

Binding effects of Mn^{2+} and Zn^{2+} ions on the vibrational properties of guanine-cytosine base pairs in the Watson-Crick and Hoogsteen configurations

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Abstract The binding effects of Mn^{2+} and Zn^{2+} ions on the vibrational properties of guanine-cytosine base pairs have been performed using density functional theory investigations. The calculations were carried out on Watson-Crick and Hoogsteen configurations of the base pairs. We have found, that in Watson-Crick configuration, the metal is coordinated to N7 atom of guanine while, in the case of Hoogsteen configuration, the coordination is at N3 atom of guanine. We have pointed out the vibrational bands that can be used to detect the presence of metallic ions in the Watson-Crick and Hoogsteen structures. Our results show that the vibrational amplitudes of metallic atoms are strong for wavenumbers lower than 600 cm^{-1} . Also, we predict that the distinction between Watson-Crick and Hoogsteen configurations can be seen around 85, 170 and 310 cm^{-1} .

Keywords DFT · Guanine-cytosine · Hoogsteen base pair · Metal cation · Watson-Crick base pair

Introduction

The influence of metallic cations on the structural and physical properties of DNA have not yet been completely clarified [1, 2], although DNA-metal cation interactions and their influence on DNA structure have been investigated extensively by a variety of techniques [2–5]. Therefore,

the active biological role of divalent metal ions (Mt^{2+}) in the functions of genetic apparatus has been attracting permanent interest in the interaction of these ions with nucleic acids [6]. These ions are known to be involved in DNA functioning and their role has been thoroughly investigated in the last years. Some of the metals interact directly with DNA, while others act as cofactors for a number of nuclear proteins [7].

Mn^{2+} ions are able to change the enzymatic activity of some nuclear proteins. To understand the molecular mechanisms implied by this activity, it is necessary to have a picture of the metal ion interaction with DNA. It was demonstrated that manganese is involved in interactions with phosphate oxygens and with the bases of DNA [7]. In a model proposed by Polyanichko et al. [7] for the interaction between manganese ions (Mn^{2+}) and DNA, mediated by water, is suggested that a destabilization of the double helix occurs, as well as partial breaking of the hydrogen bonds between the base pairs.

Transition metals such as Mn and Co, at high ionic strength are found to be mutagenic and carcinogenic and also change the handedness of DNA [8]. The effects of manganese(II) ions on calf thymus DNA structure, in the presence of Na(I) ions were investigated by us using vibrational spectroscopy [2, 9]. Raman bands of the bases at 305, 1336, 1376, 1488 and 1578 cm^{-1} are consistent with metal binding to N7 atom of guanine. To a lesser extent, binding of Mn^{2+} to adenine is suggested [2, 9].

The influence of Zn^{2+} ions on DNA structure have been previously studied by us, at room temperature, by means of Raman spectroscopy [10]. We have found that metal binds to N7 of guanine and, to a lesser extent, to N7 of adenine [10]. By analyzing the appearance of the difference bands of the bases near 1490 and 1581 cm^{-1} , we have found that the process starts at 50 mM Zn^{2+} ions.

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Besides, there is no consensus on the precise nature of the changes in DNA structure, that occur when pH is lowered. It seems that a subtle interplay between chemical parameters (pH, ion type and concentration) may result in appreciable changes in DNA conformation [11].

In this paper we present a theoretical vibrational analysis of the Watson-Crick and Hoogsteen GC base pairs in interaction with the metallic ions Mn^{2+} and Zn^{2+} . Our analysis is based on the DFT calculation. Our aim is to predict the values of the vibrational frequencies where specific signatures for the ion's presence in the vibrational spectra are present.

