Chapter 15

INTERACTION OF METAL CATIONS WITH NUCLEIC ACIDS AND THEIR BUILDING UNITS

A comprehensive view from quantum chemical calculations

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Abstract: This chapter summarizes the main achievements in quantum chemical modeling of metal-nucleic acid interactions. The topics discussed cover a wide range of problems taken from bio-inorganic chemistry starting from the nature of metal-nucleobase and metal-phosphate interactions, through joint binding modes to the phosphate and nucleobase moieties in nucleosides, up to the mechanism of action of platinum-based metallodrugs. The examples shown are of biological relevance and are aimed at understanding experimentally known phenomena.

Key words: DNA, RNA, nucleic acids, metals, quantum chemistry, DFT

1. INTRODUCTION

After the four basic elements, O, C, H, N, sodium and potassium are the most frequented components of the human organism.[1] Under physiologic conditions, these two metals serve as charge compensating species and provide with the proper osmotic pressure in the living cell.[2]

Nucleic acids (NA) are negatively charged polyelectrolytes whose charge is compensated mainly by cations condensed on their surface. While monovalent cations mostly bind in a non-specific manner, divalent metal cations are apt to occupy structurally well-defined binding positions in the

J. Šponer and F. Lankas (eds.), Computational Studies of RNA and DNA, 389–410.

RNA and DNA architectures and, thereby, to fulfill important structural and biochemical functions. They, for example, participate in the stabilization of the three-dimensional architecture or provide with bio-catalytic properties.

Although metal-NA interactions belong to an advanced branch of experimental bio-inorganic chemistry, accurate theoretical studies in this field have yet become possible in the last decade, especially due to the development of DFT-based quantum chemical techniques.[3]

In this chapter we summarize those achievements in the quantum chemical investigations of metal-NA interactions which pointed beyond the level attainable by common experimental methods. Computational studies are able to provide a simultaneous picture of the structure, stability and electronic properties of the studied systems, which is important to understand the biochemical functions. As with all modeling approaches, one has to keep in mind the limitations introduced into the model. It is pretty challenging to relate gas phase data to properties observable in physiological DNA and RNA. In addition, site-specific binding of metal ions obeys the rules of coordination chemistry. Thus, metal cations have to be surrounded with properly situated ligand field to provide a realistic picture of their interactions. This adds another level of complexity to the models used for these systems and shows that metal cations are more sophisticated entities than to treat them as charged spheres. A proper description of metal cations must account for both the electrostatic and molecular orbital effects. As a consequence, quantum chemistry is superior to the force field techniques at describing interaction of metal cations with NAs.

2. METAL-NUCLEOBASE INTERACTIONS

The nominal charge of the polynucleotide chain is contributed by the phosphate group. Nevertheless, nucleobases themselves are excellent N and O donor ligands, forming well-defined binding sites primarily for divalent cations. Under physiological conditions, i.e. in aqueous solution, metal cations are always present in a hydrated form. Thus, the relevant model for the site-specific binding of metal cations to nucleobases must always include the hydration shell of the metal cation. Then, the hydrated metal cation may interact with the nucleobase either in the (i) outer shell, (ii) inner shell, and occasionally, (iii) bidentate fashions (Figure 15-1).

While the geometry is largely determined by the coordination chemistry of the cation, the binding strength depends on the ligand type. Nucleobases offer several binding sites of various interaction strengths. Thus, one of the main questions is to determine the binding strength depending on the

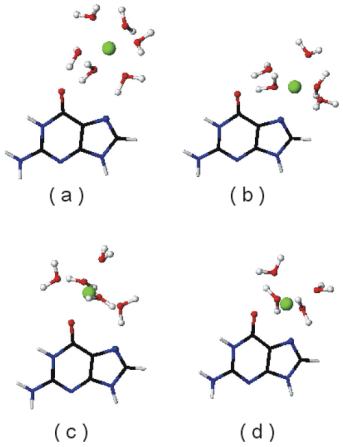


Figure 15-1. Binding modes of octahedral Mg^{2+} cations to guanine. Structures were obtained from optimizations at Becke3LYP/6-31G** level of theory. (a) Outer-shell binding; (b) inner shell binding to N7; (c) inner shell binding to O6; (d) bidentate binding mode.

metalation site for a given base and cation. Moreover, various cations exhibit different affinity towards a given type of binding.

This issue was systematically studied for the inner and outer shell binding of hydrated Mg^{2+} and Zn^{2+} cations to guanine.[4] These cations have the same charge and almost the same radius, yet, they play very different roles in chemistry and biology. The thermodynamical driving force of cation binding was estimated on the basis of three different thermodynamic cycles. As for the inner shell complexes, the computed results reveal that the N7 position of guanine has a greater affinity towards Zn^{2+} than Mg^{2+}, while the O6 position exhibits a similar propensity towards both cations. For the outer shell binding the gas-phase results were not accurate enough to unambiguously show preference towards either cations. Decomposition of the interaction energies into pairwise terms (see below) has revealed that inner shell binding of hydrated zinc to guanine can be viewed as a process in which the base is first metalated and then hydrated, as the dominant part of the total stabilization is due to the interaction between the bare cation and the nucleobase. In contrast, for the analogous complex with magnesium the metal-base term is reduced with respect to zinc, and, therefore, the complex is shifted more towards a complex of nucleobase with hydrated cation.

