Interaction of the Adenine−Thymine Watson−Crick and Adenine−Adenine Reverse-Hoogsteen DNA Base Pairs with Hydrated Group IIa (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) and IIb (Zn^{2+}, Cd^{2+}, Hg^{2+}) Metal Cations: Absence of the Base Pair Stabilization by Metal-Induced Polarization Effects

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Structures and energetics of complexes between the adenine−thymine Watson−Crick (AT WC) and adenine−adenine reverse-Hoogsteen (AA rH) DNA base pairs and hydrated (five water molecules) Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} metal cations were studied using high-level quantum chemical techniques. Binding of the cations to N7 of adenine does not enhance the strength of the base pairing through polarization effects. This is in stark contrast with the results obtained for the GG and GC base pairs. This finding and other recently published data indicate a qualitative difference between adenine-containing (AA,AT) and guanine-containing (GC,GG) base pairs. There are significant changes in the electronic structure of the guanine aromatic system upon cation binding to N7 which propagate toward the H-bonded partner. No such effect has been observed for any adenine-containing pair. The interaction between hydrated cations and adenine is much weaker than that between hydrated cations and guanine due to the low dipole moment of adenine. Furthermore, the cation and its surrounding polarized water molecules interact with the nitrogen atom of the adenine amino group which then acts as an H-bond acceptor. This can lead to destabilization of the base pairing. The zinc and magnesium groups of divalent cations have a different balance of the water−cation and base−cation interactions. Binding of the zinc-group elements to nucleobases is more efficient. Interaction of large IIa group divalent cations (Ca^{2+}, Sr^{2+}, and mainly Ba^{2+}) with the N7 site of adenine is not likely unless the amino group nitrogen atom serves as a coordination center which may disrupt the base pairing. The complexes were optimized within the Hartree−Fock approximation with the 6-31G* basis set of atomic orbitals and relativistic pseudopotentials for the cations. All atoms of the base pairs were kept coplanar. No other constraints were applied. The interaction energies have been calculated with inclusion of the electron correlation by means of the full second-order Moeller−Plesset perturbational theory.

Introduction

Quantum chemical calculations have been used for a long time to investigate nucleic acid base pairs[1] and their complexes with metal ions.[2] Most of these calculations[2] applied (at best) the Hartree−Fock approximation with a minimal basis set of atomic orbitals. The gradient geometry optimization was not available, the calculations were not corrected for the basis superposition error, and the number of metal cations considered was very limited. One of the most important conclusions from the previous studies was the observation of an enhancement of the base pairing caused by the metal ion coordination.[2a,e,g] However, as in many other areas of applied quantum chemistry, sufficient accuracy could not be achieved without applying ab initio calculations with inclusion of the electron correlation and polarized basis sets of atomic orbitals.[3,4] These techniques are of importance since molecular interactions in metal cation containing complexes represent an especially difficult task for empirical potential methods.[4b,5]

Metal ion binding to the Watson−Crick base pairs has been characterized relatively well.[4] Much less attention, however, has been devoted to the interactions between metal ions and non-Watson−Crick base pair systems.[4b] The present interest in these base-pairing patterns stems from their involvement in base-pair mismatches,[6] which can be produced by chemical damage to DNA. Some of these mismatches including the AA, GA, and AG base pairs have been characterized by X-ray and NMR techniques.[7] Furthermore, several non-Watson−Crick H-bond systems such as the Hoogsteen and reverse-Hoogsteen pairs are essential for the formation and stabilization of nucleic acid triplexes and quadruplexes. DNA triplexes have been extensively studied because of their potential applications in the control of gene expression.[8]

In this paper, we present some results of our studies on the interactions of hydrated group IIa (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) and

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group IIb (Zn^{2+}, Cd^{2+}, Hg^{2+}) divalent cations with the adenine−adenine reverse-Hoogsteen (AA rH) base pair. Also, a complex of the adenine−thymine Watson−Crick (AT WC) base pair with hydrated Mg^{2+} ion has been studied. We did not consider interactions of the other cations with the AT WC pair since the properties of these systems can be easily extrapolated from the data for the metalated AA base pairs. The AA rH base pair was of particular interest because it has been previously postulated that metal cation coordination strengthens this base pairing in DNA A·AT triplexes.[9] Preliminary calculations involving divalent zinc and magnesium ions[10] indicated that there is no such base-pairing enhancement which has been ultimately confirmed by the present results (a strong enhancement does occur in G·GC triads; cf. refs 4b, 10). Another goal of the present paper is to provide further insight in the observed differences between the divalent alkaline and transition metal ions in their complexes with nucleobases. Let us mention that in biology every cation has its specific role determined by a unique balance of interactions between the cation and various ligands in biomolecules. It is, therefore, essential to make quantum chemical studies of interactions between metal cations and biomacromolecules as broad as possible.

