

Hydrogen bonding and aromaticity in the guanine–cytosine base pair interacting with metal cations ($M = \text{Cu}^+$, Ca^{2+} and Cu^{2+})[§]

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The influence of metal cations ($M = \text{Cu}^+$, Ca^{2+} and Cu^{2+}) coordinated to the N_7 of guanine on hydrogen bonding and aromaticity of the guanine–cytosine base pair has been analysed with the help of delocalization indices using the B3LYP functional. Our analysis shows that the strengthening of the $\text{N}_1 \cdots \text{N}_3$ and $\text{N}_2 \cdots \text{O}_2$ hydrogen bonds and the weakening of the $\text{O}_6 \cdots \text{N}_4$ hydrogen bond is mainly caused by the modification of donor–acceptor (covalent) interactions rather than to a significant change of electrostatic interactions. On the other hand, the increase of the aromaticity of the guanine and cytosine six-membered rings because of the interaction with Cu^+ and Ca^{2+} is attributed to the strengthening of hydrogen bonding in the guanine–cytosine pair. The observed reduction of aromaticity in the five- and six-membered rings of guanine due to ionization or interaction with Cu^{2+} is caused by the oxidation process that removes a π electron disrupting the π electron distribution.

1. Introduction

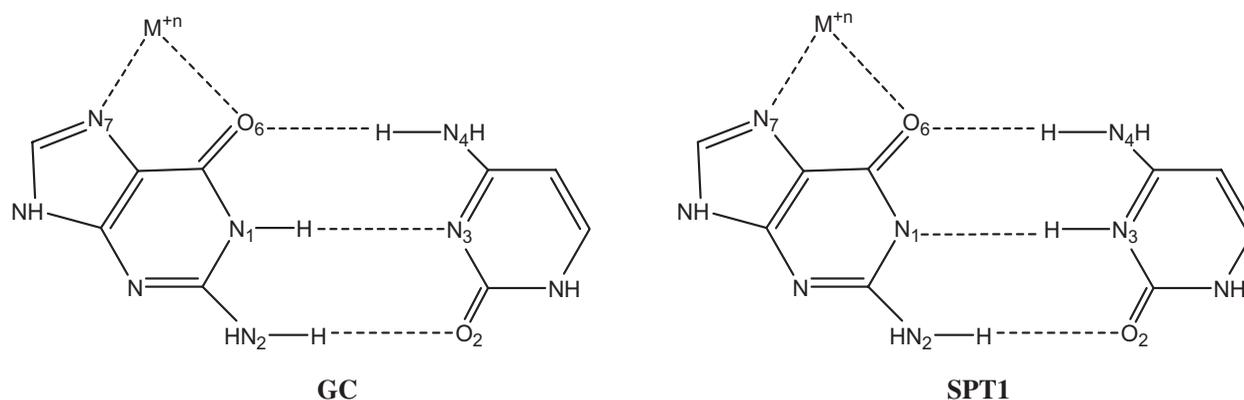
Metal cations are known to play an important role in the stabilization of DNA [1, 2]. The main effect is to neutralize the negatively charged backbone phosphate groups, but also more specific interactions take place with the bases, which modify the hydrogen bonds (H-bonds) and the stacking interactions that stabilize the double helix. The systematic study of interactions between metal ions and nucleic acids started 50 years ago, when Katz demonstrated that sensible changes in physico-chemical properties occurred upon addition of Hg^{2+} ions [3]. Nowadays, it is well established that the N_7 position of the guanine, which is well accessible in the major groove of duplex DNA and is not involved in Watson–Crick base pairing, is the preferred binding site [1, 2, 4–6].

Many studies have analysed the interaction of different metal cations to guanine [6–12] and their influence on base pairing [9–16]. Results show that metal cation binding to base pairs has a pronounced effect on structural and electronic properties of the interacting bases, the stability of the guanine–cytosine Watson–Crick base pairing being enhanced [9–15] mainly by polarization [11, 14]. Moreover, some theoretical results have shown that the presence of the metal cations interacting at the N_7 position of guanine promotes the proton transfer from N_1 of guanine to the N_3 acceptor site of cytosine yielding the so-called SPT1 species depicted in scheme 1 [11, 12]. Most of the theoretical studies have dealt with alkali, alkaline-earth or closed-shell transition metal cations. The effect of an open-shell metal cation, such as Cu^{2+} , has been recently considered [17]. Due to the oxidative character of Cu^{2+} , its interaction with the guanine–cytosine base pair leads to the formation of oxidized guanine, which has been previously shown to have strong influence on base pair H-bonds [18].