Electronic background of the difference between the complexes of group IIb and IIa cations is examined in Ref. 5. While interaction of bare IIa group cations with nucleobases is dominated by Coulombic interactions, the covalent d-orbital-lone pair interaction strengthens binding of IIb metals.

A more elaborated approach has pointed at the necessity to include solvation models into the calculations when binding affinities are addressed

in solution. One recent study[6] compares geometrical parameters of an outer shell complex of guanine with $[Mg(H_2O)_6]^{2+}$ optimized in three different approximations: (i) with the AMBER force field in vacuo, (ii) by B3LYP/ 6-31G(d,p) optimizations in the gas-phase and (iii) at the same level as (ii) but within the framework of the CPCM (COSMO polarized continuum) solvent model. The force field calculation results in overly long Mg - nucleobase distance and in a change of the experimentally suggested H-bonding pattern. Inclusion of the solvation model into the DFT-optimizations pushes the nucleobase away from the metal because the solvent considerably screens the electric field of the hydrated cation. Finally, this study suggests that in solution relatively accurate interaction energies can be obtained when using gas-phase optimized geometries from Becke3LYP/6-31G(d,p) calculations and computing binding energies by the CPCM method (cf. also section 3).

Other aspects of metal-nucleobase interactions will be addressed in section 6.1., in connection with platinated nucleobases.

NMR shifts and spin-spin coupling constants for the hydrated magnesium-guanine system have been reported recently.[7] This study illustrates, that the intermolecular spin-spin coupling constants ^1J(X,O6) and ^1J(X,N7) (X = Mg^{2+}, Zn^{2+}) can be successfully applied to differentiate between outer shell and inner shell binding modes.

In contrast to Mg^{2+}, which has a clear preference towards the hexa-coordinated form, in biological systems Zn^{2+} might be present also in tetra-coordinated complexes. An in-depth database analysis reveals that, in general, Mg^{2+} is more susceptible towards O-donor ligands while Zn^{2+} rather favors N- and S-donors. Mn^{2+} represents a borderline between Zn^{2+} and Mg^{2+}.[8]

There is a branch of studies considering interaction of bare metal cations with nucleobases. Although these contributions are of high value from methodological point of view, they are not discussed in the frame of this review. For details, refer to Ref. 9.

3. METAL-PHOSPHATE INTERACTIONS

The nominally -1 negative charge of each nucleotide unit in NAs is carried by the phosphate moiety. Therefore, the phosphate groups form the primary metal binding sites in NAs. To a large extent the charge of NAs is compensated by monovalent cations partly condensed on the surface of the macromolecule. Note, however, that since polar solvent efficiently screens the negative charge of phosphates, the individual phosphates for most of the time do not interact directly with an ion. In addition to this, (divalent) metal cations specifically bound to the phosphate groups may have important

catalytic functions: they can catalyze the hydrolysis of the phophodiester linkage in RNA.

Crystal database analyses show, that in contrast to the analogous carboxylates, metal binding to the phosphinyl (-PO_2^--) fragment of the phosphate group does not take place in a symmetric fashion: i.e. in most published crystal structures the electron density maxima are outside the bisector of the O = P = O angle.[10] A thorough ab initio analysis of variously charged mono- and diphosphate derivatives with mono- and divalent cations has shown that the asymmetric coordination of the metal cation is likely to be caused by the direct participation of polar particles, such as water, in the cation binding.[11] This again calls attention on the fact, that biologically relevant models must include the proper number of ligated water molecules to represent the hydration sphere of the cation.

Petrov et al. analyzed interaction of hexahydrated magnesium with dimethyl-phosphate anion and also concluded that to provide reliable estimates of the binding affinities in solution, it is absolutely essential to include sufficiently accurate solvation models into the calculations.[6]

Metal catalyzed hydrolysis of the phosphodiester bonds in NAs is a long-disputed problem of bio-inorganic chemistry. One study suggested, that the process involves the cleavage of the C5'-O bonds of the sugar rather than that of the P-O bonds of the phosphate.[12] Quantum chemical studies of hydrated dimethyl-phosphate models with alkali and alkaline earth cations point at the role of metal cations in these processes. It has been found that catalytic activity of a metal cation is due to its ability to (i) shield the negative charge of the phosphate group and (ii) simultaneously weaken the C-O bonds in the model compounds. This can be correlated with the charge abstracting and polarizing properties of the cations which change in the Mg^{2+} > Li^+ > Ca^{2+} ~ Na^+ > K^+ order. The entire mechanism of the metal-assisted phosphodiester hydrolysis in the hammerhead ribozyme was investigated by Torres et al.[13] The reaction proceeds according to an $S_N2(P)$ scenario and is initiated by deprotonation of the 2'-OH by a hydrated $Mg^{2+}OH^-$ cofactor bound to the phosphate moiety. In the forthcoming steps of the reaction the role of the metal center is twofold: (i) via its hydration shell it stabilizes the intermediates and transition state complexes formed, and (ii) donates a proton from its hydration shell to facilitate the loss of the leaving group in the rate determining step of the reaction.