The octahedral metal coordination sphere in our study includes five water molecules representing the first hydration shell and the N7 atom of adenine. We are aware that different cations may prefer different coordination patterns. It was not the aim of the present paper to investigate the details of metal ion hydration, since various aspects of this problem have been addressed in numerous specialized papers (cf ref 11 and references therein). Nevertheless, we believe that the hydration pattern used in the present work should be sufficient to account for effects of the first solvation shell. This approach does represent an improvement over the calculations on bare cations which assume unrealistic coordination numbers and result in inaccuracy of some geometric features when compared to the experimental values.[12]

Computational Methods

Systems. The system used in calculations consists of the adenine−adenine reverse-Hoogsteen (AA rH) base pairs interacting with divalent metal cations surrounded by five water molecules. The cations were initially placed near the N7 atom of adenine completing the octahedral coordination. This coordination pattern was stable during geometry optimization for all the systems studied. The cations under investigation included the following closed-shell systems: Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} (group IIa) as well as Zn^{2+}, Cd^{2+}, and Hg^{2+} (group IIb). In addition, the adenine−thymine Watson−Crick (AT WC) base pair with hydrated Mg^{2+} has been studied.

Level of Calculations. All systems were optimized using a gradient technique within the Hartree−Fock (HF) approximation. The standard split-valence 6-31G* basis set of atomic orbitals has been used for the H, C, N, O, and Mg^{2+} atoms; the other cations have been described by employing the Christiansen relativistic pseudopotentials.[13] The interaction energies were evaluated using the full second-order Moeller−Plesset perturbational method (MP2) with the same basis set and pseudopotentials as specified above. Although the interaction energies in the present system are dominated by the HF energy, we have used the full MP2 procedure rather than the commonly used and less expensive frozen-core approximation in order to include larger portion of electron correlation effects for the cations considering contributions from the inner s and p electrons (see also discussion in ref 14a). There would be no correlation effects for the cations of group IIa when using the frozen core approximation only. Comparison of full MP2 all-electron calculations and corresponding calculations using relativistic pseudopotentials is given in our previous paper and shows very good performance of the pseudopotential treatment.[4f] It should be emphasized that the use of relativistic pseudopotentials is essential for heavier elements such as mercury and barium, where nonrelativistic all electron approach cannot be applied.[4f]

Constraints. All atoms of the base pairs were held coplanar. These constraints prevent the destabilization of the pairing due to an interaction between the adenine amino group and the polarized water molecules and the cation. Our previous optimizations of the hydrated Zn^{2+} and Mg^{2+} cations interacting with A·AT triplets[10] revealed that there has been no minimum on the potential energy surface corresponding to a planar H-bonded base pair. At first, the initial planar structure was somewhat destabilized by an interaction between a water molecule and the amino group nitrogen atom. Pyramidalization of the amino group was followed by a fast disruption of H-bonding between adenines with the subsequent transition to a stacked structure. The planarity constraints are fully justified for several reasons. First, there would be other water molecules in DNA which could provide an additional shielding of the amino group. Second, a partial compensation originating from the neighboring negatively charged phosphate group is expected. Third, the energy gradients around the planar arrangement are small, so it does not seem difficult to eventually stabilize the planar pair by involving some additional contributions, such as the DNA backbone. There is no advantage in studying the actual nonplanar (probably stacking) minimum, since it is very far from any geometry allowed by the DNA backbone.

Evaluation of Interaction Energies. Two sets of calculations were carried out. First, we studied the influence of the hydrated cation on the base pair. Here the whole complex has been formally divided into three subsystems: the hydrated cation (the cation + five water molecules, M+5W), the proximal adenine (site of the cation binding) B_p, and the other (remote) base (B_r) which is adenine or thymine in this paper.

The interaction energy of this trimer, $\Delta E^{M+5W,Bp,Br}$ can be expressed in two ways,[4a−c] (a) as a difference of the electronic energy of the complex and of the monomers,

$$\Delta E^{M+5W,Bp,Br} = E^{M+5W,Bp,Br} - [E^{M+5W} + E^{Bp} + E^{Br}] \qquad (1)$$

(b) or as a sum of three pairwise dimer interaction energies and the three-body term ΔE^3.

$$\Delta E^{M+5W,Bp,Br} = \Delta E^{M+5W,Bp} + \Delta E^{M+5W,Br} + \Delta E^{Bp,Br} + \Delta E^3 = E^{M+5W,Bp} - [E^{M+5W} + E^{Bp}] + E^{M+5W,Br} - [E^{M+5W} + E^{Br}] + E^{Bp,Br} - [E^{Bp} + E^{Br}] + \Delta E^3 \qquad (2)$$

The symbol E stands for the total electronic energy of a complex or subsystem; ΔE means the interaction energy of a complex.

The enhancement of the strength of base pairing (defined as a difference between the energy which is necessary to separate the two bases in the presence and absence of the hydrated cation) is the sum of two contributions: the hydrated cation−remote adenine (or thymine) pairwise interaction $\Delta E^{M+5W,Br}$ (basically the long-range electrostatic contribution), and the three-body term ΔE^3 (here the polarization effects are included).

