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Theoretical Calculation of the NMR Spin−Spin Coupling Constants and the NMR Shifts Allow Distinguishability between the Specific Direct and the Water-Mediated Binding of a Divalent Metal Cation to Guanine

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Abstract: The calculated intermolecular and intramolecular indirect NMR spin−spin coupling constants and NMR shifts were used for the discrimination between the inner-shell and the outer-shell binding motif of hydrated divalent cations Mg^{2+} or Zn^{2+} with a guanine base. The intermolecular coupling constants ^1J(X,O6) and ^1J(X,N7) (X = Mg^{2+}, Zn^{2+}) can be unambiguously assigned to the specific inner-shell binding motif of the hydrated cation either with oxygen O6 or with nitrogen N7 of guanine. The calculated coupling constants ^1J(Mg,O6) and ^1J(Zn,O6) were 6.2 and −17.5 Hz, respectively, for the inner-shell complex of cation directly interacting with oxygen O6 of guanine. For the inner-shell coordination of the cation at nitrogen N7, the calculated coupling constants ^1J(Mg,N7) and ^1J(Zn,N7) were 5.6 and −36.5 Hz, respectively. When the binding of the cation is water-mediated, the coupling constant is zero. To obtain reliable shifts in NMR parameters, hydrated guanine was utilized as the reference state. The calculated change of NMR spin−spin coupling constants due to the hydration and coordination of the cation with guanine is caused mainly by the variation of Fermi-contact coupling contribution while the variation of diamagnetic spin−orbit, paramagnetic spin−orbit, and spin−dipolar coupling contributions is small. The change of s-character of guanine sigma bonding, sigma antibonding, and lone pair orbitals upon the hydration and cation coordination (calculated using the Natural Bond Orbital analysis) correlates with the variation of the Fermi-contact term. The calculated NMR shifts δ(N7) of −15.3 and −12.2 ppm upon the coordination of Mg^{2+} and Zn^{2+} ion are similar to the NMR shift of 19.6 ppm toward the high field measured by Tanaka for N7 of guanine upon the coordination of the Cd^{2+} cation (Tanaka, Y.; Kojima, C.; Morita, E. H.; Kasai. Y.; Yamasaki, K.; Ono, A.; Kainosho, M.; Taira, K. *J. Am. Chem. Soc.* **2002**, *124*, 4595−4601). The present data indicate that measurements of NMR intermolecular coupling constants may be used to discriminate between the specific inner- and outer-shell binding of divalent cations to nucleobases in DNA and RNA.

1. Introduction

Divalent cations play prominent roles in nucleic acids, ranging through neutralization of the anionic nucleic acids through specific stabilization of three-dimensional structures of RNA and DNA molecules up to their effect as cofactors in RNA-mediated catalysis. The active structural role of the Mg^{2+} cation was first evidenced by high-resolution X-ray crystallography of tRNA[1] followed by a number of structures such as the hammerhead and intron ribozymes,[2,3] lead-dependent ribozymes,[4] spliceosomal RNA,[5] viral pseudoknots,[6] 5S rRNA Loop E,[7] and other motifs. Dozens of hexacoordinated magnesium cations with variable binding arrangements were revealed by crystal structures of small and large ribosomal subunits.[8] The magnesium cations appear to be especially important in mediating key tertiary interactions and stabilizing non-Watson−Crick

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segments. A substantial role was also suggested for divalent cations in DNA-mediated catalysis,[9] and some noncanonical DNA forms are stabilized by divalent metal cations.[10]

The observed metal binding geometries are very variable and include essentially all possible binding patterns. In general, the cations interact with nucleic acids via either inner-shell (direct solute−cation contact) or outer-shell (only water-mediated solute−cation contacts) binding motifs. The preferable sites for binding include anionic oxygens of the phosphate groups and major acceptor positions of bases, i.e., mainly N7/O6 of guanine and exocyclic oxygens of uracil and thymine.

The complexes between divalent cations and nucleic acids can be very difficult to isolate and structurally characterize. In crystallographic studies, it is often not straightforward distinguishing between metal cations bound to a biologically relevant position and cations related primarily to the crystallization process and stabilization of the crystal unit. Therefore, the information from solution studies is highly desirable, and the role of metals in nucleic acids has been clearly evidenced by recent NMR studies.[11] NMR spectroscopy is the technique which has been used during the past decades for the structure determination of macromolecules[12] and for the detection of intermolecular interactions.[13] The experimental studies on nucleic acids can often be successfully complemented by computational approaches. Unfortunately, in contrast to many other aspects of nucleic acid structure and dynamics, binding of metal cations to nucleic acids is very difficult to predict via computer modeling tools such as the molecular dynamics approach. This is due to drastic approximations inherent to the simple pair additive force fields that do not allow proper description of the balance of solute−cation and solvent−cation interactions.[13] A dication is considered as a point double charge with no charge transfer from an electron donor. Such approximations are evidently not realistic. Further, the time scale of present simulations 1−100 ns is not sufficient to achieve any significant redistribution of hydrated divalent cations.[14] The metal cation interactions, on the other hand, can be well described using quantum chemical (QM) methods. QM calculations were in recent years intensely used to study structures and energies of complexes between nucleic acid bases and hydrated divalent cations.[15] The QM calculations, however, are not sufficient to determine the actual binding sites of metals in nucleic acids, as the calculations are feasible only for small model gas-phase clusters.

The calculations of NMR spin−spin coupling constants and NMR shifts in fragments of large biomolecules become feasible thanks to the progress which has been done in implementation of the calculation methods within the density functional theory (DFT) framework.[16] In this study, we present the first attempt to use QM methods to predict the effect of metal cation binding to nucleic acids on selected NMR parameters. In this manner, QM calculations could substantially improve the ability of the NMR experiments to distinguish between different binding patterns of the metals. As noted above, the nucleic acids−metal cation interactions are highly variable. In a given nucleic acid structural motif, the actual binding patterns of metal cations result from a delicate balance of numerous contributions. The N7 inner-shell binding is a characteristic (but not exclusive) binding for transition divalent metals, while Mg^{2+} binds in numerous ways and does not appear to have any preferred binding pattern. Thus, the possibility to distinguish between inner- and outer-shell binding to bases through NMR experiments would be very important. Therefore, the present calculations are carried out for hexacoordinated Zn^{2+} and Mg^{2+} cations and include three distinct binding motifs to the guanine base: inner-shell binding to N7, inner-shell binding to O6, and outer-shell binding to the major groove edge of guanine. These three structures belong to the leading cation-binding patterns occurring in nucleic acids. The binding motifs of guanine complexes with hydrated cation examined in this study represent only a limited number of all possible guanine−cation complexes. Nevertheless, they include the most common and biologically relevant binding patterns of small divalent cations to guanine that are well documented by the experiments. A more extended study on guanine binding motifs including not only its canonical form but also several guanine tautomers is in progress and will be published elsewhere. We assume that these three binding patterns are distinct enough to evaluate the dependence of the calculated (and measured) NMR parameters to the cation binding geometry.

2. Methods

Calculation Procedure. All molecular complexes were fully gradient optimized using the DFT method with the B3LYP exchange-correlation functional[17] and the 6-31G(d,p) atomic basis set.