Let us note here, however, that the mechanism of phosphodiester hydrolysis is strongly dependent on the local RNA architecture and often requires cooperation of remote segments of the macromolecule.[14] Sometimes, even two cations might be active in the process. Thus, exploring the mechanism of phosphodiester hydrolysis in biologically relevant systems is a very intricate and computationally challenging task. Indisputably, combined

QM/MM techniques are the hot candidates to solve t[illegible]roblem. However, no QM/MM techniques suited for reliable studies of KA were presented so far.

4. METAL-NUCLEOTIDE INTERACTIONS

Combination of the N- and O-donor sites of nucleobases with the negatively charged phosphate oxygens gives rise to versatile metal-binding modes in nucleotide structures. Due to their distinct biological relevance, characterization of metal-nucleotide interactions in the literature is restricted to the N7-binding in purine nucleotides.

Hydrated zinc and magnesium group divalent cations bind to the N7 position of purine nucleotides to form very strong H-bonds between the cation and anionic oxygen atoms of the phosphate group (see Figure 15-2a). Thereby the water shell exerts a screening of the negative charge concentrated on the phosphate moiety and at the same time influences the backbone geometry. The pairwise nucleotide-cation interaction provides the major contribution to the stabilization of these complexes, which can be characterized with a high non-additivity (17-28% of the total interaction energies). The non-additivity term expresses the shielding of the metal-nucleotide electrostatic attraction by the water shell and is larger for Zn^{2+} than for Mg^{2+}.[15]

As non-additivity and polarization effects are absented from standard force fields, the force fields are not suitable to capture the energetics of the metal - nucleotide interactions with a satisfactory accuracy. The SIBFA polarizable molecular mechanics force field of Gresh provides a partial remedy for this problem. It is parametrized to describe interaction of hydrated Zn^{2+} and Mg^{2+} cations with 5'-guanosine-monophosphate and properly includes polarization and charge transfer terms necessary to treat this kind of systems.[16]

Due to the high non-additivities, the pairwise interaction energies are not representative enough for the strength of the metal-nucleobase and metal-phosphate interactions. In contrast, the geometrical parameters well reflect the balance of all contributing effects and can be used to indirectly evaluate binding selectivity in these systems. From systematic changes in the interatomic distances, electronic energies and interaction energies the binding selectivity of divalent cations towards N7-binding varies in the following order: $Cu^{2+} >> Zn^{2+} = Cd^{2+} > Mg^{2+}$. In fact, the binding selectivity

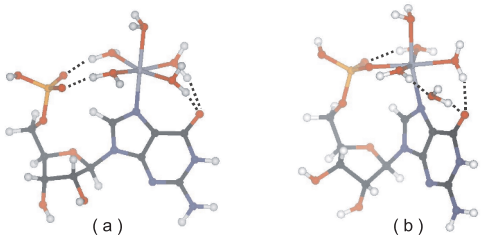


Figure 15-2. Binding of penthydrated Zn^{2+} to the N7 site of 5'-guaninosine-monophosphate. Optimized structures obtained from HF calculations with the 6-31G* basis set and Christiansen's pseudopotential on Zn. (a) Inner-shell binding to N7(G), outer shell binding to the phosphate; (b) inner shell binding both to the nucleobase and phosphate. The dotted lines indicate H-bonding contacts. Note that the highly polarized water molecules from the cation's hydration shell form extremely strong H-bonds.[15] Binding of cations in NAs would be affected by the overall topology of NAs which destabilize the water bridges to phosphates.[15]

is the result of the balance between the water - cation and cation - nucleobase contributions. Let us mention here, that the binding selectivity in biological (solution) systems is determined by pretty tiny energy differences (on the scale of 3-10 kcal/mol), while the absolute gas phase binding energies are around 300 kcal/mol. This underlines the accuracy of the methods needed to evaluate such systems.[17] The gas phase data are typically dominated by the electrostatics which is eliminated in polar environment. However, specific non-electrostatic effects well reflected by the QM calculations are often fully transferred into solution.[15,17]

Simultaneous inner-shell binding to N7(G) and the phosphate oxygen (see Figure 15-2b) attenuates the water-phosphate contacts. On the other hand, inner shell binding to the phosphate does not change the above established ranking of cations related to their selectivity towards N7-binding.[17]

It has been found that inner shell binding of guanine to hexahydrated cation-phosphate complexes substantially weakens the metal-phosphate outer shell binding. Thus, the phosphate group recognizes when water is replaced by guanine in the hydration shell of the cation. However, it is unable to recognize the Mg^{2+} to Zn^{2+} substitution, assuming the metals adopt the same coordination mode.[17] These findings are of key importance at unraveling the principles of metal-phosphate recognition in NAs.

5. INTERACTION OF METAL CATIONS WITH BASE PAIRS

It has been suggested long ago, that coordination of metal cations to the N7 position of the purine base enhances the stability of GC and AT base pairs.[18]

The enhancement is due to polarization effects and classical electrostatic attraction between the cation and the remote base. A very convenient approach has been found to asses the polarization contribution to the base pair enhancement for the interaction of cations with base pairs.[19] The following formulae decomposes the total interaction energy:

$$\Delta E_{total} = \Delta E_{Me\text{-}B1} + \Delta E_{B1\text{-}B2} + \Delta E_{Me\text{-}B2} + \Delta E_3 \quad (1)$$

where $\Delta E_{Me\text{-}B1}$ is the pairwise interaction energy between the metal (Me) and the nucleobase directly bonded to it (B1); $\Delta E_{B1\text{-}B2}$ stands for the pairwise base-base (B1 - B2) interaction energy (see Figure 15-3), and $\Delta E_{Me\text{-}B2}$ expresses interaction between the hydrated cation and the remote base (mostly long-range electrostatics). Finally ΔE_3 is the three-body (nonadditivity) term that reveals how the three interacting species cooperate when forming the complex, thus, polarization of the base pairing by the N7 metal binding. ΔE_3 and $\Delta E_{Me\text{-}B2}$ term contribute to the enhancement of the base pairing.