Second, to reveal the balance of water−cation and base−cation interactions, the following calculations were done: the remote adenine has been neglected (without reoptimization), the interaction energy between hydrated cation and proximal base ($\Delta E^{M+5W,Bp}$) has been decomposed to six individual pairwise

TABLE 1: Selected Geometrical Parameters of Optimized Hydrated Cation−Adenine−Adenine (Thymine) Complexes (in Angstroms)

M^{2+}	M···N7[a]	M···W[b]	N6···X[c]	N1···X[d]	MN7C5	MN7C5C6[e]	H(W)···N6[f]	H(N6)···O(W)[g]
Mg[h]	2.16	2.12−2.14	3.06	3.19	131.8	19.9	2.39	2.37
Mg[i]	2.16	2.12−2.14	2.88	3.10	132.0	17.5	2.42	2.36
Ca[h]	2.54	2.45−2.46	3.05	3.26	131.0	26.8	2.43	2.51
Sr[h]	2.72	2.62−2.63	3.05	3.26	129.4	32.9	2.40	2.52
Ba[h]	2.91	2.79−2.82	3.05	3.26	126.3	40.7	2.36	2.52
Zn[h]	2.04	2.17−2.19	3.05	3.19	135.9	12.3	2.48	2.40
Cd[h]	2.30	2.37−2.40	3.06	3.19	129.8	23.4	2.45	2.44
Hg[h]	2.25	2.40−2.54	3.06	3.19	129.4	19.3	2.57	2.44

[a] Cation−base distance. [b] Cation−water distances. [c] Base pairing distance, X = N7 for AA and O4 for AT. [d] Base pairing distance, X = N6 for AA and N3 for AT. [e] Dihedral angle between the cation and adenine plane. [f] Water···amino group (acceptor) interaction. [g] Amino group···water H-bond. [h] AA rH base pair. [i] AT WC base pair.

terms (five water−base contributions, $\Delta E^{Bp,W}$, and metal−base interaction, $\Delta E^{Bp,M}$) and the many-body term, ΔE^{Mb}.

$$\Delta E^{Bp,M+5W} = \Delta E^{Bp,M} + \Sigma\Delta E^{Bp,W} + \Delta E^{Mb} \qquad (3)$$

The many-body term defined in this way shows the total nonadditivity of interactions, i.e., the difference between the fully pairwise additive treatment and the fully nonadditive approach. ΔE^{Mb} could be further decomposed to the three-body, four-body, etc. contributions. However, we do not see any practical reason to do so for our purposes.

According to the perturbational theory of molecular interactions,[14b] the ΔE^3 term includes first-order exchange and induction (SCF-deformation) nonadditivities at the SCF level. The induction term is the most important contribution in our case. The MP2 noadditivity includes some additional second-order terms but not the dispersion nonadditivity since this contribution first appears at the MP3 level. For more details, see the review by Chalasinski and Szczesniak.[14b]

Cation-containing clusters are characterized by large nonadditivities of interactions and these effects are often described in the literature as "polarization" of the ligands by the cations.[2,9,11] It means that dipole moments of the ligands are larger due to the induction (polarization) effects and there might be further charge-transfer between cation and ligands. This sharply increases the interligand repulsion within the coordination sphere of the cation. On the other hand, it stabilizes interactions between the coordinated ligands and other molecules forming H-bonds with the ligand.[2,9,11] All these effects are included in the many-body terms. To be consistent with terminology used in relevant preceding papers,[2,9,11] we call the ΔE^3 term as a "polarization enhancement" of the base pairing. However, the exact definition of the enhancement is given by eq 2 above.

All interaction energies were calculated using the optimized geometries of the complex and were corrected for the basis set superposition error in the basis set of the whole complex. The deformation energies of monomers are not presented, since the deformation energies are an order of magnitude smaller than the interaction energies.[4] The largest monomer deformation energy concerns the proximal adenine and ranges from +1.5 kcal/mol for the Ba^{2+} complex to +3.2 kcal/mol for the Zn^{2+}- and Hg^{2+} complexes.

All calculations were done using the Gaussian94 suite of programs.[15] The optimizations used the tight option. This means that the following thresholds have been used: maximum force, 0.000015; RMS force, 0.00001; maximum displacement, 0.00006; RMS displacement, 0.00004 (all values in au).

Results

Structures. Table 1 summarizes the main structural characteristics of the complexes. Figures 1 and 2 present the numbering schemes and stereoviews of the $Mg(H_2O)_5AT$ and $Zn(H_2O)_5$-AA complexes, respectively. The cation−water distances are shorter than the cation−base distances for group IIa ions, while the opposite ratio is found for group IIb. This is a consequence of the different balance of water−cation and base−cation contributions for groups IIa and IIb. The distance between N7