Interaction energy (ΔE) for guanine (G)···ion (X) (eq 1) and G···X···water (W) (eq 2) complexes were determined as follows:

$$\Delta E = E_{G,X}^{G\cdots X} - (E_{G,X}^{G} + E_{G,X}^{X}) \quad (1)$$

$$\Delta E = E_{G,X,W}^{G\cdots X\cdots W} - (E_{G,X,W}^{G} + E_{G,X,W}^{X} + E_{G,X,W}^{W}) \quad (2)$$

where the upper index means the system considered and lower index indicates the basis set; e.g. $E_{G,X}^{G\cdots X}$ means the energy of the G···X

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complex determined in the basis set of the G···X dimer. In the case of larger complexes, the interaction energy was determined analogously. Evaluation of interaction energies along eqs 1 and 2 ensures the complete elimination of the basis set superposition error (BSSE).[18]

The indirect NMR spin−spin coupling constants ^nJ(A,B), where n is the number of bonds connecting nuclei A and B, were determined using the coupled perturbed DFT method[19] with the B3LYP exchange−correlation functional. The J constants are calculated as a sum of the diamagnetic spin−orbit (DSO), paramagnetic spin−orbit (PSO), Fermi contact (FC), and the spin dipolar (SD) contributions.[20]

The NMR isotropic shieldings σ(A) were calculated using the GIAO method[21] with the B3LYP functional. The NMR shift $\delta(A) = \sigma(A)^{hydrated} - \sigma(A)^{ion}$ is calculated as a shift of the shielding calculated for the guanine complex containing the ion from the shielding calculated in hydrated guanine.

The NMR parameters were calculated for the isotopes ^{1}H, ^{13}C, ^{15}N, ^{17}O, ^{25}Mn, and ^{67}Zn; however the notation without isotope specification is used in the text.

The atomic basis set used in calculation of NMR parameters was the (9s,5p,1d/5s,1p) [6s,4p,1d/3s,1p] basis for carbon, nitrogen, and oxygen and the (5s,1p) [3s,1p] basis for hydrogen developed by Kutzelnigg.[22] For the divalent ions Mg^{2+} and Zn^{2+}, the (14s,6p) [5s,2p] Ahlrichs basis set[23] was used.

The hydration of guanine was modeled by adding explicit molecule(s) of water, which interact via hydrogen bonds with guanine. This hydration model was used for the reference calculation of NMR shifts. The complex of hydrated guanine was further embedded into the dielectric cavity simulating the continuum water solvent using the COSMO method.[24] This model of solvent was used as a hydration model for calculation of the NMR spin−spin coupling constants in a way described elsewhere.[25]

The natural bond orbital (NBO) analysis[26] was used for monitoring the sp^n hybrid character of sigma bonding (σ), sigma antibonding (σ*), and lone pair (LP) orbitals in different complexes of guanine with water and with cation. Further, the perturbation $E(2)$ energy was calculated for describing the hyperconjugation effects.

All calculations were done with the Gaussian 98.7 program package[27] except the calculation of indirect NMR spin−spin coupling constants, when the COLOGNE 99 program package[28] was used.

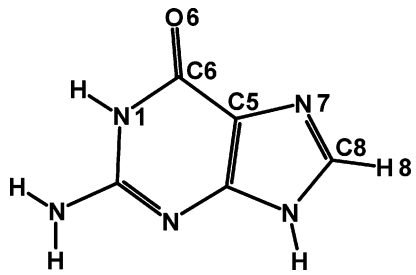

Figure 1. Guanine base with numbering of atoms used as NMR probes.

3. Results and Discussion

The variation of calculated indirect NMR spin−spin coupling constants and NMR shifts of hydrated guanine and complexes of guanine interacting with the divalent cations Mg^{2+} and Zn^{2+} were used for elucidation of the ion binding motif. In particular, we calculate the NMR spectroscopy parameters to distinguish between *the direct inner-shell configuration* of the metal cation with oxygen O6 or nitrogen N7 of guanine, shown in Figure 3a and b, respectively, and *the outer-shell configuration* of the metal ion with the contact between guanine and the ion shielded by water solvent shown in Figure 3c.

The nuclei C6, O6, C5, N7, C8, and H8 of guanine depicted in Figure 1 were chosen as the NMR probes in this work. When the oxygen O6 or the nitrogen N7 of guanine occurs in direct interaction with the ion, the geometry parameters as well as the electronic charge distribution of hydrated guanine may change substantially. When the geometry and electron distribution of guanine differ upon its interaction with water and the ion, then the change of NMR parameters can be attributed to the specific binding motif of ion and should be also detectable experimentally. We designed the molecular complexes of guanine with an ion in such a way that the abovementioned NMR probes are saturated either by explicit water molecules or by a hydrated ion.

Each of the complexes containing the divalent cation has the net charge +2 electron (e^-). The magnitude of net charge may influence NMR parameters significantly due to the polarization of molecular orbitals and the charge transfer between the ion and DNA base. The net charge compensation of the complex of DNA base with the divalent cation could occur due to the proximity of the phosphate group in DNA molecule. Thus, we further estimated the effect of the charge screening on the NMR parameters for the complexes of guanine with the directly coordinated ion. For this purpose, we optimized the geometry of the complexes shown in Figure 3a and b with one water molecule of the hydration shell replaced by the OH^- anion. We have shown before that this model complex shows a very similar effect on base pairing structures and energies as larger nucleotide complexes where the hydrated metal cation is screened by the full sugar−phosphate segment.[15c]

The NMR experiments for NA molecules are usually performed in the native environment of water solvent. To calculate the reliable shifts of guanine NMR parameters due to the direct coordination of metal cations Mg^{2+} and Zn^{2+}, we calculated for the first time the shifts of J and δ NMR parameters using the hydrated guanine as the reference state. The use of isolated guanine as the reference system would bias any comparison with experiments. The explicit hydration of guanine is shown in Figure 2a and b.

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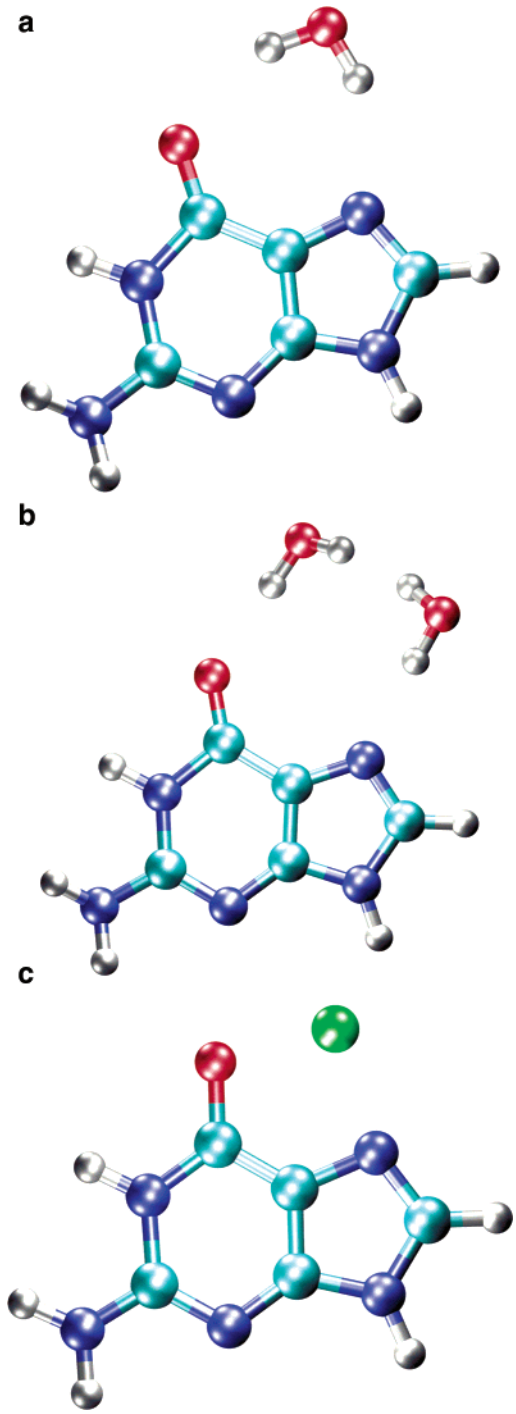

Figure 2. (a) Guanine interacting with water molecule. (b) Guanine interacting with two water molecules. (c) Guanine interacting with bare cation (green).

The second-order interaction energy $E(2)$, the natural atomic charge, and the analysis of the sp^n hybrid character of the sigma bonding orbitals, sigma antibonding orbitals, and the lone pair orbitals were calculated in the framework of NBO analysis.