Table 15-1 compares the strength of various intermolecular contacts in the metalated GC pair, for bare and pentahydrated cations. While the pairwise GC interaction is not affected by the presence of the cation's first hydration shell, the three-body term becomes strongly attenuated in the hydrated systems due to screening and polarization re-distribution (compare the entries of bare and hydrated Ca^{2+} and Ba^{2+}). Further, the pairwise $\Delta E_{Me\text{-}B1}$ terms of analogous systems with Mg^{2+} and Zn^{2+} illustrate the differences (section 2) in the cation binding affinity of the N7(G) position. The polarization enhancement (ΔE_3) depends also on the polarity of the base

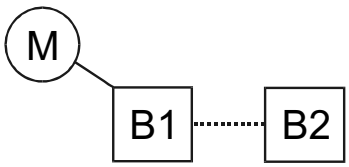


Figure 15-3. Schematic model of metalated base pairs. B1, B2 = nucleobases, M = pentahydrated metal.

Table 15-1. Interaction energies (kcal/mol) for various metalated base pairs computed at the MP2/6-31G(d)//HF/6-31G(d) level*.

Me	B1	B2	$\Delta E_{\text{Me-B1}}$	$\Delta E_{\text{Me-B2}}$	$\Delta E_{\text{B1-B2}}$	ΔE_3	ΔE_{total}
-	G	C	–	–	–26.2	–	–
Mg^{2+}	G	C	–198.7	–	–26.0	–	–243.8
$[Mg(H_2O)_5]^{2+}$	G	C	–89.3	–1.5	–26.4	–8.1	–125.4
$[Mg(H_2O)_5]^{2+}$	PG	C	–237.7	–0.7	–23.5	–5.9	–303.9
Zn^{2+}	G	C	–237.2	–	–	–	–285.4
$[Zn(H_2O)_5]^{2+}$	G	C	–93.8	–1.5	–26.4	–8.7	–130.4
Ca^{2+}	G	C	–133.9	–3.0	–25.8	–10.1	–172.7
$[Ca(H_2O)_5]^{2+}$	G	C	–82.6	–1.7	–26.3	–5.2	–115.8
Ba^{2+}	G	C	–118.3	–2.0	–25.6	–9.6	–156.1
$[Ba(H_2O)_5]^{2+}$	G	C	–71.2	–7.7	–23.2	–2.1	–104.1
-	G	G	–	–	–17.7	–	–
$[Mg(H_2O)_5]^{2+}$	G	G	–89.8	–9.5	–19.9	–10.4	–129.6
$[Mg(H_2O)_5]^{2+}$	PG	G	–274.3	–8.5	–12.9	–7.0	–302.7
-	A	U	–	–	–12.3	–	–
$[Mg(H_2O)_5]^{2+}$	A	U	–59.6	–9.8	–9.3	–0.4	–79.1
$[Mg(H_2O)_5]^{2+}$	PA	U	–244.3	–9.0	–8.1	+1.9	–259.5

* Me = metal entity; B1 = proximal base interacting with the cation (PN stands for nucleotide), B2 = remote nucleobase, $\Delta E_{\text{Me-B1}}$, $\Delta E_{\text{Me-B2}}$ = pairwise metal - nucleobase interaction energy terms; $\Delta E_{\text{B1-B2}}$ = pairwise nucleobase-nucleobase interaction energy; ΔE_3 = three-body term; ΔE_{total} = total interaction energy as defined in equation 1. Data are taken from Refs. 19 and 20.

pair, thus it is quite substantial for the GC and GG base pairs and pretty small for the AU pair. When the proximal base is replaced by a nucleotide, the metal binding is improved because of the favorable electrostatic contacts between the cation and the anionic phosphate group. On the contrary, this reduces the three-body term.

No significant differences were observed in the electron topology of the GC, GG and AU base pairs metalated with pentahydrated Mg^{2+} at N7.[20] NBO was not able to detect any significant charge transfer effects caused by the cation coordination. Therefore, the polarization base pair enhancement upon cation binding is best interpreted on the basis of "the electrostatic response of the complexes to the presence of the cation".[20]

6. NUCLEIC ACIDS AND METALLODRUGS

Introduction of cisplatin in cancer treatment ignited a series of experimental[21] and, later, theoretical studies aimed at describing its mechanism of action. Clinical application of platinum compounds is restricted due to their high cytotoxicity and the experimental research was recently shifted towards other

less toxic DNA-binder metal-based drugs. These studies also benefit from findings of theoretical investigations on square planar platinum complexes, which are also of interest for the basic bioinorganic chemistry.