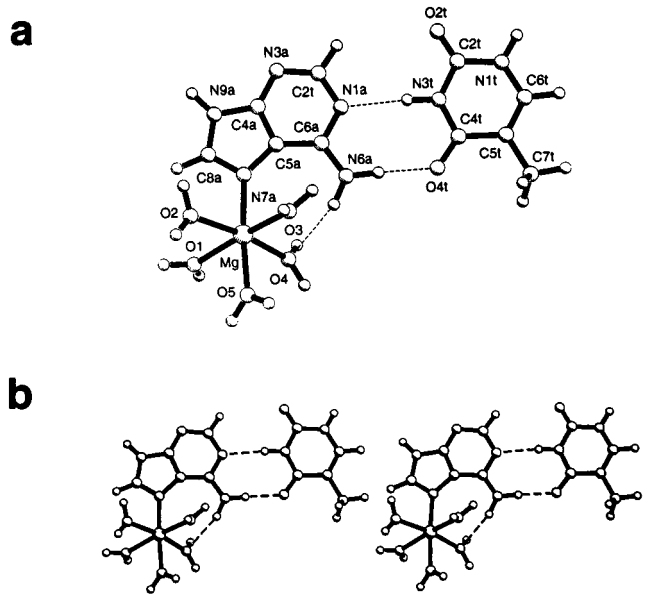

Figure 1. $Mg(H_2O)_5AT$ complex: (a) numbering scheme; (b) stereoview.

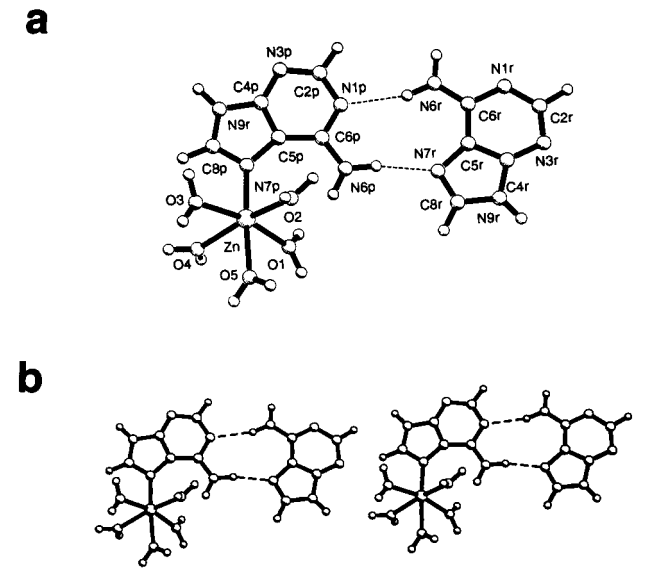

Figure 2. $Zn(H_2O)_5AA$ complex: (a) numbering scheme; (b) stereoview.

TABLE 2: Interaction Energies in the Hydrated Cation−Adenine−Adenine (Thymine) Complexes (in kcal/mol)[a,d]

M^{2+}	$\Delta E^{M+5W,Bp}$	$\Delta E^{M+5W,Br}$	$\Delta E^{Br,Bp}$	ΔE^{3}	ΔE^{T}
Mg[b]	−46.0 (−43.6)	−2.7 (−2.4)	−10.5 (−7.2)	−0.1 (−0.1)	−59.2 (−53.2)
Mg[c]	−45.8 (−43.4)	−8.8 (−9.5)	−11.4 (−9.0)	−0.5 (−0.4)	−66.5 (−62.3)
Ca[b]	−33.5 (−32.8)	−2.6 (−2.3)	−10.5 (−7.2)	0.0 (−0.1)	−46.7 (−42.5)
Sr[b]	−28.9 (−28.1)	−2.8 (−2.4)	−10.5 (−7.3)	0.0 (−0.1)	−42.1 (−42.5)
Ba[b]	−28.1 (−24.3)	−2.9 (−2.5)	−10.3 (−7.3)	0.0 (0.0)	−41.4 (−34.1)
Zn[b]	−53.7 (−50.1)	−2.6 (−2.3)	−10.6 (−7.2)	0.0 (−0.1)	−66.9 (−59.8)
Cd[b]	−45.9 (−42.4)	−2.7 (−2.3)	−10.6 (−7.2)	−0.1 (0.0)	−59.1 (−51.9)
Hg[b]	−55.3 (−48.8)	−2.6 (−2.3)	−10.6 (−7.3)	+0.2 (+0.1)	−68.4 (−58.4)

[a] $\Delta E^{M+5W,Bp}$, pairwise interaction energy between hydrated cation and proximal adenine; $\Delta E^{M+5W,Br}$, pairwise interaction energy between hydrated cation and remote adenine or thymine; $\Delta E^{Br,Bp}$, pairwise base pairing energy; ΔE^{3}, three-body term; ΔE^{T}, total interaction energy (sum of the previous contributions). [b] AA rH base pair. [c] AT WC base pair. [d] The calculations were carried out at the MP2/6-31G* level. Values in parentheses represent the Hartree−Fock component of the interaction energy.

and the cation increases sharply with the atomic number for the alkaline metal cations, whereas all the transition metal cations are tightly bound to the base. The Hg−N7 distance is shorter than the Cd−N7 distance, resulting from relativistic effects which are significant for Hg^{2+}.[4a] These structural trends are similar to those reported before for interactions between hydrated cations and guanine.[4c] However, a significant difference is observed upon replacing guanine with adenine. The carbonyl oxygen atom O6 of guanine is frequently involved in the coordination sphere, mainly of the large IIa group cations. In contrast, the planar adenine amino group is in a repulsive interaction with the metal cation through its hydrogen. As a result, all cations are shifted away from the adenine plane, as evidenced by the MN7C5C6 dihedral angles between the cation and adenine rings (Table 1). The deviation is most significant for larger group IIa cations (starting with Ca^{2+}). These cations prefer coordination numbers higher than six and exhibit a strong repulsion toward the amino group hydrogen atom. There is literally not enough space for these cations near the N7 position of planar adenine.