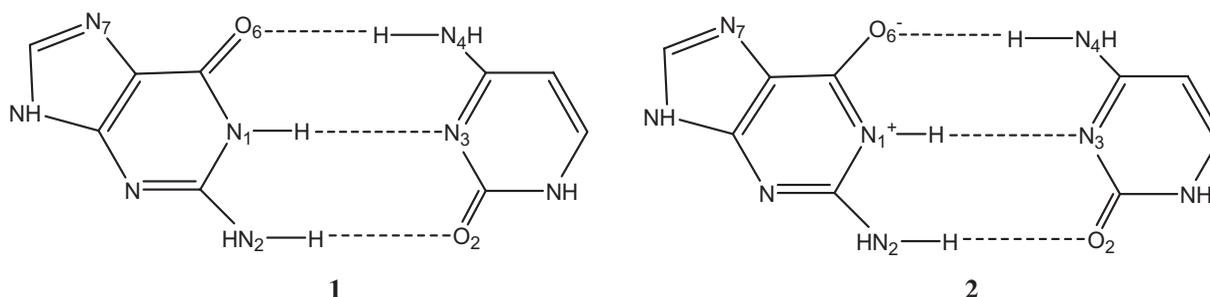
The effect of transition metal cations on the local aromaticity of Watson–Crick base pairs has not been analysed yet. Among aromatic heterocyclic compounds

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[§] Dedicated to Professor Nicholas C. Handy as a proof of our admiration for his brilliant contributions in the field of quantum chemistry.



Scheme 1.



Scheme 2.

[19], Watson–Crick base pairs have particular importance. In spite of this, the aromaticity of nucleobases and Watson–Crick base pairs has been analysed in only two works by Cyrański *et al.* [20] and by Box and Jean-Mary [21]. In their studies, the authors concluded that the formation of a Watson–Crick base pair enhances the aromatic character of the nucleobases as a consequence of H-bond formation [20, 21]. Box and Jean-Mary [21] justify this result noting that hydrogen bonding stabilizes the charge separation required for six-membered rings (6-MRs) of nucleobases to become aromatic as can be seen in scheme 2. A previous work has shown that the interaction of metal cations with the guanine–cytosine Watson–Crick base pair increases the $N_1 \cdots N_3$ and $N_2 \cdots O_2$ H-bond interactions and decreases that of the $O_6 \cdots N_4$ H-bond (see scheme 1) [17]. Not only H-bonds are modified, but also a significant charge polarization, which may affect the aromaticity of the base pair, occurs. In this sense, it remains to be seen how the interaction with metal cations alters the aromatic character of the guanine–cytosine base pair.

Thus, the goal of the present work is to study the effect of a metal cation ($M = Ca^{2+}$, Cu^+ and Cu^{2+}) on the cytosine–guanine base pair with two purposes. First, we want to discuss the electronic reorganization caused

by the interaction of the metal cation in terms of electron pairing with special emphasis on the three hydrogen bonds present in this system. Second, we aim to monitor the aromaticity changes due to the interaction of metal cations with the cytosine–guanine DNA base pair. To this end, three probes of local aromaticity based on structural, magnetic and electron delocalization properties will be considered.

2. Method of calculation

The full set of calculations has been performed at the B3LYP [22] hybrid density functional theory (DFT) level using the Wachters basis set [23] for Cu, the Blaudeau *et al.* [24] basis set for Ca, and the 6-31G(d,p) basis set for C, N, O and H (hereafter referred to as basis B1 as in [17]). The geometries taken from a previous article by Noguera *et al.* [17], who carried out full geometry optimizations with the GAUSSIAN 98 program [25] at the B3LYP/B1 level of theory, have been used throughout.

To analyse the nature of the H-bond interactions, calculations of delocalization indices (DIs) within the framework of the Atoms in Molecules (AIM) theory

[26, 27] have been carried out by means of the AIMPAC collection of programs [28] using the following expression [29–31]:

$$\delta(A, B) = 2 \sum_{i,j} S_{ij}(A)S_{ij}(B). \quad (1)$$

The summations in equation (1) run over all the occupied molecular spin-orbitals. $S_{ij}(A)$ stands for the overlap between molecular spin-orbitals i and j within the basin of atom A , which is defined from the condition of zero-flux gradient in $\rho(r)$ [26, 27]. The DI, $\delta(A, B)$, corresponds to the number of electrons delocalized or shared between atoms A and B . For a given A–B bond, $\delta(A, B)$ reflects its degree of covalency. Indeed, previous workers using indices that are equivalent to the DI defined in equation (1) have identified it with a *covalent* bond order [32–35]. As an example, $\delta(\text{H}, \text{H}) = 1.0$ for the covalent bond in H_2 , while for the more ionic bond in LiF , $\delta(\text{Li}, \text{F}) = 0.18$, both obtained at the HF/6-311++G(2d, 2p) level [30].

