to 337° instead of 360° characteristic for sp^2 nitrogens. The amino group hydrogen atom proximal to the metal adduct has a dihedral angle of 44° with respect to the adenine ring. We would like to emphasize that the partial pyramidalization of the adenine amino group is structurally important but energetically a rather subtle effect. It has no relation to basicity of the amino group; thus, amino group pyramidalization does not mean that a protonation of this site is to be expected. The calculated intrinsic protonation energy of the N6 adenine nitrogen is −215.1 kcal/mol, well below (in absolute value) the protonation energies of any of the ring nitrogen sites. Obviously, after any ring nitrogen site is protonated, the amino group adopts a purely sp^2 hybridization. Just for a comparison, protonation energy of the aniline amino group is −222.2 kcal/mol. When protonating the N1 position, the amino group can no longer remain pyramidal and the NH_3 group is pushed by the electrostatic field away the protonation site, so this local minimum disappears completely. The optimal protonated structure thus shows a strong ionic hydrogen bond between the adenine amino group and the OH^- ligand (Figure 2c), with an N−O distance of 2.61 Å and with an elongation of the N6−H covalent bond by about 0.05 Å (see discussion in ref 16). This greatly stabilizes the protonation of N1 with respect to other structures. Interestingly, when protonating N3, the metal adduct adopts a perpendicular orientation with respect to the nucleobase (several starting geometries were attempted converging toward the same structure) and none of its ligands interacts with the amino group (Figure 2d). By scrutinizing the electronic density distributions, in three out of the four cases (Figure 3a−c) we can observe a delocalization of the electronic density between the N6 amino group and the platinated adduct. However, upon protonation of the N3 nitrogen of the adenine (Figure 3d), the amino group and the platinated adduct become isolated.

When metalating the N1 position by **5b**, the metal adduct forms a hydrogen bond with the amino group via its OH^- ligand for all three adenine forms (neutral, N3 protonated, and N7 protonated). There again exists a local minimum for nonprotonated adenine with NH_3 donating a proton in an amino-acceptor interaction with the adenine amino group. This is, however, 4.5 kcal/mol less stable than the structure with an N6−H6···O hydrogen bond.

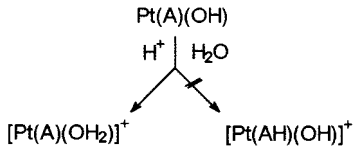
Finally, for N3 metalation, the situation is as follows. For neutral adenine, **5b** forms an H-bond between the N9−H9 and the OH^- group while the NH_3 group is situated close to the C2 adenine position. Protonation of the N1 position pushes this NH_3 ligand away and the Pt entity rotates by 180°, bringing the NH_3 ligand close to N9 and the OH^- ligand close to the C2 adenine position. In contrast, a protonation of the N7 position on the other side of the adenine ring leads to a spontaneous proton transfer of the H9 adenine hydrogen to the OH^- ligand, thus leading to a formation of an aqua ligand and a rare tautomer form of adenine. This process is not so surprising, as the OH^- is a good H-acceptor and the protonation of the adenine ring necessarily substantially acidifies the hydrogen attached to the N9 adenine position. For this case with a proton transfer, we present the absolute protonation energy in brackets in Table 4. We do not provide relative value of the protonation energy, as it is not directly comparable with the other numbers.

Unfortunately, for the species with charges 0 and −1, *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**) and $[Pt(OH)_3]^-$ (**7**), we have seen, after attempting the adenine protonation, proton transfers from either the amino group or a ring nitrogen to one of the OH^- ligands. In other words, the calculations yielded spontaneously aqua ligands at the Pt and a neutral adenine mostly in some rare tautomeric form. Thus the OH^- ligands showed a much larger gas phase proton affinity as compared with the adenine moiety. In addition, in most cases, when attempting protonation of the ring, the metal entity underwent rotation about the Pt nucleobase bond in order to optimize the interaction. Despite several attempts and starting from different initial geometries, we were not able to unify the metal adduct geometries for protonated and nonprotonated adenines. In two cases, the proton shift leading to an aqua ligand occurred even with the initial structure (i.e., before attempting a protonation; see a further discussion below), causing a spontaneous deprotonation of adenine. Thus, protonation energies for *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**) and $[Pt(OH)_3]^-$ (**7**) adducts are given in brackets in Table 4. We do not report the relative values of protonation energies, since the actual effects of the metal binding on ring position basicities (see Table 3) are much smaller compared to the effects associated with the strong intramolecular H-bonds, proton-transfer processes to the OH^- ligands of the adduct, and metal entity reorientation. As the proton-transfer processes were not the primary aim of this work, we decided not to investigate the remaining compound *trans*-$[Pt(NH_3)(OH)_2]$ (**6a**), which also shows the proton shifts. We plan to address this issue in more detail in a future study.