Computational models

DFT calculations

We have carried out calculations of the vibrational properties of GC base pairs of DNA in Watson-Crick and Hoogsteen geometry configurations, using the *SIESTA* code [12, 13]. *SIESTA* employs pseudopotentials and expands the wavefunctions of valence electrons using linear combinations of numerical atomic orbitals. Periodic boundary conditions are imposed on the system; therefore, we used a large simulation cell (i.e., $25 \times 25 \times 25 \text{ \AA}^3$) in order to get rid of the interactions between periodically repeated images of the molecule. We employed a double-zeta polarized (DZP) basis set with an energy cutoff of 120 meV for all atoms. The value for the energy shift was optimized by using the *Simplex* method provided by *SIESTA* and described in ref. [14]. We used as reference several geometric configurations extracted from the experimental data for the B-DNA structure. Precisely, we optimized energy shift for isolated bases, as well as for CG base pairs. The value of 120 meV is close to the average for the results obtained. For the exchange and correlation, we have used the PBE exchange correlation functional [15]. No scaling factors have been used for the vibrational frequencies reported in this work.

Setup of the geometrical model

Metal ions can bind RNA and DNA molecules unspecifically, by neutralizing the negative charges on the sugar-phosphate backbone, but can also bind specifically at nucleotide base atoms such as N7 of A and G purines [16]. Transition metal ions have the tendency to coordinate almost exclusively to the N7 position of purine bases, especially guanine [16, 17]. Theoretical studies have revealed that metal binding reinforces the stability of the base pairing, while the solvent has the opposite effect (i.e., reduces the metal stabilizing effect on the base pairing). It was also shown that the first coordination

sphere of the metal is saturated by water molecules, severe geometric deformations occur when a naked cation is coordinated to the N7 atom of purines.

For this reason, we initiated our analysis by investigating the interaction between the pentahydrated aquaions $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$ and $[\text{Zn}(\text{H}_2\text{O})_6]^{2+}$ and the GC base pairs, respectively [16]. In order to compute the force constants matrix we relaxed the geometrical structures up to a maximum gradient of 0.01 eV/\AA . The vibrational analysis is performed with the resulting structure, by using the frozen phonons method. A total charge of $2+$ was assigned to the system for Watson-Crick simulations, while for Hoogsteen calculations we have used a $3+$ charge in order to take into account the protonation effect.

Data analysis

Usually, the analysis of the vibrational properties of a molecule is rationalized in terms of normal modes of vibration [18]. This is a powerful approach, allowing for an accurate description of the vibrational eigenvectors in terms of geometrical representations. Its disadvantage is that it requires the analysis of a large amount of data in order to provide the global information on the vibrational spectra of the molecule. Precisely, a number of $3N-3$ geometrical representations of the vibrational eigenmodes are needed, where N represents the number of atoms in the molecule.

The vibrational density of states (VbDOS) provides an alternative way to analyze the vibrational properties of a molecule, and is more suitable for a global comparison. For the present study, we followed the straightforward definition of the VbDOS given in [19]:

$$I_{\Gamma}(\omega) = \sum_{\mathbf{N} \in \Gamma} \sum_i |A_i^{\mathbf{N}}(\omega)|^2, \quad (1)$$