The out-of-plane shift of the cation is accompanied by a pronounced asymmetry of its hydration shell. An H atom of one of the water molecules is directed toward the negatively charged lone electron pair of N6. The O···N6 distances are 2.4−2.6 Å. The O atom of the other proximal water molecule serves as an H-acceptor of the adenine amino group. The water···lone pair interaction can be quite strong since the water molecules are highly polarized by the presence of the cation. This interaction could be further enhanced by allowing the amino group to be pyramidal. However, this has been prevented by the geometrical constraints used, otherwise the base pairing would be disrupted. (Another possibility is a direct covalent binding of the cation to N6 after replacement of one of the hydrogen atoms.[16] Such interactions will be adressed in future studies.)

The ability of amino groups of bases to act as hydrogen acceptors was proposed following the observation of numerous mutual close interstrand amino group contacts in B−DNA crystal structures[17] contradicting the concept of repulsive amino−amino interstrand steric clashes.[18] Such interactions have also been predicted by ab initio calculations.[17b,19] Small-molecules database studies revealed several examples of such interactions and they seem to exist also in protein−DNA complexes.[19c] The action of an amino group as an H-acceptor is frequently associated with a lowering of symmetry of the system,[17b] which is also the case for the adenine−hydrated cation system. As shown elsewhere, the optimized structures are close to the C_s symmetry for the GC and GG base pairs[4b,c] with the cation being coplanar or almost coplanar with the guanine molecule and the base pair retaining the planar arrangement without imposing any geometrical constraints.

Let us briefly comment on the remaining structural parameters. The structure of the base pair is only slightly different from that of the isolated base pair and does not depend much on the cation. The water−cation separations are almost equidistant for all the cations expect Hg^{2+} where they range from 2.4 to 2.54 Å. The reason for this difference is not clear; the optimization was completed successfully as for all the other systems.

Energetics of the Hydrated Cation−Adenine−Adenine (Thymine) Complex. The individual interaction energy terms of the hydrated cation−proximal adenine−remote adenine (thymine) trimer are summarized in Table 2. The values in parentheses represent the HF component of the interaction energies which are in most cases close to the MP2 values. Nevertheless, the electron correlation effects are not negligible and enhance the difference between IIa and IIb cations.

Three of the contributions in the AA complexes are basically independent of the cation: the pairwise base pair energy (around −10.5 kcal/mol, comparable to isolated base pair), the hydrated cation−remote adenine interaction (−2.5 to −3 kcal/mol), and the three-body term (0 kcal/mol). The last result means that there is no three-body enhancement of the base pairing due to the cation binding (see method section). The result for the AT pair is the same with the following exception: the long-range attraction between hydrated cation and the thymine amounts to −9 kcal/mol due to a favorable orientation of thymine with respect to the cation. The same behavior has been noticed when assuming the bare cation.[4a] This observation probably explains the contradiction between our results and some older quantum chemical studies predicting large polarization enhancement of the AT WC base pairing.[2g] In these studies, the three-body term has not been separated from the other contributions. Therefore, it is very likely that the reported base-pair enhancement for AT WC pair was mainly due to simple electrostatic attraction between cation and thymine with no substantial three-body contribution. In any case, the high-level ab initio data rule out any possibility of a substantial polarization enhancement of AT and AA base pairs due to cation binding to N7. There is no base pairing enhancement for the AA pair because here even the "classical" cation−remote base attraction is weak. On the other hand, calculations for the bare and hydrated cations interacting with guanine−cytosine (guanine) base pairs consistently predict very significant enhancement of the base pairing due to the three-body term.[4a,c] Thus, there seems to be a qualitative difference between adenine- and guanine-containing base pairs. While the first group shows no three-body stabilization, the second group has significant attractive three-body contribution. These effects also correlate with the nonadditivity of base stacking. The base stacking is additive when considering two consecutive AT base pairs, and there might be very weak nonadditivity (few tenths of kcal/mol) for some configurations

TABLE 3: Interaction between Bare Cation and Planar A, Bare Cation and G, Hydrated Cation and Planar A, and Hydrated Cation and G[a]

M^{2+}	M···A	M···G	M^{hydr}···A	M^{hydr}···G
Mg	−107.9	−198.7	−46.0	−89.8
Ca	−61.5	−133.9	−33.5	−86.5
Sr	−48.9	−116.6	−28.9	−80.1
Ba	−51.4	−118.8	−28.1	−70.8
Zn	−152.9	−237.2	−53.7	−93.6
Cd	−116.6	−192.6	−45.9	−87.7
Hg	−141.1	−208.0	−55.3	−91.9