6.1 Platinated Nucleobases

A systematic study on the proton affinities of platinated adenines has revealed that the *gas-phase* protonation energies depend primarily on the overall charge and to a much lesser extent on the location of the metal.[22] The charge-dependence is enormous, yet it appears to be completely abolished in *aqueous solution,* where the corresponding pKa values are essentially independent on the charge of the metal adduct in the range of charges +3 to –1. In contrast, the subtle variations in the interplay between different cation binding and protonation sites stem from molecular orbital effects and appears to be translated from the gas phase to solution. Figure 15-4 summarizes the computed gas-phase proton affinities as a function

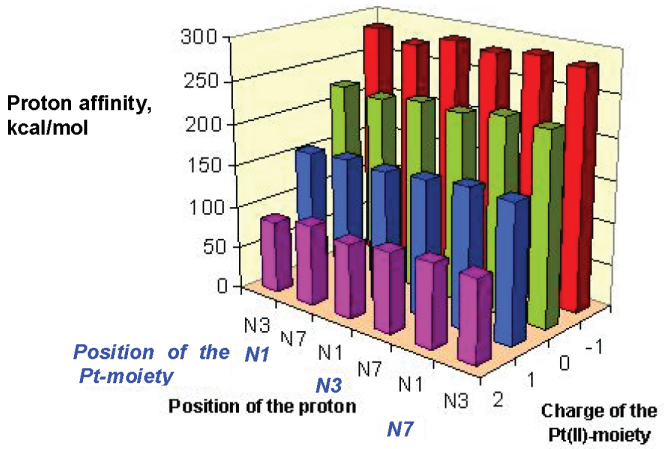


Figure 15-4. Gas phase proton affinity of platinated adenines for different combinations of platinum binding and protonation sites, as a function of the total charge of the metal adduct. Computed results were obtained at Becke3LYP level of theory using the 6-31G* basis set and the lanl2dz pseudopotential on Pt.

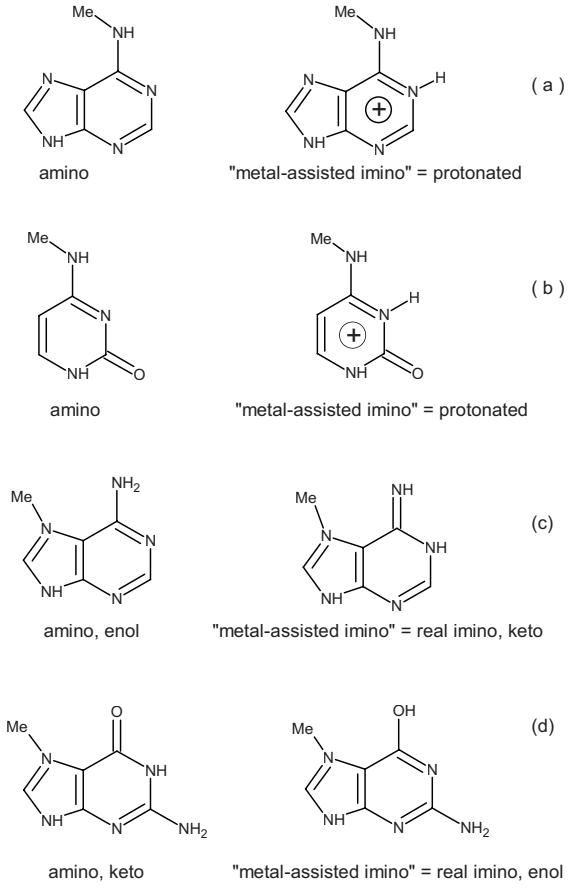


Figure 15-5. Metal-assisted tautomeric forms of (a) N6-metalated adenine, (b) N4-metalated cytosine, (c) N7-metalated adenine, and (d) N7-metalated guanine. The amino metalation affects the tautomerism by non-electrostatic effects and the tautomerism is thus seen in bioinorganic experiments. The N7 metalation results in primarily electrostatic influence on tautomerism which is likely fully eliminated in polar environments.

of the total charge as well as the position of the proton and platinum moiety. For a given platinum and proton position one can recognize an almost linear relationship between the proton affinity and the charge of the platinum adduct. On the other hand, for a given total charge of the metal adduct the maximum is reached if the platinum is at N7 and the proton binds to N1. This is in qualitative agreement with experimental observations from solution studies and at the same time illustrates that site-dependence of the protonation energy stems from molecular orbital effects, because it is independent on the total charge of the metal-adduct.

The improvement of the protonation energy is larger for the N4-platinated cytosine than for N6-mercurated adenine (Figure 15-5a and b) and can be correlated with the shift of the π-electron density from the nucleobase to the metal center in the major tautomeric forms. Thus, metal-assisted tautomerism (protonation) of these otherwise charge-neutralized systems is unambiguously dominated by molecular orbital (nonelectrostatic) effects and this is why it is seen in all solution and x-ray experiments. Metal-induced shift of the protonation equilibria and thereby stabilization of the rare tautomeric forms may give rise to stabilization of mispairs in DNA.[23] This was studied for amino-metalated base pairs. For example, stabilization of the N1-protonated form of adenine by N6-metalation with Hg^{2+} gives rise to the formation of stable AH^+.C and AH^+.G base pairs. Even more considerable stabilizing effect is proposed for the CH+.G Hoogsteen base pair platinated at N4(C), where metalation enhances the stability of the mispair by ca. 20 kcal/mol. Interbase proton transfer processes may also be accelerated by amino-platination. For example, proton transfer from the N1 position of guanine to N3 of cytosine results in the formation of a $CH^+.G^-$ ion pair. The energy difference between the ion pair and canonical forms of this base pair is ca. 24 kcal/mol in the gas-phase. Due to the stabilization of the protonated (i.e. "rare tautomeric") form in N4-platinated cytosines, however, this energy difference can be reduced to the half by metalation.[23]

N7-metalation also changes the gas-phase tautomeric equilibria of purine bases. However, the effect vanishes for neutral metal adducts.[24] Charged metal-adducts stabilize the major (amino) and minor (imino) forms of guanine and adenine, respectively (Figure 15-5c, d). As the shift of tautomeric equilibria in these gas-phase structures is due to electrostatic effects, they are not expected to occur in solution or crystal.