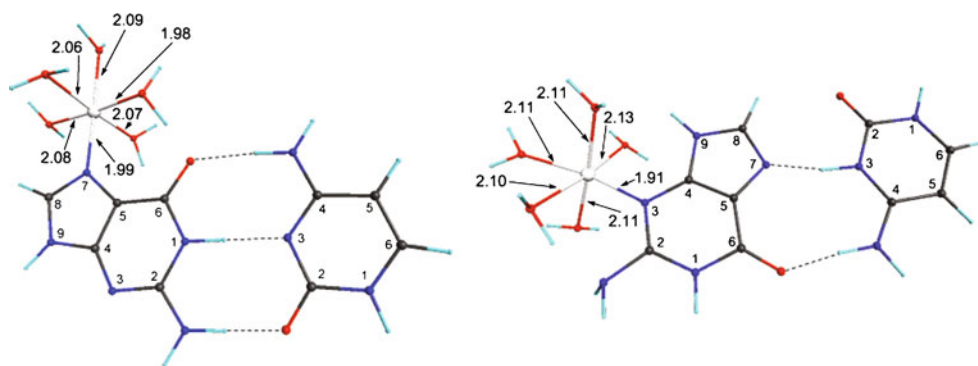
where $|A_i^{\mathbf{N}}(\omega)|$ is the i th component of the vibrational eigenvector ($i=1, 2, 3$) of the modes with vibrational frequency ω . Here, Γ refers to a group of atoms that are indexed by \mathbf{N} . For plotting purposes, we use a smearing function with a Lorentzian shape and a width of 5 cm^{-1} , as well as a discretization step of 1 cm^{-1} . By using the VbDOS, we can select the modes where a specific atom (in our case the metallic atom) has important contributions to the vibrational eigenvector.

Results

Effect of the manganese (II) ion on the vibrational properties

The spin singlet, as well as high spin electronic states, were taken into account for our simulations. The structural

Fig. 1 The relaxed structure of the guanine-cytosine base pair in the Watson-Crick configuration (left) and Hoogsteen configuration (right), in the presence of Mn^{2+} atom coordinated to the N7 (Watson-Crick) and to the N3 (Hoogsteen) atoms of guanine, respectively. Metal-water distances are indicated in Å



relaxation lead us to the conclusion that the high spin states are energetically more stable compared to singlet states. In the case of Watson-Crick base pair, the total energy of the hextet state is 2.87 eV lower compared to that of the singlet state. This is a direct consequence of the number of d electrons (i.e., $3d^5$ electronic level in the Mn atom).

The total energy of the spin polarized Hoogsteen structure is 1.99 eV lower than the energy of the structure relaxed based on singlet state calculations; this shows that the high spin state is the fundamental state for the Hoogsteen base pairs with Mn^{2+} ion. In this case we also found a spin hextet as ground state.

The two relaxed structures are presented in Fig. 1. We note that the experimental average value for the hydrated Mn^{2+} cations in dilute aqueous solution is 2.19 Å [20]. The average values resulting from our calculations are 2.25 Å for Watson-Crick configuration and 2.21 Å for Hoogsteen (i.e., about 2 % longer than the experimental value). These values are in agreement with the well known over-estimation of the bond lengths by the GGA exchange correlation functionals.

We note, in the case of Hoogsteen configuration, that the metallic ion is located slightly outside of the guanine's plane: the angle between guanine's plane and the N7–Mn bond is about 18.5°. The main cause of this deformation is the interaction taking place between the N1 atom of the guanine and one of the water molecules coordinated to the metallic atom.

In the next step of our study, we project the total VbDOS for the two configurations of the GC base pairs over the metal atom. Calculations were done in the wavenumber range between 0–4000 cm^{-1} . The first conclusion we draw from these results is that the metal atom vibrates with large amplitudes only for wavenumbers ranging from 0 to 400 cm^{-1} (see Fig. 2). While this is a clear hint for the presence of specific Raman large scattering amplitudes, we have to remember that the metal's presence can also influence the Raman intensity in a more indirect manner. Precisely, the presence of manganese(II) may lead to important modification of system's polarizability, which will be reflected in the Raman intensity at wavenumbers larger than 400 cm^{-1} .

Let us compare now the vibrational bands characteristic for the vibration of the metallic ion in each of the two

configurations. First, we note a large difference in the vibrational amplitudes of the metallic ion at 44 cm^{-1} and 83–87 cm^{-1} , respectively. While the peaks in this region have similar amplitudes for the Hoogsteen type configuration, in the Watson-Crick base pair we have a depletion of the VbDOS peak at 45 cm^{-1} , while the amplitude of the peak at 83 cm^{-1} is roughly double, compared to the peaks present at similar wavenumbers in the Hoogsteen configuration.