[a] All energies in kcal/mol. The cation and its hydration shell are considered as one subsystem. MP2/6-31G*//HF/6-31G* level.

of sequences with alternating AT and GC pairs. However, the nonadditivity can be significant (up to 10−20% of the stacking energy) for two consecutive GC base pairs, with further enhancement for longer tracts of GC pairs.[3i] It looks as if the different ability of AT compared to GC base pairs to exhibit various nonadditive interactions constitutes yet another qualitative difference between these two pairs. This could contribute to various aspects of the DNA structure and function. Note, e.g., papers rationalizing unusual properties of the GG stacks with respect to the oxidation of guanine.[20]

The remaining term in the eq 2 is the hydrated cation−proximal adenine interaction energy $\Delta E^{M+5W,A}$. This contribution varies significantly with the cation and dominates the total stabilization energy of the (M+5W)−adenine−adenine trimers. The (M+5W)−proximal adenine interaction is much weaker for the large cations (Ca^{2+}, Sr^{2+}, Ba^{2+}), which reflects the destabilizing role of the amino group−cation interaction, discussed above.

A comparison of the interaction energy of bare and pentahydrated cations with adenine and guanine compiled from the present and previous data (Table 3) reveals several interesting trends. First, the M−adenine interaction is systematically much weaker than the M−guanine contribution. This is due to the molecular dipole−ion interactions. Guanine has a large dipole moment of 6.5 D; adenine shows only a weak one (ca 2.5 D). This explanation is complementary to the explanation based on the so-called basicity used by experimentalists. (Hypoxanthine has the same orientation of molecular dipole as guanine, though the dipole is by about 20% weaker. Thus it has somewhat reduced affinity to bind cations. The lowest ability to bind a cation has 2-amino adenine with a dipole moment of ca. 1 D only. Due to the very low polarity, 2-amino adenine cannot form any strong H-bonded base pair; the triply bonded 2-amino adenine···thymine base pair is 2 times less stable than GC WC.[3c]) Second, the hydrated cation−base interaction is weaker than the cation−base interaction itself. In the case of guanine, the hydration seems to almost cancel the pronounced difference between IIa and IIb groups of cations. It should be noted, however, that bases can still rather effectively discriminate between these two groups of cations, even between Zn^{2+} and Mg^{2+}. This is achieved through the different balance of cation−base and cation−water contributions. It can be visualized after decomposing the $\Delta E^{M+5W,Bp}$ interaction energy to the individual terms, as discussed in detail in ref 4c. The system with Zn^{2+} behaves more like a hydration of a metalated base, while that with Mg^{2+} is closer to the interaction between a base and a hydrated cation.[4c] The same different balance of water−base and cation−base interactions influences also the adenine−hydrated cation complexes; however, the adenine amino group makes the IIa vs IIb difference more visible. In fact, adenine differentiates between Mg^{2+} and the rest of IIa group. When replacing guanine by adenine, the hydrated cation−base attraction is reduced to 50−60% for Mg^{2+} and for the IIb group, and to 35−40% for Ca^{2+}, Sr^{2+}, and Ba^{2+}.

TABLE 4: Decomposition of the $\Delta E^{M+5W,A}$ Term for Selected Cations (kcal/mol)[a]

	$\Delta E^{M+5W,A}$	$\Delta E^{M,A}$	$\Sigma\Delta E^{A,W}$	ΔE^{Mb}
Zn^{2+}	−54.1 (−50.3)	−148.4 (−134.6)	+10.4 (+14.1)	+83.9 (+70.2)
Mg^{2+}	−46.4 (−43.8)	−111.8 (−108.4)	+9.7 (+13.6)	+55.7 (+51.0)
Ca^{2+}	−33.9 (−33.0)	−60.4 (−60.2)	+4.0 (+6.0)	+23.1 (+21.6)

[a] MP2/6-31G*//HF/6-31G* level. Values in parentheses represent the Hartree−Fock component of the corresponding terms. $\Delta E^{M,A}$, pair cation−adenine interaction energy; $\Sigma\Delta E^{A,W}$, sum of pair water−adenine terms; ΔE^{Mb}, many-body term.

In the preceding paragraphs, we emphasized that two water molecules form H-bonds with the amino group, the first one as an H-donor, the other one as an H-acceptor. Even these water molecules have a weakly repulsive pair interaction $\Delta E^{W,A}$ with the adenine. However, this should in no case be interpreted as evidence against some H-bonding contribution between the water and amino group. The water molecules are highly polarized, and the actual interaction can thus be very different from the picture obtained from the pairwise contributions. Elongation of the O−H bond by 0.003−0.004 Å in the O−H···N6 contacts supports the presence of a weak hydrogen bond.