It is worth noting that calculations of DIs at the DFT level of theory cannot be performed exactly because the electron-pair density is not available at this level of theory [36]. As an approximation, DFT DIs are obtained using the expression for the calculation of the DIs at the Hartree–Fock (HF) level of theory (equation (1)) substituting the HF molecular spin-orbitals by the Kohn–Sham molecular spin-orbitals derived from the DFT calculations. The values of DIs obtained using this approximation are reasonable and generally closer to the HF values than to the correlated results [36].

With respect to the aromaticity study, we have used the geometry-based harmonic oscillator model of aromaticity (HOMA) measure [37, 38], the magnetic-based nucleus independent chemical shift (NICS) index [39] and the electronic-based *para*-delocalization index (PDI) [40, 41] computed at the B3LYP/B1 level as indicators of aromaticity. The use of differently based indices of aromaticity is recommended because of the multidimensional character of this phenomenon [42]. The HOMA has been obtained from equation (2):

$$\text{HOMA} = 1 - \frac{\alpha}{n} \sum_{i=1}^n (R_{\text{opt}} - R_i)^2, \quad (2)$$

where n is the number of bonds considered and α is an empirical constant (for CC and CN bonds $\alpha = 257.7$ and 93.52, respectively) chosen in such a way that $\text{HOMA} = 0$ for a model non-aromatic system, and $\text{HOMA} = 1$ for a system with all bonds equal to an optimal value R_{opt} (1.388 and 1.334 Å for CC and CN bonds, respectively) [38], assumed to be achieved for fully aromatic systems. R_i stands for a running bond

length. The PDI index is defined as the average of all DIs of *para*-related carbons in a given 6-MR [40]. Finally, the NICS index is obtained as the negative value of the absolute shielding computed at a ring centre or at some other interesting point of the system [39]. Rings with large negative NICS values are considered aromatic. The more negative the NICS values, the more aromatic the rings are. The Gauge-Independent Atomic Orbital (GIAO) method [43] has been employed to perform the calculations of NICS with the help of the GAUSSIAN 98 program [25]. All open-shell calculations have been carried out using an unrestricted formalism.

3. Results and discussion

3.1. Structure, base pair interaction energies and metal–ligand interactions

Figure 1 contains the molecular structures of the systems studied [17]: GC, $[\text{GC}]^+$, $\text{Ca}^{2+}\text{-GC}$, $\text{Cu}^+\text{-GC}$ and $\text{Cu}^{2+}\text{-GC}$, including the metal–ligand bond lengths and H-bond distances. Scheme 1 shows the labels of the atoms involved in the H-bond interactions. From these structures, it is already seen that ionization or interaction with a metal cation strengthen the $\text{N}_1 \cdots \text{N}_3$ and $\text{N}_2 \cdots \text{O}_2$ H-bond interactions and weaken that of $\text{O}_6 \cdots \text{N}_4$, the effects being more pronounced for the divalent cations than for the monovalent one. As a result, the energetic stabilization due to the base pair GC formation increases from 25.5 kcal mol^{−1} to 35.5, 42.1, 44.3 and 63.3 kcal mol^{−1} for $\text{Cu}^+\text{-GC}$, $[\text{GC}]^+$, $\text{Ca}^{2+}\text{-GC}$ and $\text{Cu}^{2+}\text{-GC}$, respectively [17]. The changes in H-bond interactions in these species will be treated in more detail in the next section.

As previously pointed out [17, 44], the interaction of the metal cation with guanine occurs through binding to both the N_7 and O_6 atoms, thus showing a bidentate coordination. This bidentate binding is generally stronger than the unidentate coordination [44, 45]. The $\delta(\text{M}, \text{O}_6)$ and $\delta(\text{M}, \text{N}_7)$ values of table 1 can be used to analyse the nature of the metal–ligand interaction for the different metal cations studied.