Next, we see differences which are rather small between the two vibrational densities (let us remember here that an error of a few percent, typically 2 %, is expected to affect the position or the amplitude of the peaks). We note, for example, the presence of a peak at 169 cm^{-1} and the large amplitude of the peak at 253 cm^{-1} in Watson-Crick configuration, compared to the similar peak at 246 cm^{-1} in the Hoogsteen configuration.

Finally, one of the most distinctive peaks in the Hoogsteen configuration is present at 336 cm^{-1} . Its equivalent in the Watson-Crick configuration is shifted to 310 cm^{-1} (see Fig. 2).

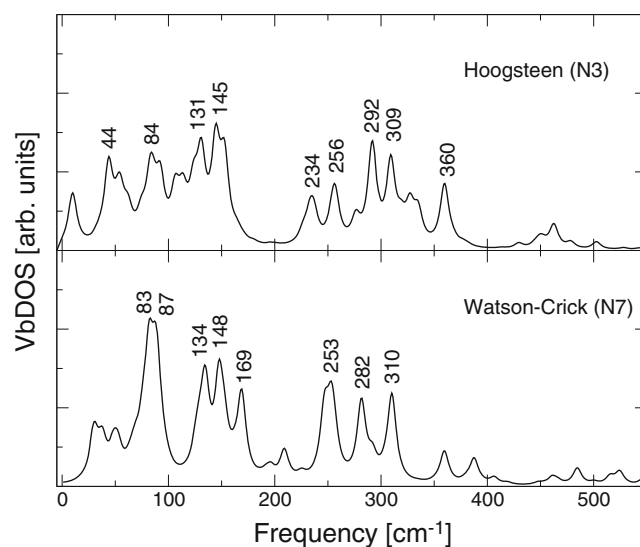
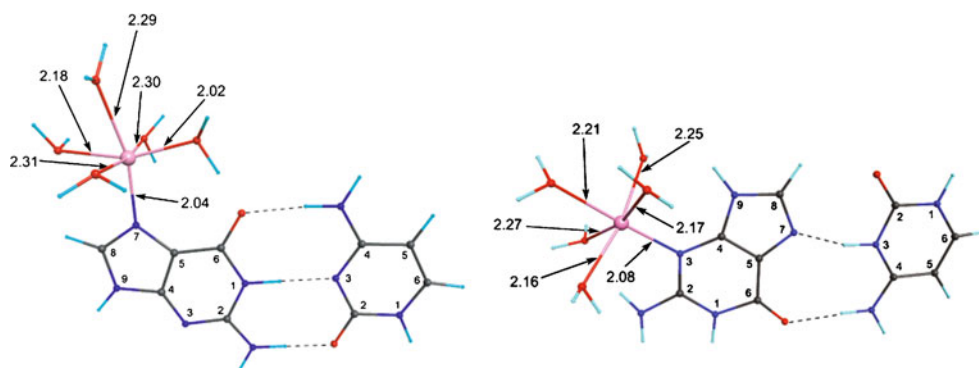


Fig. 2 Vibrational density of states projected over the Mn^{2+} ion in Watson-Crick and Hoogsteen base pairs. We have restricted our plot to the 0–550 cm^{-1} . For larger wavenumbers, the VbDOS projected over the Mn^{2+} ion is negligible

Fig. 3 The relaxed structure of the guanine-cytosine base pair in the Watson-Crick (left) and Hoogsteen (right) configurations, in the presence of Zn^{2+} atom coordinated to the N7 of guanine (Watson-Crick) and N3 in cytosine (Hoogsteen). Metal-water distances are indicated in Å