3.1. Calculation of NMR Parameters for the Hydrated Guanine and for the Complexes of Guanine with the Non-hydrated Mg^{2+} and Zn^{2+} Ion. Figure 2a and b show the guanine molecule hydrated by one and two water molecules (**2a** and **2b**, respectively), which represents one of our reference structures. Figure 2c shows the complex of guanine with a bare ion (**2c**). The coordination number of Mg^{2+} in biomolecules is strictly 6 (hexacoordination), while coordination of the Zn^{2+} ion is more flexible and varies between 4 and 6.[29] Nevertheless, for comparative reasons, the first part of our analysis was carried out assuming binding of bare cations to the nucleobase (Figure 2c). This simplified molecular model is used to highlight the limit case of the ion binding effects on the NMR parameters. The calculated geometry and NMR parameters of guanine, hydrated guanine, and the complex of guanine with Mg^{2+} and Zn^{2+} ions are shown in Table 1.

Table 1. Bond Distance, R, Isotropic Shieldings, σ, and Spin−Spin Coupling Constants, J,[a] Calculated for the Guanine in Vacuo and Hydrated Guanine and Guanine in Direct Contact with the Bare Mg^{2+} or Zn^{2+} Ion

	G[b]	G···H_2O[c]	G···$2H_2O$[d]	G···Mg^{2+}[e]	G···Zn^{2+}[f]
			Geometry		
R(N1−C6)	1.441	0.009	0.013	0.074	0.078
R(C5−C6)	1.439	0.001	0.003	0.049	0.052
R(C6−O6)	1.218	−0.005	−0.006	−0.073	−0.081
R(C5−N7)	1.383	0.001	0.001	−0.009	−0.004
R(N7−C8	1.307	−0.001	−0.002	−0.024	−0.026
			NMR Shielding		
σ(C5)	58.5	58.6	59.3	66.2	67.4
σ(C6)	24.7	23.3	23.1	17.0	16.4
σ(O6)	−54.1	−42.5	−31.5	147.3	123.9
σ(N7)	−40.7	−31.7	−26.6	70.8	76.8
σ(C8)	47.7	46.5	45.1	30.9	30.3
			NMR Spin−Spin Coupling		
^1J(C5,N7)	3.8	2.6	1.6	−2.9	−6.2
^1J(C6,N1)	−5.8	−7.8	−8.6	−20.8	−22.2
^1J(C6,C5)	103.5	101.2	101.0	87.1	87.6
^1J(C6,O6)	33.3	32.9	31.9	33.3	36.8
^1J(N7,C8)	2.6	1.0	0.3	−10.8	−12.4

[a] Bond distance in Å, shieldings in ppm, and coupling constants in Hz. The bond length shortening (+) or lengthening (−) is relative to the bond distance calculated for guanine in vacuo shown in the first column. [b] Guanine in vacuo, Figure 1. [c] Guanine hydrated by one water molecule, Figure 2a. [d] Guanine hydrated by two water molecules, Figure 2b. [e] Guanine in direct contact with Mg^{2+} ion, Figure 2c. [f] Guanine in direct contact with Zn^{2+} ion, Figure 2c.

3.1.1. Geometry. The variation of guanine geometry upon hydration and complexation at oxygen O6 and nitrogen N7 with the Mg^{2+} or Zn^{2+} ion is shown in Table 1. The C6—O6 bond length of guanine lengthens gradually upon the hydration and coordination of the Mg^{2+} or Zn^{2+} ion, while the N1—C6 and C5—C6 bond lengths gradually shorten. Formation of the C6═O6···HOH hydrogen bond (H-bond) is reflected by the change of sp^n hybridization[30] of the C═O bond; the p-character of the (C6—O6) σ orbital increases what results in weakening and elongation of the bond length. The (C6—O6) σ orbital of guanine is the linear combination of the two orbitals localized at carbon C6 and oxygen O6. The calculated sp^n hybrid character of the orbital localized at oxygen O6 is 1.52, 1.54, and 1.98 for guanine, hydrated guanine in Figure 2b, and the guanine complex with the Mg^{2+} ion (G···Mg^{2+}), respectively. The sp^n character of the orbital localized at carbon C6 is 1.82, 1.85, and 2.15 for the same series of complexes. Thus the s-character of the (C6—O6) σ bond decreases gradually upon the hydration and coordination of the Mg^{2+} ion since the sp^n hybrid character of both orbitals localized at carbon C6 and oxygen O6 simultaneously increases.

The interaction of the bare ion with guanine shown in Figure 2c gives rise to the new mesomeric state of guanine. The guanine

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double bond C6═O6 becomes a single bond, whereas the N1−C6 bond of guanine becomes a double bond. This remarkable change of the guanine electronic structure represents the limit case of the ion interaction with guanine. The same mesomeric structure of guanine as found for the complex **2c** was also found for the similar N7/O6 inner-shell complex with the Mg^{2+} ion hydrated by four water molecules. Formation of the mesomeric structure upon a simultaneous N7/O6 inner-shell binding of a dication in DNA was actually anticipated before.[31] To our knowledge, however, the simultaneous inner-shell binding of small dications to oxygen O6 and nitrogen N7 of guanine was not found so far by X-ray crystallography, and thus the likelihood of their occurrence in nucleic acids should be negligible compared to the binding motifs studied primarily in this paper, especially for the zinc dication. The N7/O6 simultaneous inner-shell binding can nevertheless be expected, e.g., for the large barium dication due to its coordination number of 8.[32]

Hydration and complexation of guanine with an ion leads to the increase of s-character of the (N1−C6) σ bonding orbital which results in the strengthening of the bond and its contraction. The variation of the sp^n hybrid character of the (C5−C6) σ orbital in the complexes of guanine with water and ion is rather ambiguous when compared to the variation that of σ orbitals (C6−O6) and (N1−C6). The NBO analysis unveils the increase of s-character of the orbital localized at carbon C5, while the calculated s-character of the orbital localized at carbon C6 decreases. Consequently the C5−C6 bond length shortens less than the N1−C6 one (Table 1). The C5−N7 bond of guanine shortens upon hydration, while complexation with an ion results in lengthening. Detail NBO analysis of the (C5−N7) σ orbital unveils the smooth decrease of s-character of the orbital localized at carbon C5 upon hydration and complexation. The sp^n hybridization amounts to 2.10, 2.11, and 2.34 in guanine, hydrated guanine, and complex G···Mg^{2+}, respectively. The sp^n hybridization of the orbital localized at nitrogen N7 was 2.28, 2.22, and 2.35 for the same series of complexes. Also, the calculated weight of the orbital localized at nitrogen N7 increases by 56.5%, 56.9%, and 58.5% for the same series of complexes. The orbital localized at nitrogen N7 thus controls the character of the (C5−N7) σ orbital in different complexes of guanine. This probably explains the small C5−N7 bond length shortening upon hydration and the bond lengthening upon coordination of an ion to guanine.

The N7═C8 bond lengthens upon hydration and complexation with the ion. The calculated s-character of the orbital localized at carbon C8 decreases smoothly upon guanine hydration and ion complexation, while the s-character increase was calculated for the orbital localized at nitrogen N7. The weights of the orbital localized at nitrogen N7 in the linar combination of the (N7−C8) σ orbital were 57.7%, 58.0%, and 60.5% for guanine, hydrated guanine, and complex G···Mg^{2+}. The dominating part of the (N7−C8) σ orbital localized at nitrogen N7 is thus responsible for decreasing the double bond character of the guanine N7═C8 bond which explains the bond lengthening in hydrated guanine and in complex G···Mg^{2+}.