The metal–ligand distances and DIs in table 1 show that Ca^{2+} has a larger affinity for O_6^\dagger , whereas Cu^+ and Cu^{2+} show a stronger preference for N_7 . This is probably the result of the different cation hardnesses. As expected from the hard–soft acid–base

[†]The stronger preference of Ca^{2+} (and also of Na^+ , K^+ , Be^{2+} and Mg^{2+}) for the O_6 of guanine as compared to N_7 was already reported in [45] using the B3LYP/6-311G** method.

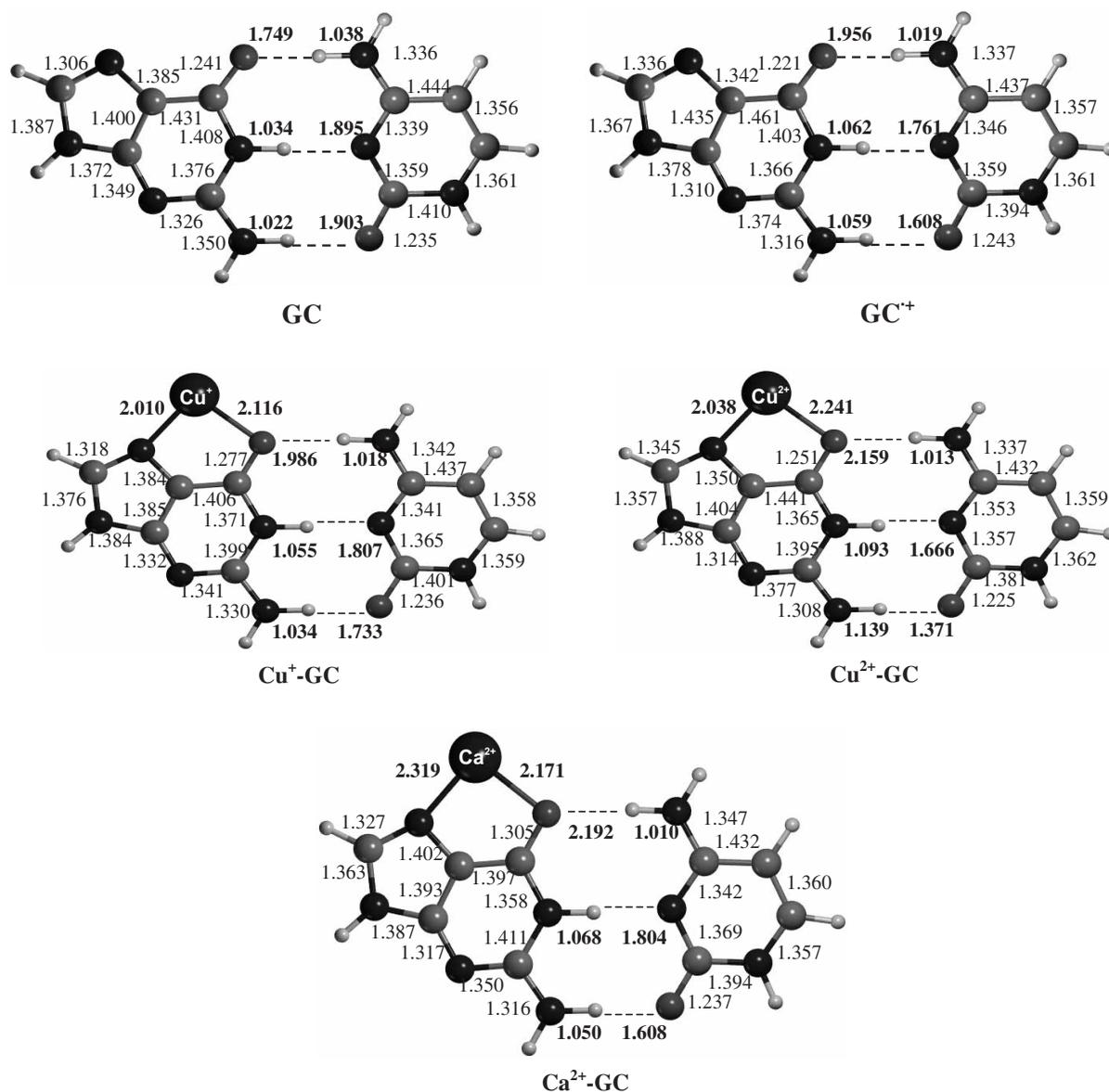


Figure 1. B3LYP/B1 optimized geometries for GC, $[GC]^+$, Cu^+-GC , $Cu^{2+}-GC$ and $Ca^{2+}-GC$. Distances are in Å.