Catalytic scenarios (cytosine deamination) brought about by platinum in N3-metalated cytosines are discussed in Ref. 25.

6.2 Interaction Strength of Platinated Base Pairs

Enhancement of the base pairing by N7 binding of $[Pt(NH_3)_3]^{2+}$ is much larger than that caused by the inner shell binding of hydrated Mg^{2+} or Zn^{2+} cations.[26] The electronic changes stimulated by Pt-coordination in the structure of nucleobases implicate even more pronounced changes in the stability of platinated base pairs.

A correlation has been found between the N1-acidity of N7 platinated guanines and the strength of the GC base pairs in the gas-phase.[27] The enhancement of the base pair is caused primarily by polarization effects, while the N1-acidity is mostly influenced by ionic-electrostatic forces with

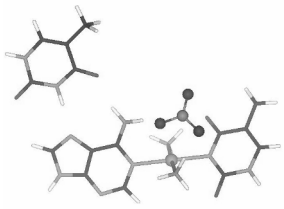


Figure 15-6. Counterion effects in platinated systems. Note, that the nitrate ion is coordinated to the platinum(II)-center in a pseudoaxial position and entirely biases the experimental outcome, since it belongs to the buffer.

some additional polarization effects. While the computed gas-phase N1-deprotonation energies can be correlated with the solution pK_a values,[28] there is no correlation with the $K_{GC,DMSO}$ association constants measured in solution. This led to the conclusion that the deviation (in the condensed phase experiment) from the expected trend is likely to be caused by environmental effects, such as unwanted (biasing) presence of counterions in the experimentally studied system.

In fact, similar observations were made for the AT Hoogsteen pair platinated at N1(A)-position.[29] While the computed interaction energy for the AT Hoogsteen pair is –14.1 kcal/mol, platination enhances its strength to –18.8 kcal/mol, primarily due to the long-range electrostatic attraction between the charged Pt^{2+} cation and thymine. In contrast, no base pairing was detected by experiment. When including, however, a nitrate anion coordinated in pseudoaxial position to Pt^{2+} (see Figure 15-6) the base pair stability dropped down to –11.6 kcal/mol, because of the compensation of the charge on Pt^{2+}. This suggests that the buffer anion determines the experimental outcome.

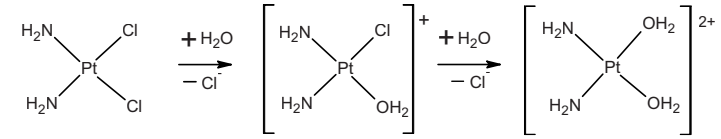
6.3 Studies on Cisplatin Binding to Nucleobases

Cisplatin is the first anticancer drug successfully employed in the clinical treatment of various kinds of cancers, such as small cell lung, ovarian, testicular, head and neck tumors. From chemical point of view it is a square planar molecule (see Figure 15-7) whose hydrolysis results in the formation of the active form suitable to bind to DNA.[30,31] Cisplatin binds to the G-reach segments of DNA in such a way to form intrastrand cross-links between two adjacent guanines. The antitumor activity of the drug is associated with the bending of the DNA as a consequence of the cross-link formation. The binding was modeled by classical MD simulation.[32] In fact, the binding is a ligand exchange reaction between the hydrated form of cisplatin and guanine, in which the water ligand is replaced by the nucleobase. The ligand exchange reaction proceeds via a trigonal bipyramidal transition state and is kinetically

controlled. A comparative study on cisplatin binding to guanine and adenine has shown, that for guanine the binding process is energetically more favored both from kinetic and thermodynamic points of view.[33] The extra stabilization of the transition state with guanine is attributed to (i) a very strong H-bond between the ammine ligands of the Pt-moiety and O6 of guanine as well as (ii) the significantly stronger electronic interaction between the Pt and the guanine ligand as compared to that between Pt and adenine. In addition, the diaqua complex has been found to exhibit a higher selectivity towards guanine.