In a previous work we have studied the localization of vibrational modes over the cytosine and guanine in the Watson-Crick and Hoogsteen configurations [19]. We have found that the vibrational modes are localized on the guanine for the modes with wavenumbers 291, 306 and 347 cm^{-1} for Hoogsteen configuration, while for Watson-Crick base pair the vibrational modes localized on guanine are at 276, 341 and 356 cm^{-1} (see the supplementary material in [19]). It can be seen, that in the presence of Mn^{2+} ion attached to guanine, the values of VbDOS peaks in the region around $250\text{--}300\text{ cm}^{-1}$ are at 292, 309 and 360 cm^{-1} for Hoogsteen configuration and near 253, 282 and 310 cm^{-1} for Watson-Crick one. All these late modes are dominated by strong vibrations of metal and surrounding water molecules, while smaller collective vibrations occurs in guanine. Firstly, we notice the qualitative agreement between the calculations with and without metal atom attached to the guanine; secondly, these results suggest that the influence of Mn^{2+} ion upon the vibrational properties of GC base pairs is stronger for Watson-Crick configuration, where the shift of the wavenumbers has values from 23 to 46 cm^{-1} (compared to 1 to 13 cm^{-1} for the Hoogsteen one).

Effect of the zinc(II) ion on the vibrational properties

The resulting geometrical parameters for the metal-water bonds are depicted in Fig. 3. The average metal-oxygen bond length resulting from our calculations is 2.21 Å. The EXAFS experimental results show that metal-oxygen first shell distance is 2.08 Å [21]. We note that the computed average value is about 5 % larger than the experimental value obtained for hexahydrated Zn^{2+} ion; this value is significantly larger than the overestimation of bond lengths in GGA approximation (i.e., about 2 %). On the other hand, by inspecting Fig. 3 we note that the metal-oxygen distances have values ranging from 2.02 close to 2.3 Å. The partial loss of the octahedral symmetry is probably responsible for the relatively large bond lengths in the present theoretical model. The electronic ground state is a spin-singlet state, unlike for manganese, where the ground state is a high-spin state.

In the case of Hoogsteen configuration we also found a spin-singlet as fundamental state; the average metal-water distance is similar to that obtained in the case of Watson-Crick (about 2.20 Å). Also, the $Zn\text{--}N3$ bond forms an angle with the plane of the GC base pair; its value is slightly lower compared to the value obtained for $Mn\text{--}N3$ bond (i.e., 14.5°).

Vibrational properties calculations were done in the wavenumber range between $0\text{--}4000\text{ cm}^{-1}$. Vibrational density of states projected on Zn^{2+} is presented in Fig. 4. The Zn^{2+} ion displays large vibrational amplitudes for relatively small wavenumbers (i.e., in the range $0\text{--}600\text{ cm}^{-1}$). This range is comparable with the similar range obtained for Mn^{2+} ion (see Fig. 2).

The comparison between VbDOS of Zn^{2+} in the Hoogsteen and Watson-Crick configuration reveals relatively small differences between the two VbDOS curves. While similar features are present in the two spectra, variations of the frequencies, as well as the variation of the VbDOS amplitudes up to 10 %, are present.

By comparing the VbDOS projected over the Zn^{2+} ion with that projected on the Mn^{2+} ion (both in Watson-Crick

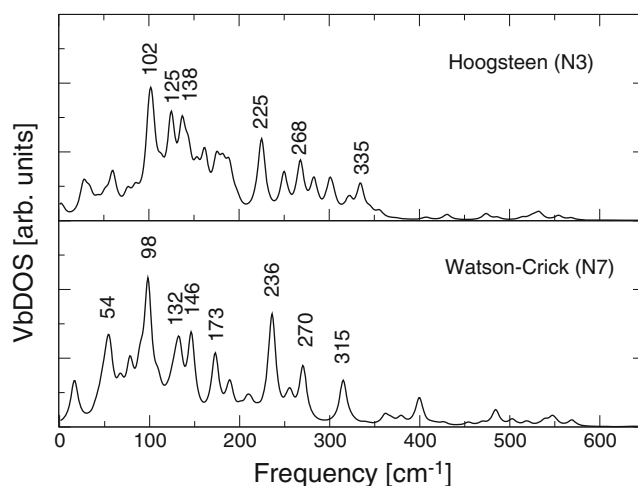


Fig. 4 Vibrational density of states projected over the Zn^{2+} ion, in the Watson-Crick and Hoogsteen base pair. We have restricted our plot to the $0\text{--}650\text{ cm}^{-1}$. For larger wavenumbers, the VbDOS is negligible