Expectations in Solution. Acid−base equilibria of adenine complexes carrying hydroxo or aqua ligands at the Pt(II) center have not been widely studied to date.[41] The proton shift from the adeninium ligand to a hydroxo ligand, as seen in the calculations with *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**) and $[Pt(OH)_3]^-$ (**7**), is not unexpected at all, if pK_a values for aqua and adeninium ligands are compared. Thus pK_a values for $Pt(II)OH_2$ groups are typically in the range 4−7,[41,44] whereas pK_a values for adeninium bound to Pt(II) are generally lower by several log

(44) Martin, R. B. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; VHCA Zürich and Wiley-VCH: Weinheim, 1999; pp 183−200.

Scheme 3



units (see above). In essence this implies that in aqueous solution protonation of a mixed adenine, hydroxo species is always expected to take place at the hydroxo ligand to give an aqua species, irrespective of the metal binding site N1, N3, or N7 (Scheme 3). Thus the gas phase trends again appear to be relevant to the solution situation.

This situation with *trans*-$[Pt(NH_3)_2OH]^+$ (**5a**) and *cis*-$[Pt(NH_3)_2OH]^+$ (**5b**) adducts is more complicated. The calculations do not suggest spontaneous proton transfers to form the aqua ligands, with exception of one case where the H9 hydrogen has been shifted away the ring (see above). It is to be noted that pK_a values for aqua ligands in *trans*-$[Pt(NH_3)_2L(H_2O)]^{2+}$ species generally are at the lower end[44] and hence the trend seen for protonation of *trans*-$[Pt(NH_3)_2A(OH)]^+$ is not unreasonable, especially if platination takes place at the N7 position. It should be noted that, when carrying out calculations for the *trans*-$[Pt(NH_3)_2OH]^+$ (**5a**) adduct, the formation of the aqua ligand could be prevented because the trans OH^- group is situated far from the adenine moiety. Therefore, during a gradient optimization and assuming the starting structure with protons residing on the ring, a proton cannot be spontaneously transferred to the OH^- ligand. To clarify this issue, we calculated the actual protonation energies of the OH^- ligands for selected adducts, by adding the proton directly to the OH^-. In the case of *trans*-$[Pt(NH_3)_2OH]^+$ (**5a**) attached to N7 of adenine, the resulting protonation energy is −157 kcal/mol; i.e., the protonation of the ring N1 position (see Table 4) is favored over the aqua ligand formation by ca. 7 kcal/mol. Thus, for this adduct we actually predict the protonation to occur at the ring. We have repeated the calculation with the *cis*-$[Pt(NH_3)_2OH]^+$ (**5b**), bound again to N7, and the resulting protonation energy for formation of the aqua ligand was again −157 kcal/mol, showing no difference between the cis and trans positions. (For the sake of completeness we should notice that the latter calculation was done assuming such an orientation of **5b** where the NH_3 ligand is in contact with the adenine amino group while OH^- or OH_2 groups are at the opposite side.) Finally, we calculated the energy of formation of the aqua ligand also for *trans*-$[Pt(NH_3)_2OH]^+$ attached to N1 of adenine. In this case the protonation energy was −161 kcal/mol. Thus (cf. with Table 4), for the N1−Pt binding, the formation of the aqua ligand becomes weakly favored (by ca. 3 kcal/mol) over the N7 protonation.