3.1.2. NMR Shieldings. The calculated NMR shieldings σ(O6) and σ(N7) of guanine are negative, and their values increase upon hydration toward the values obtained for complexation with a bare ion (Table 1). The withdrawal of electron density from the guanine molecule due to the formation of an H-bond between the water and oxygen O6 and nitrogen N7 of guanine is represented by the decrease of the calculated negative natural charge for oxygen O6 and nitrogen N7 (from −0.57 to −0.61 e^- and from −0.40 to −0.45 e^-, respectively). The increasing electronegativity at oxygen O6 and nitrogen N7 of guanine and the polarization of π electrons lead to the increase of calculated NMR shieldings σ(O6) and σ(N7) in hydrated guanine. This effect further increases upon complexation with an ion. The calculated NMR shieldings σ(O6) and σ(N7) of guanine shifts by 201.4 and 111.5 ppm upon the coordination of the Mg^{2+} ion. The large NMR shift calculated for oxygen O6 and nitrogen N7 of guanine due to the contact with the Mg^{2+} ion is accompanied by further decreases of natural charge of oxygen O6 and nitrogen N7 from −0.57 to −0.81 e^- and from −0.40 to −0.72 e^-, respectively. The changes of NMR shielding σ(O6) and σ(N7) by 11 and 5 ppm, respectively, calculated for the differently hydrated guanine **2a** and **2b** indicate the large sensitivity of guanine nuclei O6 and N7 with respect to the different models of solvent. The NMR shieldings σ(O6) and σ(N7) shift by −23.4 and 6.0 ppm when the Mg^{2+} ion is replaced by the Zn^{2+} ion. The NMR shielding σ(C5) increases, while a decrease in σ(C6) and σ(C8) shieldings was found in a series guanine, hydrated guanine, and guanine in complex with the ion. The calculated NMR shifts due to the guanine interaction with the Mg^{2+} ion are 7.7, −7.7, and −16.8 ppm for carbon C5, C6, and C8, respectively. The magnitude of NMR shift calculated for carbon C5, C6, and C8 of guanine correlates with the change of the natural charge. The natural atomic charges −0.08, 0.61, and 0.17 e^- calculated for carbon C5, C6, and C8 change to −0.10, 0.59, and 0.31 e^- upon coordination of the Mg^{2+} ion to guanine. All carbon NMR shieldings of Table 1 change by less than 1.2 ppm when Mg^{2+} is exchanged by Zn^{2+}.

3.1.3. NMR Couplings. The calculated one-bond coupling constant ^1J(C6,O6) of guanine decreases gradually upon the interaction of guanine with one and two water molecules by 0.4 and 1.4 Hz, respectively. The decrease by 1.4 Hz of the ^1J(C6,O6) coupling constant is mainly due to the decrease of its FC contribution by 1.8 Hz. Since the FC operator[20] probes the s-electrons at the sites of coupled nuclei, the decreasing s-character of the (C6−O6) σ orbital (see above) explains the decrease of the FC contribution of the ^1J(C6,O6) coupling. When the π-character of the C6═O6 multiple bond decreases the absolute value of SD contribution decreases.[19a] The SD contribution of the ^1J(C6,O6) coupling calculated for guanine and hydrated guanine **2b** is −1.8 Hz and −1.5 Hz, respectively, indicating the small weakening of the C6═O6 bond. The π-character of the C6═O6 bond in the guanine complex **2c** decreases upon complexation with the ion, and the bond becomes a single σ bond. Consequently, the bond length increases. Surprisingly, however, the value of the ^1J(C6,O6) coupling remains the same. The calculated SD and PSO contributions of the ^1J(C6,O6) coupling change from −1.8 Hz to 0.5 Hz and from 11.9 to 9.5 Hz, respectively, reflecting the change of bond multiplicity. Since the variation of the SD and PSO contribution of the ^1J(C6,O6) coupling compensates and the FC contribution is the same in guanine and in the guanine

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complex with the Mg^{2+} ion, the ^1J(C6,O6) coupling constant remains the same. The magnitude of the FC term is known to decrease with the decreasing s-character of orbitals probed at the position of coupled nuclei and with the increase of the energy gap between occupied and virtual molecular obitals.[19] The change of multiplicity of the C6═O6 bond leads to the fairly large decrease of s-character of the (C6−O6) σ orbital which is however compensated by the decrease of the calculated energy gap between the (C6−O6) σ and σ* orbitals and between the σ (C6−O6) orbital and the oxygen LP(O6) orbital. This explains the same value of the FC term calculated in guanine and in G···Mg^{2+} complex. The energy gaps between the (C6−O6) σ and σ* orbital and between the (C6−O6) σ orbital and oxygen LP(O6) orbital further decrease when the Mg^{2+} ion is replaced by the Zn^{2+} ion. This probably yields the increase of the ^1J(C6,O6) coupling constant mediated via FC contribution calculated for the G···Zn^{2+} complex. The impact of the double charged ion is crucial for the change of guanine mesomeric state. When the ^1J(C6,O6) coupling constant is calculated for the nonrelaxed geometry of the guanine complex in Figure 2c with the Mg^{2+} ion removed, its value increases to 53.5 Hz with the PSO, FC, and SD contributions 13.8, 41.7, and −1.8 Hz, respectively.

The absolute value of the calculated ^1J(N1,C6) coupling constant gradually increases with the increasing s-character of the (N1−C6) σ orbital in the hydrated guanine and upon complexation of guanine with the ion. The increase of the calculated ^1J(N1,C6) coupling constant is driven by the FC contribution decreasing from −7.8 Hz in guanine to −25.4 Hz in the guanine complex with the Zn^{2+} ion. The calculated C5−C6 bond distance of guanine shortens upon its hydration and complexation. The shortening of the C5−C6 bond indicates the increase of s-character of the (C5−C6) σ orbital which should give rise to the increase of the FC contribution of the ^1J(C5,C6) coupling. However the calculated ^1J(C5,C6) coupling constant decreases due to its FC contribution in the hydrated guanine and upon the coordination of the Mg^{2+} ion to guanine. The final sp^n hybridization of the (C5−C6) σ orbital is affected by the decrease of sp^n hybridization of the orbital localized at carbon C5 and by the increase of sp^n hybrid character of the orbital localized at carbon C6. The FC contribution of the ^1J(C5,C6) coupling constant of the guanine constant decreases by 2.3 Hz (2% of total ^1J(C5,C6)) upon hydration, and the sp^n characters of the orbital localized at carbon C5 and C6 change from 1.87 to 1.86 and from 1.62 to 1.64, respectively. In the case of complex G···Mg^{2+}, the FC decreases by 15.1 Hz when compared with its value in guanine, and the sp^n hybrid characters calculated for the orbital localized at carbon C5 and C6 are 1.82 and 1.75, respectively. The s-character increase of the (C5−C6) σ orbital at the site of carbon C6 which clearly dominates in the G···Mg^{2+} complex explains the decrease of the FC contribution of the ^1J(C5,C6) coupling even though the C5−C6 bond shortens.

The calculated ^1J(C5,N7) and ^1J(N7,C8) coupling constants of guanine decrease gradually upon hydration and complexation with the ion. The decreasing trend of these couplings is driven by the increase of the absolute value of the respective FC contributions. The calculated FC contributions are 0.4, −1.7, and −5.5 Hz for the ^1J(C5,N7) coupling and −2.0, −4.3, and −14.8 Hz for the ^1J(N7,C8) coupling in guanine, hydrated guanine **2b**, and complex G···Mg^{2+}. The NBO analysis unveils the pure decrease of the s-character of the (C5−N7) σ orbital in the complex G···Mg^{2+} (see above). The decrease of s-character of the (C5−N7) orbital due to the coordination of the Mg^{2+} ion to guanine is however compensated by lowering of the energy gap between the (C5−N7) σ and σ* orbitals and by an even more pronounced lowering of the energy gap between the (C5−N7) σ and nitrogen LP(N7) orbitals. The more effective coupling of the (C5−N7) σ orbital with the LP(N7) orbital due to the energy gap lowering explains the increase of the absolute value of the FC contribution of the ^1J(C5,N7) coupling constant. The calculated lowering of the energy gap between the (N7−C8) σ orbital and the nitrogen LP(N7) orbital in hydrated guanine and in the G···Mg^{2+} complex makes the coupling between σ and LP orbitals more effective as in the case of the C5−N7 bond. The s-character of the guanine (N7−C8) σ orbital localized at nitrogen N7 increases while the s-character of the orbital localized at carbon C8 decreases upon hydration and guanine complexation. The effect of the energy gap lowering between the (N7−C8) σ and LP(N7) orbitals is even more enhanced by the increase of the s-character of the (N7−C8) σ orbital at the site of the nitrogen N7. This explains the larger decrease of the FC contribution of the^1J(N7,C8) coupling than that of the ^1J(C5,N7) coupling in the same complex.