There are controversial views on the role of π-back-donation in cisplatin-nucleobase binding.[34] A study on hypothetical square planar Pt-complexes containing CO-ligands concludes that, even with the strongest π-acids, π-back-donation may operate exclusively in the zero oxidation state. Thus, it hardly has any role in stabilizing the cisplatin-nucleobase adducts. In addition, it is suggested, that non-coplanarity of the cisplatin and nucleobase moieties also impedes the π-interactions in these systems. On the contrary, a recent orbital analysis of guanine complexes cross-linked with cisplatin reports on a rare binding combination between the d_{xy} atomic orbital of Pt and the antibonding π*-orbital of guanine.[35]

In complex biological matrices, such as the cell, there are several potential targets of cisplatin binding. In fact, Pearson's theory predict a larger binding affinity of cisplatin towards S-donor ligands.[36] Considering the high concentration of cystein and methionine in the cell one can thus almost rule out that cisplatin reaches DNA. A comprehensive theoretical rationale on the competition of S- and N-donor ligands in cisplatin binding has been given by Deubel.[36-38] From an analysis of the Pt-L binding energies in various $[Pt(NH_3)_3L]^{2+}$ (L = ligand) complexes he has inferred that intrinsic binding affinity of cisplatin is higher towards N-donors than S-donors in the gas-phase. However, solvation effects, particularly in polar solvents, strongly alter the gas-phase trends, and eventually in water S-donors become more favorable targets than the N-donor ligands. At ε = 78.4 (water) only



cis-diamminedichloro-platinum = "cisplatin"

Figure 15-7. Cisplatin and its hydrolysis products.

methyl-guanine remains competitive with neutral S-donors.[36] The kinetic control of cisplatin binding is determined by three factors: (i) the nucleophile, (ii) the substituents of the nucleophile, (iii) the environment, i.e. solvation effects. Computed gas-phase activation energy of the nucleophilic substitution reaction of cis-$[Pt(NH_3)_2(OH_2)Cl]^+$ with NH_3 has been found to be lower than that of H_2S, showing that N-donors may be intrinsically better nucleophiles than S-donors. However, this trend can be easily reverted by substituent as well as environmental effects. While in a more polar environment, i.e. water, the scenarios with sulfur nucleophiles are kinetically more favorable in the low ε regions of chromatin binding to the guanine-reach regions of DNA is the decisive reaction mechanism.[37] Ammine loss of cisplatin derivatives via nucleophilic attack of S-donors at the platinum center is a possible way of cisplatin inactivation in the cell. Thermodynamic and kinetic aspects of this process were studied for a series of substituted $[Pt(NH_3)_4]^{2+}$ derivatives both in gas-phase as well as in aqueous solution. The substrate selectivity of the reaction is determined by the kinetic and thermodynamic trans-effect of the ligands connected to the platinum entity. The following order is established for the trans effect of ligand L in complexes with composition of $[Pt(NH_3)_3L]^{2+/+}$: S-donors > N-donors > water = 0. Similarly, cis-$[Pt(NH_3)_3MeS]^+$ has been selected as a substrate to model the effect of various nucleophiles on the kinetic and thermodynamic control of the deactivation process, i.e. ammine loss reaction. It has been shown that adenine is unable to replace ammine from the model substrate both for kinetic and thermodynamic reasons. Similarly, thiolates can be rejected due to the unfavorable activation energy of the substitution reaction. In contrast, nucleophiles like Met, Cys, and Gua may play a more significant role in the deactivation of cisplatin.[38]

Cisplatin induced depurination reaction of nucleosides has been studied by Baik et al.[39] They have described the cleavage of the glycosidic bond in N7 platinated as well as protonated forms including solvent effects in the SCRF (Self-Consistent-Reaction-Field) approach. The lack of reactivity in the platinated systems is explained by the very similar activation energy obtained for non-metalated guanosine and its platinated adduct. In contrast, protonation causes a significant stabilization of the transition state and thereby accelerates the depurination reaction.

7. CATION-Π INTERACTIONS

In principle, not only the heteroatoms of nucleobases may form binding sites for metal cations. It is for example well known that metal cations may interact with the π-electron system of aromatic compounds. The prototype of these interactions is depicted in Figure 15-8a, which shows the optimized

geometry of magnesium-hexahydrate with benzene, obtained from optimization at the HF/6-31G* level of theory. To catch a similar minimum for the cytosine - magnesium-hexahydrate system (see Figure 15-8b), however, one has to introduce geometrical constraints, as full optimization will always lead to the considerably more stable in-plane binding.[40,41] As nucleobases have no intrinsic propensity towards participating in cation-π interactions, occurrence of cation-π-like geometries in NA crystals is accidental and they are fixed by other interactions.

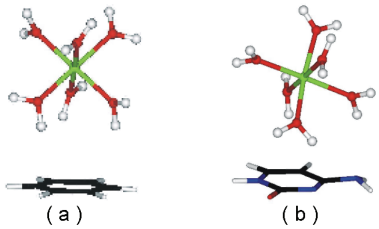


Figure 15-8. Cation - π interaction between magnesium-hexahydrate and (a) benzene, (b) cytosine. Geometry optimizations were performed at the HF/6-31G* level of theory

8. SITE-SPECIFIC BINDING OF CATIONS TO NUCLEIC ACIDS

Interaction of metal cations with NAs is, in fact, a very complex network of numerous contributing effects. All studies summarized above capture only selected aspects of this very intricate process, and, therefore their applicability to project to larger systems is limited.

The recently published approach by Petrov et al represents an interesting step towards understanding the binding of metal cations to NAs in its complexity.[42] They divide the total binding free energy into four contributions on the basis of a thermodynamic cycle approach. At first, interaction energy of the gas-phase system, describing the binding site is determined. In the second step the gas-phase system is immersed into water using e.g. a COSMO-polarized continuum model (CPCM). Interaction between the RNA and the metal cation is considered in the next step, adapting the approach introduced by Bashford and Karplus[43] for the calculation of ionization constants of titratable sites in proteins. Finally, the free energy change upon addition of diffuse electrolyte ions is calculated using non-linear Poisson-Boltzmann equations. The summation of these four contributions gives the total binding free energy in solution.