Decomposition of the Hydrated Cation−Adenine Interaction. Table 4 presents a decomposition of the $\Delta E^{M+5W,A}$ interaction energy for Mg^{2+}, Zn^{2+}, and Ca^{2+} cations. The hydrated cation−proximal adenine interaction energy consists of three different contributions. First, there is a highly attractive pairwise metal−base interaction. This contribution is largest for Zn^{2+}, as expected.[4a] The sum of the five pair-additive water··· base interactions is weakly repulsive, which is because the water molecules are primarily oriented by the cation, adopting a repulsive orientation toward the base. Finally, the many-body term containing all nonadditivites is large and highly repulsive. The repulsive nonaddivity of an amount of dozens of kcal/mol is not surprising. Similar values are known from calculations of hydration of cations and represent the additional repulsion between the water molecules in the shell due to the polarization.[11] Our present complex can be viewed as a replacement of one of the water molecules from the hydration shell by the nucleobase. Thus, the many-body term includes the screening of the cation−base interaction by the water and also the effect of the polarization on base−water interactions. These two contributions cannot be separated due to the definition of nonadditivity which is a property of the whole cluster.[4c] The large values of nonadditivity clearly indicate limited applicability of conventional pairwise additive molecular mechanics potentials. (An explicit polarization energy contribution is being implemented by several laboratories for complexes with metal cations.[5]) In principle, the pairwise additive potentials could be parametrized to provide a very good estimate of the total $\Delta E^{M+5W,Br}$ contribution. However, this is because of a mutual compensation of two large errors: the qualitative underestimation of the pairwise cation−base attraction,[4b] and the total neglect of the many-body repulsion. Thus even if the $\Delta E^{M+5W,A}$ value calculated by potentials is reasonable, the balance of various contributions would be incorrect which would consequently result in inaccuracies for the calculated dynamics of the system.[11e] Note the substantial differences in the individual terms for Zn^{2+} and Mg^{2+} in Table 4, despite the fact that both cations have similar ionic radii and the same charge.

Table 5 further illustrates the difference between Zn^{2+} and Mg^{2+} by comparing several different ways of evaluation of the interaction energies in the adenine−hydrated cation complex.

TABLE 5: Selected Interaction Energies (kcal/mol) Obtained at the MP2/6-31G* Level [f]

	Zn^{2+}	Mg^{2+}	Zn^{2+}-Mg^{2+}
$A\cdots M^{2+}$	−148.4 (−134.6)	−111.7 (−108.4)	−36.7 (−26.2)
$H_2O\cdots M^{2+}$ [a]	−94.9 (−90.0)	−82.9 (−82.4)	−12.0 (−7.6)
$M^{2+}\cdots 6H_2O$ [a,b]	−339.8 (−328.0)	−329.6 (−325.5)	−10.2 (−2.5)
$A-M^{2+}\cdots 5H_2O$ [c]	−202.2 (−200.6)	−222.3 (−219.1)	+20.1 (+18.5)
$A\cdots(M^{2+} + 5H_2O)$ [d]	−54.1 (−50.3)	−46.4 (−43.8)	−7.7 (−6.5)
$A\cdots M^{2+}\cdots 5H_2O$ [e]	−350.7 (−335.2)	−334.1 (−327.6)	−16.6 (−7.6)

[a] This system has been optimized at the HF/6-31G* level, all other energies in the table were obtained for the complexes reported above. [b] Hydration of the cation (seven subsystems). [c] Hydration of the $A-M^{2+}$ complex (subsystems: five water molecules and the metalated adenine). [d] Interaction between the hydrated cation and adenine (two subsystems). [e] Interaction energy of the whole complex (seven subsystems). [f] HF/6-31G* values are in parentheses. All interaction energies were corrected for the basis set superposition error; deformation energies of monomers are not included. The last column shows the difference between zinc and magnesium.

Table 5 corresponds to Table 4 in ref 4c showing the same analysis for guanine. The first two rows of Table 5 compare the interactions between the cations and adenine as well as between the cations and a single water molecule. Zinc shows a stronger interaction with both the water and the nucleobase. However, the zinc/magnesium difference is much more pronounced for the cation···nucleobase complex. The third row provides the hydration energies of zinc and magnesium in hexahydrated complexes. The hydration energies are rather similar, the difference of 10 kcal/mol in favor of Zn^{2+}, being in agreement with the cation−water interaction energies. The next row presents hydration energy of the cations bound to a nucleobase (hydration of a metalated base), indicating that the difference between Zn^{2+} and Mg^{2+} is sharply (by ca. 30 kcal/mol) reversed compared to hydration of an unbound cation. The reason for the observed behavior is the repulsive contribution caused by weakening of the cation−base attraction upon hydration. This contribution is much larger for Zn^{2+} . As in the case of guanine,[4c] we have found similar interaction energies when the complex is treated as an interaction between the base and a hydrated cation (the fifth row of Table 5). The last row of Table 5 shows the total interaction energies of the adenine−metal−hydration shell complex. Here, the energy difference between zinc and magnesium complexes increases only slightly with respect to the corresponding value for hexahydrated cations (row 3), again because of the larger reduction of Zn^{2+}-base interaction by hydration. The results are fully consistent with previously published data for guanine and illustrate the different balance of water−cation and nucleobase−cation interactions for Zn^{2+} and Mg^{2+}.[4c]

Reliability of the Basis Set. During the review process, one of the referees suggested that the 6-31G* basis set of atomic orbitals may not be appropriate for our calculations, especially for an estimation of polarization effects and charge distributions around the hydrogens. Therefore, we would like to discuss this point in more details.