The calculated NMR parameters exhibit a monotonic increase or decrease in a series guanine, hydrated guanine, and guanine in complex with an ion. In contrast, the ^1J(C6,O6) coupling constant calculated for the complex of guanine with the Zn^{2+} ion attached to G/O6 gives rise to the increase of the coupling constant when compared with the decrease induced by hydration. Also the guanine NMR shift δ(O6) was larger in the guanine complex with Mg^{2+} than in the complex with the Zn^{2+} ion.

The charge transfer from guanine to water or ion occurs exclusively from the LP orbitals of G/O6 and G/N7 to the σ* orbital of water or LP* orbital of ion. The E(2) energies for LP(O6) → σ*(O−H) of water or LP* of ion were 2.3, 5.8, 23.2, and 61.4 kcal/mol in G···H_2O, G···$2H_2O$, G···Mg^{2+}, and G···Zn^{2+} complexes, respectively. For N7, the energies for LP(N7) → σ*(O−H) of water or LP*(ion) were 3.5, 8.3, 18.6, and 40.9 kcal/mol for the same series of complexes. The reverse charge transfer (i.e., from ion or water to guanine) with an E(2) energy greater than 1 kcal/mol occurs only for ion complexes: core (CR) (Mg^{2+}) and CR(Zn^{2+}) → BD*(N7−C8) with E(2) values equal to 1.9 and 3.7 kcal/mol, respectively. The interaction energies calculated for G···$2H_2O$, G···Mg^{2+}, and the G···Zn^{2+} complex is 13.4, 210.2, and 252.7 kcal/mol. Note that the difference of the Mg^{2+} and Zn^{2+} interaction energies is due to specific 3d (Zn)−lone pair (base) interaction which is responsible for the preferential binding of zinc to nitrogen atoms.[13a]

3.2. Calculation of NMR Parameters for the Complexes of Guanine with Hydrated Mg^{2+} and Zn^{2+} Ions. The NMR parameters were calculated in the complexes of the hydrated Mg^{2+} ion interacting directly with the oxygen O6 (**3a**) or with the nitrogen N7 of guanine (**3b**) shown in Figure 3a and b and in the complex where the interaction of the Mg^{2+} ion with guanine is mediated through water molecules as shown in Figure 3c.

The NMR spin−spin coupling constants calculated for the hydrated guanine and for the complexes of guanine with the

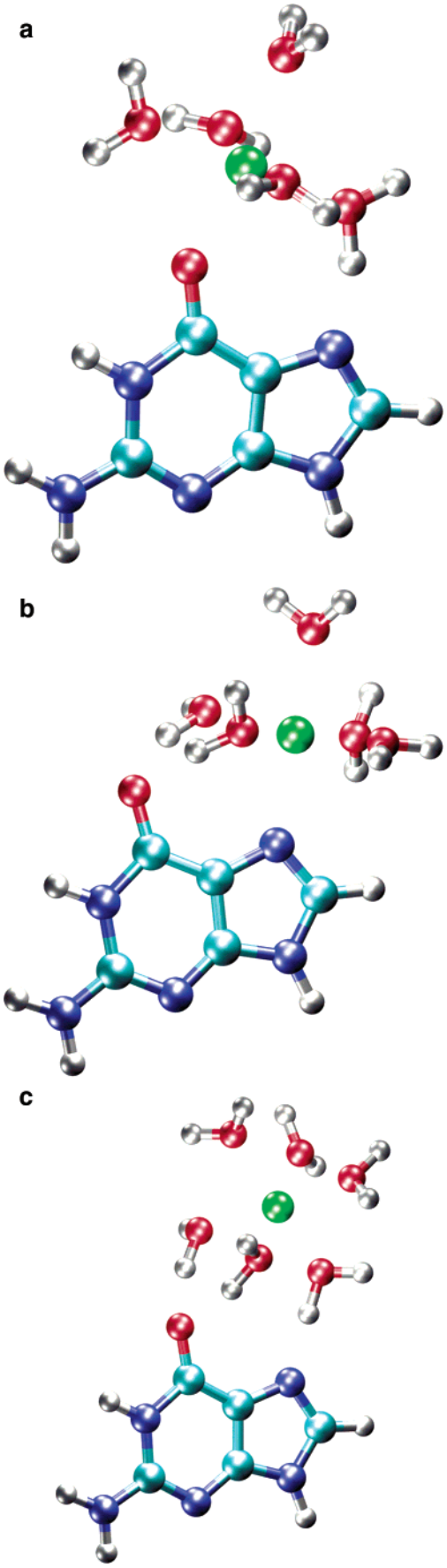

Figure 3. (a) Inner-shell coordination of cation (green) with oxygen O6 of guanine. (b) Inner-shell coordination of cation (green) with nitrogen N7 of guanine. (c) Outer-shell coordination of cation (green) with guanine.

Table 2. NMR Spin−Spin Coupling Constants[a] Calculated for the Inner-Shell Binding Complex of Hydrated Mg^{2+} Ion with Guanine and for the Outer-Shell Complex of Mg^{2+} Ion with Guanine

	G···2H_2O[b]	G/O6···$Mg^{2+/1+}$[c]	G/N7···$Mg^{2+/1+}$[d]	G···(Mg^{2+})[e]
^1J(C5,N7)	0.5	−1.6/−0.4	−3.5/−3.1	−1.8
^1J(C6,N1)	−9.7	−15.9/−14.4	−15.3/−13.3	−14.8
^1J(C6,C5)	98.1	99.2/99.2	95.8/96.8	98.8
^1J(C6,O6)	28.7	22.1/22.8	33.1/33.2	30.4
^1J(N7,C8)	−0.9	−4.2/−2.6	−5.5/−4.7	−3.6
^1J(C8,H8)	212.9	216.7/214.5	209.3/219.3	212.9
^2J(C5,N1)	−9.4	−6.7/−7.8	−7.5/−8.1	−7.1
^2J(C6,N7)	−7.3	−5.9/−6.7	−5.3/−5.8	−6.0
^2J(N7,H8)	−11.9	−10.7/−11.1	−10.5/−9.7	−10.9
J(Mg,C5)		−0.1/−0.1	−0.2/−0.3	0.0
J(Mg,C6)		−1.1/−0.9	−0.1/−0.1	0.0
J(Mg,O6)		6.2/4.6	0.0/0.0	0.0
J(Mg,N7)		0.1/0.0	5.6/4.6	0.1
J(Mg,C8)		0.0/0.0	−0.5/−0.3	0.0

[a] The coupling constants in Hz. [b] Guanine hydrated by two water molecules (Figure 2b) embedded into the dielectric cavity modeled using the COSMO approach. For details, see the Methods section. [c] Inner-shell binding of hydrated Mg^{2+} to guanine O6 (Figure 3a). The second number in each row is calculated for a hydrated cation having one OH^- anion in its coordination shell, leading thus to a total charge of $+1e^-$. For details, see the Methods section. [d] Inner-shell binding of hydrated Mg^{2+} to guanine N7 (Figure 3b). The second number in each row is calculated for a hydrated cation having one OH^- anion in its coordination shell, leading thus to a total charge of $+1e^-$. For details, see the Methods section. [e] Outer-shell configuration of hydrated Mg^{2+} ion with water-mediated contact between the Mg^{2+} ion and guanine (Figure 3c).