9. COMMENT ON THE ACCURACY OF FORCE FIELD CALCULATIONS FOR CATIONS

Force field calculations enable an explicit treatment of a very complex matrix consisting of nucleic acids, cations, counterions as well as water molecules. Systems up to 100+ nucleotides can be routinely treated with explicit solvent molecular dynamics method.[44, 45] However, a caution should be taken when evaluating results of MD simulations due to severe force field and sampling limitations.

The most remarkable shortages of force field calculations are the following: (i) they are not able to account for polarization and charge transfer effects, (ii) metal ions are treated as van der Waals spheres with a point charge in the center, disregarding their electronic structure, (iii) they fail to fully capture the competition between water molecules, monovalent and divalent cations for the binding to NAs. The profound effect of the ion on the first-shell ligand waters (which are very strong H-bond donors compared to the bulk waters) is neglected.[15-17,42,46] To overcome these problems specially tuned polarization potentials have been developed, yet they have not so far been introduced in common MD simulations.[16,47] Note that simple polarization force fields would still not capture charge transfer.

In general, performance of MD technique is much better for monovalent than divalent cations.[46,48] Monovalent ions sample rather reasonably on the time scale 10-100 ns, at least for strong binding sites. One has to be more careful with anions such as Cl^-, since anions are polarizable species. Description of divalent cations suffers from large force field imbalances while they sample entirely insufficiently in contemporary simulations. Incorrectly placed divalent ion may behave like an unguided missile in the simulation and cause solute perturbation that is not reparable on a common simulation time scale. [48]

Keeping in mind its limitations the MD technique can still be successfully exploited to study interaction of cations with NAs. For example, MD simulations were applied to examine the role of monovalent ions in stabilizing guanine quadruplexes.[49] Simulations can also be used to detect major ion binding sites in RNA, including key binding sites with very wide free energy minima. These are characterized by delocalized (dynamical) cation binding and thus difficult to capture by X-ray technique.[50]

10. CONCLUSIONS

The uniqueness of quantum chemical methods to simultaneously capture the structure, energy and electronic properties can be fruitfully exploited at studying the interaction of metal cations with NAs.

When applying quantum chemistry to metal ions, it is critically important to distinguish between ionic electrostatic effects and (molecular orbital, polarization) non-electrostatic effects. While the ionic effects are usually not visible in polar environment due to solvent screening the non-electrostatic effects are typically well expressed. Consideration of gas phase experimental or QM data without at least a qualitative inclusion of the solvent effects leads to conclusions that are not relevant outside the gas phase.

In NAs, a great variety of binding sites are available for the cations, represented by the heteroatoms of nucleobases and the charged oxygens of the phosphate groups. Electronic effects control the intrinsic propensity of a cation towards a given type of binding, which cannot be accounted for by standard force field calculations. Site-specific coordination of metal cations causes sensible changes in the protonation and tautomeric equilibria of nucleobases and base pair strengths. QM calculations represent a dedicated tool to study such physical properties. Gas-phase properties can be projected into solution either by using continuum solvent techniques or by following the charge dependence of the gas-phase results.

To understand mechanism of action of DNA-binder metallodrugs, beyond the NAs, one has to consider interaction with other components of the biological matrix.

Assessing the strength of metal-NA interactions calls for combining information from gas-phase quantum chemical calculations with other theoretical approaches used to describe solvation effects as well as polymer-cation interactions. The state-of-the-art approach[42] elaborated by Petrov et al illustrates the way of computational quantum chemistry towards more extended systems, such as RNA and DNA.

In general, the nature of metal-nucleobase, metal-phosphate and metal-nucleotide interactions is a well-explored field of computational quantum chemistry. Thus, the new directions of research should concentrate either on the metal-NA interactions or on mechanistic studies. In the later subject there are a lot of intriguing problems to investigate, e.g. the cleavage of the phosphodiester bond in catalytic RNAs and mechanism of action of metallodrugs. To describe these issues, however, one needs to employ computational methods suited to tackle extended systems, such as properly parametrized QM/MM techniques. QM/MM approaches could also be very useful in future, albeit the methods are currently used in practice only for

proteins. Therefore, there is an urgent need to continue development of plausible computational platforms for this purpose.

ACKNOWLEDGEMENTS

This contribution was supported by the Grant Agency of the Academy of Sciences of the Czech Republic grant No. 1QS500040581 (J. E. Š., J. Š.), by grants No. 203/05/0009 and 203/05/0388 (J. E. Š., J. Š.) from the Grant Agency of the Czech Republic, as well as by the Wellcome Trust International Senior Research Fellowship GR067507 (J. Š.). J. L., J. Š. and J. E. Š. acknowledge for the financial support from NIH grant S06 GM008047, NSF-CREST grant HRD-0318519 and ONR grant N00034-03-1-0116. J. V. B. is very grateful for the financial support from MSMT-NSF grant 1P05ME784. IBP and IOBC are supported by the grants AVO Z5 004 0507 and Z4 055 0506, Ministry of Education of the Czech Republic.

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