It should be noted that the relatively low (in absolute value) protonation energies of the *trans*- and *cis*-$[Pt(NH_3)_2(OH)]^+$ adducts are not inconsistent with the high proton affinity of the OH^- ligands deduced (see the proton shifts) in calculations carried out with *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**) and $[Pt(OH)_3]^-$ (**7**) entities. The hydrogen-acceptor capability of the OH^- ligand attached to the Pt(II) is obviously substantially dependent on the *total* charge carried by the metal adduct. Thus it is greatly enhanced for neutral (**6**) and anionic (**7**) adducts compared with a positively charged metal entity (**5**). To show this, we have protonated the OH^- ligand trans with respect to adenine for the *cis*-$[Pt(NH_3)(OH)_2]$ (**6b**) attached to N7. The direct formation of the aqua ligand is associated with a protonation energy of −256.4 kcal/mol, which is considerably better than any of the numbers shown on the corresponding fourth row of Table 4 (despite that these numbers also include formation of aqua ligands via proton uptake from the adenine moiety). We can again reiterate that for neutral and anionic complexes the formation of an aqua ligand is favored over protonation of the adenine moiety.

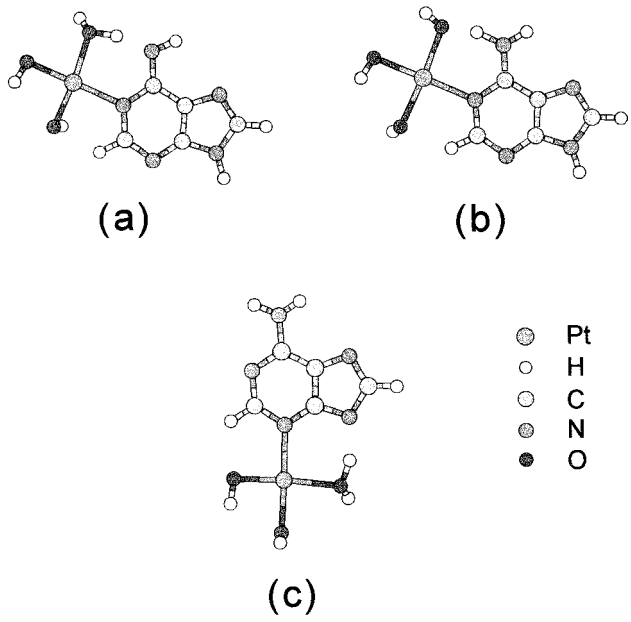


Figure 4. Optimized structures of selected metalated adenine complexes $[Pt(OH)_3]^-$ (**7**). (a) N1 metalated proton shifted form; (b) N1 metalated canonical form; (c) N3 metalated global minimum.

Proton Shift from Neutral Adenine to Pt−OH. Some interesting structures, obtained when investigating the neutral and anionic species with OH^- ligands, deserve a special attention. Of the initial *non*protonated models differing in the position of the Pt binding site, there are two cases where a proton shift from N−H (adenine) to OH^- has occurred, viz., with N1 and N3 platinated adenine and the $[Pt(OH)_3]^-$ (**7**) moiety. If $[Pt(OH)_3]^-$ (**7**) is attached to the adenine N1 position, a proton shift takes place from the N6 amino group to the neighboring OH group at the platinum as shown in Figure 4a. It implies formation of an adenine monoanion (Figure 4a) and a $Pt(H_2O)$ group. Although the high pK_a of the exocyclic amino group (estimated 12−14) and the lower pK_a of an aqua ligand seem to be unfavorable for this proton shift, the observed[45] Pt(II) migration from N1 to N6 in alkaline medium would be consistent with such a preequilibrium. We also note that our previous observations on the migration of a Pt(IV) entity from N1 to N4 of cytosine via an N1, N4 chelate were tentatively rationalized on such a mechanism, hence adenine deprotonation by a Pt−OH group.[46] Let us finally notice that, in this particular case, we have been able to localize the canonical adenine form (Figure 4b) as a secondary minimum on the potential energy surface, being only 0.8 kcal/mol less stable than the proton-shifted form.