Table 3. NMR Shift[a] Calculated for the Guanine in Direct Contact with the Hydrated Mg^{2+} Ion and for the Outer-Shell Hydrated Mg^{2+} Ion Complex with Guanine

	G/O6···$Mg^{2+/1+}$[b]	G/N7···$Mg^{2+/1+}$[c]	G···(Mg^{2+})[d]
δ(C5)	−5.4/−3.1	−4.4/−4.2	−4.4
δ(C6)	3.3/2.8	6.6/5.5	5.7
δ(O6)	−133.1/−92.8	−75.0/−61.3	−106.5
δ(N7)	−35.0/−20.6	−48.3/−37.0	−32.9
δ(C8)	6.9/3.7	5.3/8.7	6.5
δ(Mg)[e]	3.1/11.3	6.7/14.7	7.1

[a] The NMR shifts in ppm relative to the hydrated guanine (Figure 2b). [b] Inner-shell binding of hydrated Mg^{2+} to guanine O6 (Figure 3a). The second number in each row is calculated for a hydrated cation having one OH^- anion in its coordination shell, leading thus to a total charge of $+1e^-$. [c] Inner-shell binding of hydrated Mg^{2+} to guanine N7 (Figure 3b). The second number in each row is calculated for a hydrated cation having one OH^- anion in its coordination shell, leading thus to a total charge of $+1e^-$. [d] Outer-shell configuration of Mg^{2+} ion with no direct contact between Mg^{2+} ion and guanine (Figure 3c). [e] NMR shift obtained as shielding of Mg^{2+} ion in cluster of six molecules of water in the absence of guanine base minus the shielding of Mg^{2+} ion in complex with guanine.

hydrated Mg^{2+} ion are shown in Table 2. The coupling constants of hydrated guanine shown in the first column of Table 2 were calculated for the complex **2b** embedded into the dielectric cavity simulating the continuum water solvent using the COSMO method. The polarization of the complex **2b** by continuum solvent leads to the further saturation of the calculated NMR coupling constants as can be seen from the NMR coupling constants calculated for the complexes **2a,b**, shown in Table 1. The continuum solvent described by the COSMO method was successfully used for the calculation of the hydration effect on NMR coupling constants in the DNA hairpin molecule.[25] The calculated NMR shifts for the complexes of guanine with the hydrated Mg^{2+} ion **3a**−**c** are shown in Table 3. The NMR shifts were calculated as shifts from the NMR shieldings obtained for the hydrated guanine **2b**, shown in Table 1.

The interaction energies calculated for the complex of the hydrated Mg^{2+} ion interacting directly with the oxygen O6 of

guanine, with the nitrogen N7 of guanine, and for the complex with the outer-shell configuration of the Mg^{2+} ion amount to 377.6, 368.5, and 390.4 kcal/mol, respectively. The small variation of the interaction energy calculated for the three complexes indicates the near equivalency of all complexes with the little preference for the complex of Mg^{2+} ion coordinated at the nitrogen N7 of guanine.

When the effect of the Mg^{2+} ion on the calculated NMR parameters of guanine in the outer-shell ion complex is negligible and the hydration model of guanine is complete, the difference between the *J* constants shown in the first and in the last column of Table 2 is negligible. Also, the calculated NMR shifts for the outer-shell ion configuration shown in the last column of Table 3 should approach zero in this case. However, some of NMR parameters calculated for the hydrated guanine and for the guanine complex with the outer-shell Mg^{2+} ion differ. In particular, the ^1J(C5,N7), ^1J(N1,C6), and ^1J(N7,C8) couplings and the δ(O6) NMR shift indicate the possible non-negligible effect of the outer-shell ion on NMR parameters of guanine or the unsaturated hydration of guanine. When the Mg^{2+} ion was removed from the complex **3c** and the geometry of guanine and the water cluster of the complex **3c** was kept rigid, the calculated ^1J(C5,N7), ^1J(N1,C6), ^1J(N7,C8) and ^1J(C6,O6) coupling constants change to 2.8, −11.8, 1.4, and 39.5 Hz, respectively. Work is in progress to clarify whether the interaction of the outer-shell ion with guanine is shielded entirely by water solvent or not. For comparison of the calculated NMR parameters with experiment, the guanine complex with the Mg^{2+} ion coordinated in the outer solvent shell was taken as a reference system rather than the complex of hydrated guanine **2b**.

3.2.1. NMR Coupling Constants. The direct interaction of the Mg^{2+} ion with oxygen O6 of guanine in the complex **3a** gives rise to the increase of the calculated coupling constant ^1J(O6,Mg) from 0, when the interaction is water shielded, to 6.2 Hz. Similarly, the calculated ^1J(N7,Mg) coupling constant increases from 0 to 5.6 Hz upon the direct coordination of Mg^{2+} ion to nitrogen N7 of guanine. The scalar coupling constant between the nitrogen N7 of guanine base of hammerhead ribozyme and the Cd^{2+} ion was not observed by Tanaka, although the large NMR shift measured for nitrogen N7 of guanine toward the high field was attributed to the coordination of Cd^{2+} ion to nitrogen N7 of guanine.[11f] It was argued by Tanaka that if the coupling between N7 and Cd^{2+} ion is present, its value would be smaller than 20 Hz due to an NMR signal line width resolution of 22 Hz. Also the direct coupling between the inosine nitrogen N7 and Hg^{2+} ion was not observed, although a large NMR shift toward the high field measured for the nitrogen N7 indicated the coordination of the Hg^{2+} ion to the nitrogen N7 of inosine.[33] The fact that the coupling between the Cd^{2+} and guanine N7 was not observed by Tanaka might be possibly explained by its small value which is supported by the modest value of the direct coupling constant ^1J(N7,Mg) calculated here. When the Mg^{2+} cation binds simultaneously to oxygen O6 and nitrogen N7 (Figure 2c), the calculated coupling constants ^1J(O6,Mg) and ^1J(N7,Mg) were 2.8 and 15.5 Hz, respectively. For the complex **2c** with the Mg^{2+} cation hydrated by four water molecules (figure not shown), the ^1J(O6,Mg) coupling constant was 2.6 Hz, while the ^1J(N7,Mg) coupling constant decreased to 4.7 Hz. The two-bond coupling constant ^2J(Mg,C6) decreases from 0 to −1.1 Hz due to the direct contact of the Mg^{2+} ion with the oxygen O6 of guanine (Figure 3a), and the ^2J(Mg,C8) coupling constant decreases from 0 to −0.5 Hz upon coordination of Mg^{2+} ion to nitrogen N7 of guanine (Figure 3b).

Also the calculated intramolecular coupling constants of guanine change due to the direct contact of the Mg^{2+} ion with guanine. The calculated coupling constant ^1J(C6,O6) decreases by 11.0 Hz upon the coordination of the Mg^{2+} ion to oxygen O6 of guanine compared to the value calculated for the outer-shell configuration of the ion. For the Mg^{2+} ion coordinated to nitrogen N7 of guanine, it increases only by 2.7 Hz. The calculated decrease or increase of the ^1J(C6,O6) coupling constant correlates with the calculated sp^n hybrid character values of the (C6,O6) σ orbital at the site of carbon C6 which were 1.54, 1.76, and 1.74 for the complexes **3a**, **3b**, and **3c**. In the case of direct contact of the Mg^{2+} ion with oxygen O6 of guanine, the trend is further enhanced by the decrease of the energy gap between the (C6−O6) σ bonding orbital and the oxygen LP(O6) orbital. The coupling constants ^1J(C5,N7) and ^1J(N7,C8) of guanine decrease due to the coordination of the Mg^{2+} ion to the nitrogen N7 by 1.7 and 1.9 Hz, respectively, compared with the couplings in the complex with outer-shell ion configuration. When the Mg^{2+} ion is coordinated to oxygen O6 of guanine, the ^1J(N7,C8) coupling constant decreases only by 0.6 Hz and the ^1J(C5,N7) coupling constant increases only by 0.2 Hz compared to the couplings obtained for the outer-shell ion complex. For the coupling constants ^1J(C5,N7) and ^1J(N7,C8), the calculated decreases of the energy gap between the σ and σ* orbitals and σ and LP(N7) orbitals dominate the modest variation of the sp^n hybrid character of (nitrogen−carbon) σ orbitals which gives rise to the increase of the absolute value of both couplings.