Table 1. Metal–ligand delocalization indices (δ), distances (r), and AIM atomic charges of the atoms most involved in the metal–ligand interaction. δ and q in electrons and r in Å.

System	$\delta(M, N_7)$	$\delta(M, O_6)$	$r(M, N_7)$	$r(M, O_6)$	$q(N_7)$	$q(O_6)$	$q(M)$
$Ca^{2+}-GC$	0.290	0.381	2.319	2.171	-1.378	-1.253	1.716
Cu^+-GC	0.554	0.418	2.010	2.116	-1.293	-1.202	0.701
$Cu^{2+}-GC$	0.685	0.577	2.038	2.241	-1.265	-1.168	0.763

(HSAB) principle [46, 47], a hard Lewis acid such as Ca^{2+} interacts better with O_6 as a Lewis base than with N_7 , the former being harder than the latter. On the other hand, the interaction of softer Lewis acids

such as Cu^+ and Cu^{2+} with N_7 is, to a certain extent, driven by the larger availability of the lone pair to be shared in N_7 as compared to the lone pair of O_6 . The different kind of interaction is reflected by the DIs

values, which show more electron-shared interactions for Cu^+ and even more markedly for Cu^{2+} than for Ca^{2+} .

Table 1 also includes the AIM atomic charges of atoms O_6 , N_7 and the metal cation. The results show that the Ca^{2+} -GC species presents a smaller charge transfer between the metal cation and the base pair when compared to Cu^+ -GC and Cu^{2+} -GC systems. This fact together with the above-mentioned DI values allows us to attribute a large electrostatic character to the interaction between Ca^{2+} and the GC base pair, whereas the larger charge transfer together with the higher DI values show that the corresponding interaction in Cu^+ and Cu^{2+} has a noticeable covalent character. Finally, atomic charges and DIs reveal that the interaction of Cu^+ and Cu^{2+} with GC has a similar character, which is probably a consequence of the formation of a Cu^+ - GC^+ complex in the latter species.

3.2. Hydrogen bonding analysis

The H-bonds play a key role in the formation of base pairs such as GC. It must be mentioned that H-bond interactions in base pairs have already been analysed in a previous paper through first-order density descriptors obtained from the AIM theory, such as the density and the Laplacian of the density at the H-bond critical point [48]. Here, we aim to analyse the H-bonds in the GC base pair and related species using the DIs of equation (1) derived from the second-order density [29–31]. These H-bonds are commonly believed to be predominantly electrostatic phenomena [49]: proton-acceptor atoms have negative charges that favourably interact with the positively charged protons they face. Apart from the electrostatic interaction, there is also a donor-acceptor (covalent) interaction between the lone pair of the proton-acceptor atoms of one base pointing towards and donating charge into the unoccupied σ^* orbital of an O-H or N-H group of the other base [50]. These covalent interactions are normally considered to have a minor contribution to the final H-bond interaction. However, a recent experimental work [51] and several theoretical studies [8, 52, 53] that analyse a number of H-bonds using energy decomposition schemes [54] have shown that covalent interactions can represent a significant percentage (*ca.* 30 to 40%) of the total attractive interactions. A problem with the energy decomposition scheme for the analysis of the bonding mechanism in DNA base pairs, with two or three H-bonds occurring simultaneously, is that the electrostatic and covalent terms of the H-bond interactions cannot be separated into contributions for each individual H-bond. In a previous work [52], we have

shown that the intermolecular DI between proton and proton-acceptor atoms is strongly correlated to the orbital interaction energy term (the covalent contribution) of the H-bond as obtained from an energy decomposition scheme. Since DIs can be calculated separately for each H-bond, this measure can help us to classify the character of each one of the three H-bonds present in the GC base pair. In this work, the relative importance of the covalent term for each H-bond interaction will be accounted for by using the corresponding DIs, while the changes of the electrostatic character of each individual H-bond will be assessed in an approximate way through the AIM atomic charges of the proton and proton-acceptor atoms.