Similarly, if $[Pt(OH)_3]^-$ (**7**) is linked to the N3 position, the weakly acidic proton at N9 scrambles to the nearest OH^- ligand of the platinum, forming an aqua ligand there (Figure 4c). In this second case we were unable to find any gas phase minimum energy structure corresponding to a form without proton shift, which is not surprising considering the high acidity of H9.

(45) Arpalahti, J.; Klika, K. D. *Eur. J. Inorg. Chem.* **1999**, 1199.
(46) (a) Lippert, B.; Schöllhorn, H.; Thewalt, U. *J. Am. Chem. Soc.* **1986**, *108*, 6616. (b) Schöllhorn, H.; Beyerle-Pfnür, R.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1986**, *108*, 3680.

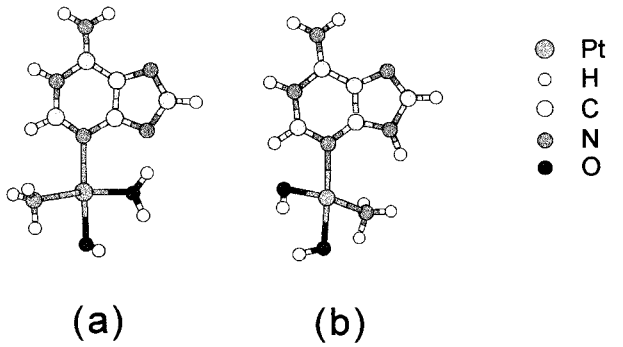


Figure 5. Isomeric forms of N1 protonated adenine with *cis*-[Pt(NH_3)($OH)_2$] (**6b**) ligand at N3 position. (a) Proton shifted; (b) canonical form.

After protonation, the proton-shifted aqua form becomes energetically more favorable than the intended protonated adenine form in all cases when considering **6b** and **7**. In addition, except for one case, the canonical form could not be located even as the second (local) minimum. The only exception is the N1 protonated form of adenine carrying the neutral *cis*-[Pt(NH_3)($OH)_2$] (**6b**) at N3. There we have been able to identify two stable isomeric forms. In the more stable one (Figure 5a) a proton shift takes place from the N9 position to the neighboring OH^- group, yielding a complex in which the adenine is neutral, yet in a tautomeric form different from the preferred one, which carries the proton at N9. The hydrogen could still be stabilized at the N9 position as the secondary minimum, however, with a high energy penalty of 16 kcal/mol. In this energetically less favored isomeric form (Figure 5b), the contact between the OH^- group and the N9 site is prevented by initially rotating the *cis*-[Pt(NH_3)($OH)_2$] (**6b**) unit compared to the optimal position of the nonprotonated, N3-metalated structure. We note that N7 protonation of adenine carrying *cis*-[Pt(NH_3)($OH)_2$] (**6b**) at N3 destabilizes the hydrogen at N9 to such an extent that the above-mentioned energetically less stable isomeric form does not occur. Again, a rare metalated form of neutral A is generated, having the proton at N7 rather than N9.

It should be interesting to test experimentally (in water) tautomerization reactions of A by using a Pt(II) entity carrying an OH^- (acceptor) and OH_2 (donor) ligands cis to the purine nucleobase. While biologically not relevant (N9 position blocked by sugar), the scenario observed for the parent nucleobase A may very well apply to N7 metalated adenosine or AMP. We should nevertheless point out that the proton shifts in our calculations occurred primarily because we attempted to enforce a ring protonation in order to study adenine protonation energies. As noted above, a direct protonation of the OH^- ligand is in general more favorable, though it does not rule out that in some cases the most stable structure will consist of a rare adenine tautomer. Complete evaluation of all possible options is beyond the scope of this study and will be carried out soon.