The coupling constant ^1J(C5, C6) decreases by 3.0 Hz upon the contact of the Mg^{2+} ion with nitrogen N7 compared with the value calculated for the outer-shell ion complex, while it increases only by 0.4 Hz in the case when the ion is attached to the oxygen O6 of guanine. The sp^n hybrid character of the (C5,C6) σ bond at the carbon C6 increases to 1.66 when the ion is attached to nitrogen N7, while it decreases to 1.60 for the contact of the ion with oxygen O6 compared to the value 1.62 calculated for the outer-shell coordination of the ion to guanine. When the Mg^{2+} ion is coordinated to oxygen O6 of guanine, the ^1J(N1,C6) coupling constant decreases by 1.1 Hz compared to the value calculated for the outer-shell ion coordination, while, in the case of ion contact with the nitrogen N7, the coupling decreases only by 0.5 Hz.

The calculated coupling constant ^1J(C8,H8) remains the same for the hydrated guanine and for the outer-shell complex of the Mg^{2+} ion. The direct contact of the Mg^{2+} ion with oxygen O6 of guanine in the complex **2a** causes the increase of ^1J(C8,H8) coupling by 3.8 Hz, while, in the case of ion contact to nitrogen N7, the coupling decreases by 3.6 Hz. The variation of the calculated ^1J(C8,H8) coupling constant could be explained by the variation of the calculated sp^n hybrid character of the (C8,H8) σ orbital since the energy gap between the (C8,H8) σ and σ* orbitals remains similar in all complexes of guanine. The calculated sp^n hybrid character 1.91 of the orbital localized at carbon C8 in the complex with outer-shell coordination of

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the Mg^{2+} ion increases to 1.92 and decreases to 1.89 when the Mg^{2+} ion is coordinated to oxygen O6 and nitrogen N7 of guanine, respectively. Since the orbital localized at hydrogen H8 is of 100% s-character in all complexes, the hybrid character of the (C8−H8) σ orbital is determined by the change of the orbital localized at carbon C8. The increase or decrease of the s-character explains the variation of the ^1J(C8,H8) coupling constant with respect to the ion coordination. The increase from 215 to 220 Hz was measured for the ^1J(C8,H8) coupling constant of guanine in the d(TGGT) oligonucleotide upon the interaction of Pt^{2+} with the guanine.[34] However, the increase of the ^1J(C8,H8) coupling constant measured in the d(TGGT) oligonucleotide cannot be related directly to the coordination of the Pt^{2+} ion to O6 of guanine since (a) a large NMR shift was measured for N7 of guanine,[34] (b) the negative phosphate group of d(TGGT) can affect the coupling value as indicated by calculation for complex **2b** with the total charge $+1e^-$, and (c) the C8−H8 group of guanine is not fully hydrated in our theoretical models which could also affect the coupling value.[25]

The variation of the calculated two-bond coupling constants shown in Table 2 is smaller than 1 Hz for all complexes of guanine containing an ion. Nevertheless the calculated two-bond coupling constants slightly decrease in the complexes of guanine with a directly coordinated ion except the ^2J(C5,N1) coupling. The larger difference ranging from 1 to 2.3 Hz was calculated between the two-bond couplings of hydrated guanine and the couplings of guanine with outer-shell ion coordination.

3.2.2. NMR Shifts. The NMR shifts of guanine nuclei due to the direct coordination of cation discussed in this section are calculated as a NMR shift of nucleus in the respective inner-shell complex (Table 3) minus the NMR shift of nucleus in the outer-shell complex (Table 3). The NMR shifts discussed in the text are thus considered with respect to the outer-shell ion configuration in contrast to the shifts related to the hydrated guanine shown in Table 3.

An NMR shift of −15.4 ppm was calculated for the nitrogen N7 of guanine due to the direct coordination of the Mg^{2+} ion at nitrogen N7 in complex **3b**. When the Mg^{2+} ion is coordinated at oxygen O6 of guanine, the δ(N7) NMR shift is only −2.1 ppm. The calculated δ(N7) nicely corresponds to the shift by 19.6 ppm toward the high field measured by Tanaka for nitrogen N7 of guanine in the hammerhead ribozyme upon the coordination of the Cd^{2+} ion to nitrogen N7 of guanine.[11f] The similar values of NMR that shift toward the high field were also measured by Buchanan and Stothers for the nitrogen N7 of guanosine upon the addition of the Hg^{2+} and Zn^{2+} ions[35] and for the nitrogen N7 of inosine upon the addition of the Hg^{2+} and Zn^{2+} ions.[33] The NMR shifts −26.6 ppm and +31.5 ppm were calculated for the oxygen O6 of guanine due to the direct contact of the Mg^{2+} ion with the oxygen O6 in the complex **3a** and upon contact with the nitrogen N7 of guanine, respectively.

The calculated shift δ(C5) is 0 and −1.0 ppm upon the coordination of the Mg^{2+} ion with the nitrogen N7 and with the oxygen O6 of guanine. NMR shifts of −2.50 and −1.77 ppm were measured for carbon C5 of guanine bases in the d(TGGT) oligonucleotide upon coordination of Pt^{2+} with nitrogen N7 of guanine by Mukundan.[34] The calculated δ(C6) values were −2.4 and 0.9 ppm for coordination of the Mg^{2+} ion to O6 and N7 of guanine, respectively. NMR shift δ(C8) values were +0.2 ppm and −1.2 ppm for the interaction of the Mg^{2+} ion with oxygen O6 or nitrogen N7 of guanine. Mukundan measured the resonance of carbon C8 shifted by +0.24 and +1.07 ppm for guanine bases in the d(TGGT) molecule due to the coordination of Pt^{2+} at N7 of guanine.[34] Tanaka measured the carbon C8 shift of +0.5 ppm in guanine of the hammerhead ribozyme due to the binding of the Mg^{2+} ion to the nitrogen N7 of guanine.[11f] The small variation of calculated carbon NMR shifts for C5, C6, and C8 due to the direct contact of an ion is of the similar order as the experimental NMR shifts. The theoretical model must be further improved, e.g., by inclusion of the base pairing effect and complete hydration of complex, to account for such subtle NMR shifts.

The NMR shifts of −4.0 and −0.4 ppm were calculated for the Mg^{2+} ion directly interacting with oxygen O6 or nitrogen N7 of guanine, respectively, which is in agreement with the decrease of NMR shift for the Hg^{2+} ion interacting with nitrogen N7 of inosine as observed by Buchanan.[33]