Table 2 contains the DIs for the three H-bonds involved in the GC base pair: $\text{N}_2 \cdots \text{O}_2$, $\text{N}_1 \cdots \text{N}_3$ and $\text{O}_6 \cdots \text{N}_4$, for all systems analysed. In particular, for each H-bond we have listed two DIs, referred to the two interactions of the hydrogen atom: one with the proton-donor (chemically bonded) atom and one with the proton-acceptor (non-bonded) atom. Thus, $\delta(\text{N}_2, \text{H})$, $\delta(\text{N}_1, \text{H})$ and $\delta(\text{N}_4, \text{H})$ correspond to the DI for the proton and the chemically bonded proton-donor atom; while $\delta(\text{H}, \text{O}_2)$, $\delta(\text{H}, \text{N}_3)$ and $\delta(\text{H}, \text{O}_6)$ correspond to the DI for the proton and the proton-acceptor atom. First focusing on the bonded DI values for GC, $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$ follow the same tendency with the introduction of a metal: $\text{GC} > \text{Cu}^+ > \text{Ca}^{2+} \geq [\text{GC}]^+ > \text{Cu}^{2+}$. As expected, the interaction of Cu^+ and Ca^{2+} with GC favours the N_1 - N_3 and N_2 - O_2 H-bond interactions due to both charge density polarization (the proton becomes more positively charged) and stabilization of the unoccupied σ^* orbital of the N-H groups, thus also favouring the covalent interactions. Occupation of the $\sigma_{\text{N-H}}^*$ orbital leads to larger N-H bond distances and smaller $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$ indices [55]. The reduction of $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$ indices due to ionization or interaction with metal cations is due to both a lengthening of the N-H bonds (as deduced from the GC^{wm} values[†]) and charge density polarization (compare the GC^{wm} values with the M^{+n} -GC results). As found previously [17], the effects are more pronounced for a divalent cation such as Ca^{2+} than for the monovalent one, Cu^+ . The effects on DIs due to the interaction of GC with Ca^{2+} are similar to those observed after ionization of GC to yield $[\text{GC}]^+$. Because the interaction of Cu^{2+} with GC leads to the formation of Cu^+ - $[\text{GC}]^+$ species [17], $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$

[†]The so-called GC^{wm} species is the GC base pair system at the M^{+n} -GC geometry but without the metal cation.

Table 2. Delocalization indices (δ) and AIM atomic charges of the atoms most involved in the three hydrogen bonds of the GC base pair. δ and q in electrons.

System	$N_2-H \cdots O_2$				$N_1-H \cdots N_3^a$				$N_4-H \cdots O_6$				
	$\delta(N_2, H)$	$\delta(H, O_2)$	$q(O_2)$	$q(H)$	$\delta(N_1, H)$	$\delta(H, N_3)$	$q(N_3/N_1)$	$q(H)$	$\delta(N_4, H)$	$\delta(H, O_6)$	$q(O_6)$	$q(H)$	
GC	0.633	0.090	-1.249	0.521	0.604	0.115	-1.264	0.512	0.565	0.119	-1.235	0.555	
[GC] ⁺	0.505	0.149	-1.243	0.583	0.529	0.150	-1.275	0.547	0.646	0.077	-1.149	0.517	
Ca ²⁺	GC	0.515	0.144	-1.237	0.579	0.526	0.146	-1.271	0.553	0.720	0.050	-1.253	0.468
	SPT1	0.646	0.082	-1.203	0.515	0.127	0.560	-1.314	0.541	0.625	0.087	-1.263	0.528
	GC ^{wm}	0.557	0.138	-1.242	0.549	0.585	0.130	-1.311	0.513	0.636	0.068	-1.144	0.533
	SPT1 ^{wm}	0.690	0.081	-1.210	0.476	0.149	0.508	-1.326	0.566	0.533	0.122	-1.161	0.580
Cu ⁺	GC	0.570	0.117	-1.245	0.554	0.548	0.138	-1.268	0.542	0.659	0.073	-1.202	0.508
	SPT1	0.663	0.077	-1.209	0.505	0.167	0.503	-1.312	0.556	0.528	0.138	-1.211	0.571
	GC ^{wm}	0.596	0.114	-1.246	0.535	0.583	0.129	-1.318	0.517	0.608	0.088	-1.188	0.543
	SPT1 ^{wm}	0.690	0.076	-1.217	0.480	0.182	0.475	-1.313	0.566	0.478	0.164	-1.200	0.592
Cu ²⁺	GC	0.400	0.223	-1.216	0.605	0.486	0.179	-1.275	0.559	0.693	0.050	-1.168	0.489
	SPT1	0.583	0.094	-1.212	0.558	0.118	0.578	-1.314	0.533	0.644	0.068	-1.183	0.522
	GC	0.469	0.206	-1.214	0.566	0.561	0.159	-1.342	0.511	0.626	0.072	-1.235	0.539
	SPT1 ^{wm}	0.677	0.090	-1.201	0.480	0.145	0.509	-1.363	0.569	0.562	0.102	-1.251	0.571

^aFor SPT1 systems it corresponds to $N_1 \cdots H-N_3$.

indices for the $\text{Cu}^{2+}\text{-GC}$ are smaller than those of $[\text{GC}]^+$ and $\text{Ca}^{2+}\text{-GC}$.