Comparison of the DFT/Becke3LYP Results with the ADF Method. As mentioned above, we have reevaluated part of the results with the DFT method combined with the ZORA relativistic approach as implemented in the ADF code. The main motivation for this extension of the calculations was to get some insight into the error margins of the calculated results. The ADF results are summarized as Supporting Information in the Web edition of the journal. For nonmetalated adenine, the ADF code provides systematically slightly lower (in absolute value) gas phase protonation energies compared with the Becke3LYP calculations. The difference is, however, less than 3%. Both methods provide close to identical relative values of gas phase protonation energies. The Becke3LYP absolute values are very close to values obtained with conventional techniques including electron correlation,[10,11] which represent current reference values for protonation energies of isolated adenine.

Comparison of the two methods for the platinated adenines can be summarized as follows. Both methods clearly show that protonation occurs in general at the N1 position after N7 metalation, closely followed by a protonation of the N3 position. Both methods identically predict that attachment of a neutral Pt adduct to the ring nitrogens reduces the proton affinity of the remaining ring nitrogen sites by few kilocalories per mole compared to a nonmetalated base. Both methods also consistently show that binding of +1 Pt adduct to the ring has a considerably smaller effect on the protonation energies compared to attachment of a proton (cf. Table 3 vs Table 2 of this paper and Table 3 vs Table 2 in the Supporting Information). Finally, both methods predict very similar relative values of protonation energies for adenine that is metalated by a neutral Pt adduct. On the other hand, as for nonmetalated adenine, the ADF code provides systematically smaller absolute values of the gas phase protonation energies. Further, for Pt adducts carrying charges of +2, +1, and −1, we noticed that the two methods provide for some protonation sites different relative protonation energies, with the maximum difference between the two methods reaching as much as 5 kcal/mol. We do not want, at this stage, to suggest which of the two techniques is more accurate. Rather, the reader can consider the differences between the two data sets as a solid example of the error margins of this type of calculation for gas phase proton affinities for nucleobases and metalated nucleobases. It is obvious that the ADF ZORA method has a more sophisticated treatment of the relativistic effects. At the same time, the Becke3LYP calculations provide better values for a bare adenine, indicating this method has a better basis set and better approximation of the electron correlation effects. Nevertheless, the observed differences are not large and do not affect any conclusion of the present paper. Note also that the results of the present paper are based on performing around 200 gradient geometry optimizations; thus the level of calculations is appropriate. We nevertheless plan to carry out high-level reference calculations for few selected structures soon.

Concluding Remarks

Protonation of nucleic acid bases and its influence by a nucleobase metalation are important processes that can affect certain aspects of nucleic acid structure and function. Therefore, protonation of nucleic acid bases, nucleosides, nucleotides, and other model compounds have been widely studied by condensed phase and solid phase experiments. In the present paper, we have systematically calculated gas phase protonation energies of adenine and platinated adenines using the DFT technique and compared them with available condensed phase experimental data. The analysis shows that while in the gas phase the pure electrostatic effects associated with the charge on the metal entity are the dominating contribution, in the condensed phase situation these electrostatic effects are mostly eliminated. Despite this, the comparison shows that the intrinsic gas phase trends correlate surprisingly well with the available condensed phase data, although we obviously suggest some caution concerning a quantitative interpretation of the data. It concerns items such as the preferred site for a protonation for a given metal entity, difference of the effect of protonation and metalation on the basicity of nucleobases, and others. Therefore, the gas phase data provide a very useful complement to the condensed phase and X-ray experiments. Gas phase studies appear to be capable

of providing valuable qualitative predictions in cases when unambiguous condensed phase or X-ray data are not available. Further, a systematic comparison of intrinsic proton affinities of metalated as well as nonmetalated nucleobases with condensed phase pK_a values is important to understand the effect of solvent and counterions on the metal-assisted proton shift processes. Knowledge of the intrinsic gas phase trends may also be very useful for a qualitative assessment of the magnitude and direction of proton shift processes in nucleic acids. In nucleic acids, the metalated nucleobases may be exposed to the solvent screening effects to a different (presumably smaller) extent compared to experiments on model systems.

We suggest that the present paper should be considered as a comparison of proton shift processes in nucleobases in two entirely different environments rather than as a comparison between theory and experiment.

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Supporting Information Available: Listings of protonation energies of isolated adenine in neutral and single protonated forms as well as of platinated adenine species with Cl^- ligands obtained from ADF calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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