The change of calculated NMR coupling constants and NMR shifts when the total charge of the inner-shell complex of $+2e^-$ is reduced to $+1e^-$ is shown in Tables 2 and 3, respectively. The absolute values of the one-bond coupling constants decrease or remain the same when the total charge of the complex is reduced to $+1e^-$, except the increase of the ^1J(C6,C5) coupling constant by 1 Hz in the complex **3b**. The two-bond coupling constants slightly decrease in the complexes with the total charge $+1e^-$, except the increase of the ^2J(N7,H8) coupling constant by 0.8 Hz in the complex in Figure 3b. The largest variation of the coupling constant due to the charge reduction was calculated for ^1J(C8,H8) and ^1J(Carbon,N7) coupling constants. The ^1J(C8,H8) coupling constant increases by 10.0 Hz in complex **3b** with the total charge $+1e^-$ compared with the complex with the charge $+2e^-$. The calculated sp^n hybrid character of the (C8−H8) σ bond at C8 changes from 1.92 in complex **3b** with the charge $+2e^-$ to 1.86 in the complex with the charge $+1e^-$ which explains the increase of the ^1J(C8,H8) coupling constant. On the contrary, the ^1J(C8,H8) coupling constant decreases by 2.2 Hz in complex **3a** when the charge changes from $+2e^-$ to $+1e^-$ which correlates with the increase of the sp^n hybrid character of (C8−H8) σ bond at C8 from 1.89 to 1.91. The calculated coupling constants ^1J(C5,N7) and ^1J(N7,C8) increase by 1.2 and 1.6 Hz in complex **3a** when the charge of the complex is reduced from $+2e^-$ to $+1e^-$. The increase of those coupling constants in complex **3a** is driven by the decrease of the absolute value of the FC term which is caused by the increase of the energy gap between the σ and σ^* (carbon−nitrogen) orbitals and the gap between the σ and LP orbitals rather than by the change of the s-character of the σ (carbon−nitrogen) orbitals. The absolute values of the calculated NMR shifts shown in Table 3 decrease upon the change of the total charge from $+2e^-$ to $+1e^-$ in complexes **3a** and **3b**, except the shift δ(C8) in complex **3b** and the δ(Mg) shift. The change of carbon NMR shifts due to the change of total molecular charge is smaller than 3.5 ppm excluding the fairly large change calculated for oxygen O6 and nitrogen N7 of guanine. The δ(O6) increases by 40.3 and 13.7 ppm when the total charge changes from $+2e^-$ to $+1e^-$ in complexes **3a** and **3b**, respectively. The δ(N7) increases by 14.4 and 11.3 ppm in

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(35) Buchanan, G. W.; Stothers, J. B. *Can. J. Chem.* **1982**, *60*, 787.

Table 4. Change of NMR Spin−Spin Coupling Constants and NMR Shifts[a] When Interchanging an Mg^{2+} with a Zn^{2+} Ion in Complexes with Guanine

	G/O6···ion^{2+} [b]	G/N7···ion^{2+} [c]	G···ion^{2+} [d]
	NMR Spin−Spin Coupling		
Δ^1J(C5,N7)	0.5	2.2	0.6
Δ^1J(C6,O6)	−3.2	0.3	0.2
Δ^1J(N7,C8)	0.3	1.6	−0.9
Δ^1J(C8,H8)	−0.5	−1.7	−0.7
	NMR Shielding		
$\Delta\delta$(C5)	−1.1	−1.1	−0.4
$\Delta\delta$(O6)	−4.1	4.3	0.9
$\Delta\delta$(N7)	−3.1	−10.0	−2.5

[a] The change of parameter was calculated as a difference between the parameter in complex with Mg^{2+} ion minus the parameter calculated in complex with Zn^{2+} ion. The NMR parameters varying less than 1 Hz for *J* couplings and 1 ppm for the NMR shift in all three complexes were not included. The change of direct couplings between the ions and oxygen O6 and nitrogen N7 of guanine is discussed in text. [b] Inner-shell binding of hydrated cation to guanine O6 (Figure 3a). [c] Inner-shell binding of hydrated catioin to guanine N7 (Figure 3b). [d] Outer-shell configuration of cation (Figure 3c).

complexes **3a** and **3b**, respectively, when the total charge changes from $+2e^-$ to $+1e^-$. Let us reiterate that the +1 complexes were utilized to estimate the upper limit of screening of the system for example by the negative charge of the sugar−phosphate backbone.

The change of selected NMR parameters due to the replacement of the Mg^{2+} ion by the Zn^{2+} ion in the complexes **3a**, **3b**, and **3c** is shown in Table 4. The opposite sign of the magnetogyric ratio of ^{25}Mg and ^{67}Zn nuclei gives rise to the opposite sign of the direct coupling constant between the ion and oxygen O6 or nitrogen N7 of guanine. The values of the calculated ^1J(Zn,O6) and ^1J(Zn,N7) coupling constants were −17.5 Hz and −36.5 Hz, respectively, when the Zn^{2+} ion is coordinated at oxygen O6 or nitrogen N7 of guanine.

4. Conclusion

The indirect NMR spin−spin coupling constants and the NMR shifts calculated for the complexes of the guanine base interacting with the hydrated cations Mg^{2+} or Zn^{2+} can be used for the discrimination between the inner-shell and the outer-shell coordination of the cation to the guanine base. To obtain a reliable change of NMR parameters induced by the inner-shell coordination of the cation with guanine, the calculations were for the first time carried out assuming a hydrated rather than an isolated guanine as the reference state. The stepwise hydration of guanine presented in this study clearly shows the relevance of the inclusion of a water solvent reference in particular for the nuclei involved in direct interactions with surrounding molecules as O6 and N7. It was demonstrated that the calculated change of NMR parameters due to the presence of an ion in the environment of water is more reliable when the effect of hydration is taken into the account.

The intermolecular spin−spin coupling constants ^1J(X,O6) and ^1J(X,N7) (X = Mg^{2+}, Zn^{2+}) respond selectively to the direct coordination of the cation with either oxygen O6 or nitrogen N7 of guanine. The calculated coupling constants ^1J(Mg,O6) and ^1J(Zn,O6) are 6.2 and −17.5 Hz, respectively, in the complex of guanine with an ion coordinated at O6 of guanine. When the ion is coordinated at N7 of guanine, the coupling constants ^1J(Mg,N7) and ^1J(Zn,N7) are 5.6 and −36.5 Hz, respectively. The intermolecular couplings approach zero when the interaction between the cation and guanine is shielded by water, i.e., in the outer-shell complex. The two-bond intermolecular coupling constant ^2J(Mg,C6) decreases from 0 to −1.1 Hz due to the inner-shell coordination of the Mg^{2+} ion to O6, and the ^2J(Mg,C8) coupling constant decreases from 0 to −0.5 Hz upon complexation of the Mg^{2+} cation to N7.

The intramolecular one-bond coupling constants ^1J(C5,C6), ^1J(C5,N7), ^1J(N7,C8), and ^1J(C8,H8) decrease, and the coupling constant ^1J(C6,O6) increases upon the complexation of a cation with N7. The decrease of the coupling constant ^1J(C6,O6) and the increase of the coupling constant ^1J(C8,H8) were calculated when the cation is coordinated to O6 of guanine. The variation of calculated NMR spin−spin coupling constants due to the interaction of water molecules or hydrated metal cation(s) with guanine is driven mainly by the variation of the FC term. The changes of the FC term were explained on the basis of the calculated sp^n hybrid character of sigma bonding, sigma antibonding, and lone pair orbitals and by considering the energy gap between the orbitals in different complexes.

The calculated two-bond intramolecular coupling constants vary less than 1 Hz when calculated for inner-shell and-outer shell complexes.

The NMR shift δ(N7) of −15.3 and −14.8 ppm calculated for nitrogen N7 of guanine due to the inner-shell contact of Mg^{2+} and Zn^{2+} cations, respectively, is comparable with the NMR shift of 19.6 ppm toward the high field measured for N7 of guanine upon the complexation with Cd^{2+} cation.[11f] The same trend was measured for the NMR shift of guanine N7 upon complexation with Hg^{2+} and Zn^{2+} cations.[35] When the cations Mg^{2+} and Zn^{2+} bind to O6 of guanine, the calculated shifts δ(N7) are only −2.1 and 6.3 ppm, respectively.

The interaction energies amount to 377.6 and 368.5 kcal/mol for the inner-shell complex with the Mg^{2+} ion coordinated to O6 and to N7, respectively. For the outer-shell complexes of the Mg^{2+} cation, the interaction energy was 390.4 kcal/mol. This is in agreement with literature data.[15b] The comparable interaction energies are not surprising, and as stated above, all binding motifs investigated in this paper are commonly seen in nucleic acids, depending on the balance of other contributions. The possible presence of the binding motif with a cation coordinated to oxygen O6 of guanine can be verified by measuring the ^1J(Mg,O6) coupling constant.

The present study shows that the NMR method complemented by quantum chemical theory has high capability to detect selectively the specific coordination of metal cation with nucleic acid base.

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Supporting Information Available: The geometry parameters of all complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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