We agree that the 6-31G* basis set is known to be insufficient for some applications. Nevertheless, for the present system this basis set provides quite good results. We study large molecular clusters where there are many closely spaced atomic centers with atomic orbitals (the number of the contracted basis functions in our calculations is around 450). This significantly improves the flexibility of the basis set. Accordingly, the actual performance of the 6-31G* basis set for our extended cluster is much better than it would be, for example, for a water dimer. This is well-known for the H-bonded base pairs where the results obtained with a medium-size basis set were tested against data obtained with much larger basis sets and actually a very good agreement has been noticed.[3f,m] To bolster our position, we re-evaluated the HF/6-31G* interaction energies for the complex between the adenine−adenine pair and the hydrated Mg^{2+} using the much larger 6-311+G(2d,2p) basis set. The basis contains 890 contracted basis functions for this system. We have obtained the following interaction energies: −43.4 kcal/mol, −2.4 kcal/mol, −6.2 kcal/mol, and −0.1 kcal/mol for $\Delta E^{M+5W,Bp}$, $\Delta E^{M+5W,Br}$, $\Delta E^{Br,Bp}$, and ΔE^3, respectively. These values are very similar to the corresponding SCF values in the first row of Table 2 (data in parentheses). The largest difference is found for the base pairing (1 kcal/mol), which has also been observed in our previous study.[3m] The other three terms are identical. There is no indication that the 6-31G* basis set is substantially deficient for any value reported in Table 2. The reason for the three-body term being close to 0 kcal/mol is not related to the choice of the basis set different from 6-31G*. This happens because this term vanishes for the present system. The 6-31G* basis set provides large three-body terms for guanine-containing base pairs.[4c,10]

Nevertheless, it has been reported that the hydration energies of cations are overestimated with the 6-31G* basis set by ca. 10−15%.[11g] This would still be an acceptable accuracy for our purposes, since the effect of adding a second hydration shell around the cation would change the hydration energy by about 30%.[11f] In addition, we did not study the hydration energy of cations in the present paper in detail. Nevertheless, we have estimated the accuracy of the 6-31G* basis set for this contribution. The calculations were again made for the hydrated Mg^{2+} complex with adenine taken from the previous optimization, and the interaction energy has been evaluated as the energy difference between the complex and the seven monomers (cation, adenine, and five water molecules). We have obtained SCF values of −316.0 and −327.6 kcal/mol for the 6-311+G(2d,2p) and 6-31G* basis sets, respectively. This means a difference below 4%. However, we have obtained a much larger difference, above 8%, when the interaction energy has not been corrected for the basis set superposition error, since this error is 20 kcal/mol for the 6-31G* basis set but only 4 kcal/mol for the 6-311+G(2d,2p) basis set. This leads to the uncorrected interaction energies of −320.0 kcal/mol for the 6-311+G(2d,2p) basis set and −347.0 kcal/mol for the 6-31G* basis set. On the basis of this result, we would like to suggest that a significant part of the reported deficiency of the 6-31G* basis set (and other medium-size basis sets) for evaluation of the hydration energies of cations compared to large basis sets can be ascribed to the common practice of neglecting the counterpoise correction for hydrates of cations.

Biological Significance. The AA base pair in the reverse-Hoogsteen orientation is a part of the basic unit of triple-helical DNA consisting of A·AT base triplets. Stability of these triplexes has been studied by several groups.[21] As all purine·purinepyrimidine triplexes, the A·AT triplexes require multivalent cations for their stabilization.[22] Previous investigations[9] suggested that the binding of divalent metal cations to the N7 site of the third strand purine bases results in a polarization of the H-bond system and subsequent strenghtening of the purine−purine base pairing. In fact, this enhancement has been confirmed for the G·GC triplet.[10] However, three-body (polarization) effects cannot be considered as an important factor for the stability of the A·AT base triplet. In this case, as well in

the case of mixed G·GC and A·AT triplexes, other factors such a specific DNA conformation could be responsible for their stability.

Conclusions

Interaction of hydrated cations with the N7 site of adenine does not induce any polarization stabilization for the base pairing with another adenine or thymine. The base pairing enhancement observed for the AT WC base pair is solely due to the classical electrostatic attraction between the hydrated cation and the thymine.

The amino group of adenine acts as an H-bond acceptor for a polarized water molecule from the first hydration shell of the cation. The interaction of cations with adenine is substantially weaker than that with guanine due to the lower polarity of adenine. The adenine amino group further destabilizes binding of large cations to N7. As for guanine, cations of group IIb exhibit stronger binding to the N7 site of adenine which leads to a different balance of the cation−base and water−base contributions for IIa and IIb cations.

The observed absence of significant polarization effects could be important for our understanding of the structure of A·AT and mixed G·GC/A·AT DNA triplexes. A closer investigation of the role of the phosphate groups as well as of the overall conformation of these triplexes may be necessary to account for their formation and stability.

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