For the same reasons that ionization or the presence of the metal cation increase the strength of the $\text{N}_1\cdots\text{N}_3$ and $\text{N}_2\cdots\text{O}_2$ H-bond interactions, they reduce that of the $\text{O}_6\cdots\text{N}_4$ H-bond. Thus, not surprisingly, $\delta(\text{N}_4, \text{H})$ behaves almost in an opposite way to $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$: $\text{Ca}^{2+} > \text{Cu}^{2+} > \text{Cu}^+ > [\text{GC}]^+ > \text{GC}$. This opposite result can be attributed to the fact that in this case cytosine acts as a proton-donor. As compared to GC, changes in $\delta(\text{N}_4, \text{H})$ are larger than those in $\delta(\text{N}_2, \text{H})$ and $\delta(\text{N}_1, \text{H})$, as expected from the fact that this H-bond is the closest to the metal site, thus being more influenced by electronic polarization and reorganization.

As compared to the DIs of the bonded interaction mentioned above, the DIs of a non-bonded interaction between proton and the proton-acceptor atoms follow an opposite trend. In general, the stronger the bonded interaction, the weaker the non-bonded one. Moreover, for the $\text{N}_1\cdots\text{N}_3$ and $\text{N}_2\cdots\text{O}_2$ H-bonds, the ordering given by the $\delta(\text{H}, \text{O}_2)$ and $\delta(\text{H}, \text{N}_3)$ DIs perfectly agrees with that obtained from the guanine-cytosine interaction energies ($\text{Cu}^{2+}\text{-GC}$: $63.3 > [\text{GC}]^+$: $44.3 > \text{Ca}^{2+}\text{-GC}$: $42.1 > \text{Cu}^+\text{-GC}$: $35.5 > \text{CG}$: $25.5 \text{ kcal mol}^{-1}$) [17]. It is also seen that these DIs for non-bonded interactions reflect the strength and distances of the corresponding H-bonds. This is proven by means of representing the three distances $\text{O}_6\cdots\text{N}_4$, $\text{N}_1\cdots\text{N}_3$ and $\text{N}_2\cdots\text{O}_2$ (see figure 2), in front of the corresponding $\delta(\text{H}, \text{X})$ non-bonded DIs. A good correlation is achieved, showing that the larger the DI, the shorter the H-bond distance. It is seen how ionization or the metal cation interaction strengthens those H-bonds of GC in which guanine

acts as proton-donor and weakens the one in which it acts as proton-acceptor. Thus, for all $\text{M}^{+n}\text{-GC}$ systems, compared to GC, $\delta(\text{H}, \text{O}_6)$ decreases, whereas the other two, $\delta(\text{H}, \text{O}_2)$ and $\delta(\text{H}, \text{N}_3)$, increase. The same trend is followed by the $[\text{GC}]^+$ system. As before, changes due to divalent metal cations (Ca^{2+} and specially Cu^{2+}) are larger than those found in the monovalent Cu^+ . The reduction of $\delta(\text{H}, \text{O}_6)$ and the increase of $\delta(\text{H}, \text{O}_2)$ and $\delta(\text{H}, \text{N}_3)$ indices due to ionization or interaction with metal cations is due to both a lengthening of the $\text{O}_6\text{-H}$ and a shortening of the $\text{O}_2\text{-H}$ and $\text{N}_3\text{-H}$ bonds (see figure 1 and compare the GC and GC^{wm} values) and charge density polarization (as can be inferred from comparison of the GC^{wm} values with the $\text{M}^{+n}\text{-GC}$ results).

The DIs corresponding to the non-bonded interactions for the GC species (table 2) points out that, taking GC as the reference, the largest increase in the $\delta(\text{H}, \text{O}_2)$ and $\delta(\text{H}, \text{N}_3)$ occurs for the oxidizing Cu^{2+} metal cation. In particular, $\delta(\text{H}, \text{O}_2)$ and $\delta(\text{H}, \text{N}_3)$ change from 0.090 to 0.223 and from 0.115 to 0.179, respectively, when going from GC to $\text{Cu}^{2+}\text{-GC}$. Thus, the interaction with proton and proton-acceptor atoms is strongly strengthened. Interestingly, the change in $\delta(\text{H}, \text{O}_2)$ from GC to $\text{Cu}^{2+}\text{-GC}$ is more pronounced than that of the corresponding $q(\text{O}_2)$ and $q(\text{H})$ atomic charges (table 2), indicating that the increase of the H-bond strength due to Cu^{2+} is mainly because of better covalent interactions rather than due to electrostatic effects. The same conclusions can be drawn from the $\delta(\text{H}, \text{N}_3)$ values in GC.