configuration) we note that they share similar features. Nevertheless, the vibrational bands with important values of VbDOS for Zn^{2+} are slightly shifted toward larger wavenumbers (with about 5 cm^{-1}). The most important difference between the two configurations is the presence of a large peak in the Zn^{2+} -projected VbDOS at 236 cm^{-1} , which has no correspondent in the VbDOS of Mn^{2+} . Therefore, this may represent useful information allowing the detection of Zn^{2+} in the structure of Watson-Crick GC base pairs. In the Hoogsteen configuration, the most striking differences between the VbDOS of Mn^{2+} and Zn^{2+} occurs around 300 cm^{-1} .

We recall that in the case of metal-free base pairs, the vibrational modes are localized on the guanine at the wavenumbers 291, 306 and 347 cm^{-1} (Hoogsteen configuration), and at 276, 341 and 356 cm^{-1} (Watson-Crick configuration), respectively [19]. In the presence of Zn^{2+} ion, the VbDOS displays peaks at 268 and 335 cm^{-1} (Hoogsteen configuration); smaller peaks are also present between these values, in particular at 303 cm^{-1} . For Watson-Crick base pair, the wavenumbers of the VbDOS peaks are at 270 and 315 cm^{-1} . In this case, the qualitative agreement with the results obtained for free base pairs is less obvious than for Mn^{2+} . We conclude, that the presence of Zn^{2+} leads to a more important perturbation of the vibrational spectrum at low frequencies, as compared with Mn^{2+} . This result is partially explained by the larger mass of the Zn^{2+} ion.

Conclusions

We present a DFT investigation of the vibrational properties of GC Watson-Crick and Hoogsteen base pairs in the presence of Mn^{2+} and Zn^{2+} ions. We have tested the formation of bonds at binding sites; we have found that the most stable bonds are formed at the N7 atom of guanine (i.e., in Watson-Crick configuration) and at the N3 atom of cytosine, respectively (i.e., in Hoogsteen configuration).

For Mn^{2+} , we have found that the high-spin states are the most stable in GC Watson-Crick base pair, as well as in the Hoogsteen one. For Zn^{2+} , we have found that the fundamental state is a spin-singlet one. The vibrational density of states projected over the metallic atoms suggests the bands to be used as markers for the detection of the Mn^{2+} and Zn^{2+} ions, respectively, by means of vibrational spectroscopy. Metal's vibrational amplitudes are important for wavenumbers lower than 400 cm^{-1} . Also, the distinction between the metal's vibrational amplitudes in Watson-Crick and Hoogsteen configurations, may be seen at 84 cm^{-1} and 169 cm^{-1} , where larger amplitudes are present in the Watson-Crick configuration. In the Hoogsteen type configuration, the metal presents a strong vibration at 336 cm^{-1} , while the equivalent in the Watson-Crick VbDOS is found close to 310 cm^{-1} .

As a distinctive band for the presence of the Zn^{2+} ion we note the large VbDOS peak at 236 cm^{-1} , which has no equivalent in the VbDOS spectrum of the Mn^{2+} ion. By comparing the VbDOS for the two GC base pairs configurations coordinated to the Zn^{2+} , we have found that while the vibrational density of states characteristic to the Hoogsteen configuration is clearly different from that of the Watson-Crick one, it is difficult to spot clear differences between the two. Instead, we have noted differences of about 10 % for both position and intensity, throughout the spectrum.

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