We note in passing that polarization by Cu^+ and Cu^{2+} reduces the absolute charge on O_6 , while Ca^{2+} has the

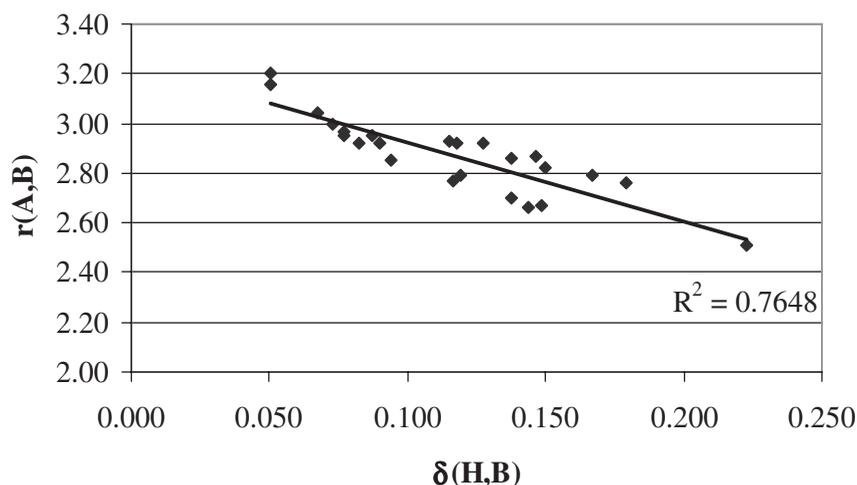


Figure 2. Representation of hydrogen bond distances ($r(\text{A}, \text{B})$) versus delocalization indices between the proton and the proton-acceptor atoms ($\delta(\text{H}, \text{B})$). Distances in Å and δ in electrons.

unexpected opposite effect as can be seen in table 2[†]. The special behaviour of Ca²⁺ can be attributed to its larger hardness which inhibits the charge transfer and favours electrostatic interactions. A decomposition of charge in its σ and π components for the Ca²⁺–GC system shows that the σ donation of charge from GC to Ca²⁺ is overcompensated by polarization of the π electronic cloud. Because of that the weakening of the O₆···N₄ H-bond must be attributed to a greater extent to a reduction of covalent interactions (as pointed out by the decrease in the $\delta(\text{H}, \text{O}_6)$ value) rather than to a decrease of electrostatic interactions of the H-bond.

It is also enlightening to analyse the DIs for the H-bonds of the SPT1 species. The SPT1 species corresponds to the most favourable single-proton-transfer process between N₁ and N₃ atoms (see scheme 1) [17]. The metal cation stabilizes the M⁺ⁿ···G–H···C H-bonds in GC and SPT1 species by, first, reinforcing the positive charge of the proton bonded to the guanine base and therefore making stronger the electrostatic part of the H-bond interaction, and second, lowering the energy of the $\sigma_{\text{N-H}}^*$ orbital thus also favouring its covalent component. For analogous reasons, the M⁺ⁿ···G···H–C H-bonds are disfavoured. It is interesting to analyse the change in strength of the N₂···O₂ and O₆···N₄ H-bonds when going from M⁺ⁿ–GC to M⁺ⁿ–SPT1 for the different metal cations considered. It must be noted that the N₂···O₂ H-bond length increases from M⁺ⁿ–GC to M⁺ⁿ–SPT1 [17]. This is translated into a decrease of the $\delta(\text{H}, \text{O}_2)$ values of M⁺ⁿ–SPT1 when compared to the corresponding M⁺ⁿ–GC values. This effect is mainly due to geometry relaxation rather than charge polarization as it can be realized from the comparison between the M⁺ⁿ–SPT1 and M⁺ⁿ–SPT1^{wm} values. This apparent reduction in the covalent component of the N₂···O₂ H-bond interaction due to proton transfer is followed by a similar decrease in its electrostatic part due to the reduction in absolute value of the charges on O₂ and the proton involved in this H-bond. On the other hand, the N₄···O₆ H-bond is reinforced during the proton transfer that transforms M⁺ⁿ–GC to M⁺ⁿ–SPT1. In this case, both the covalent and the electrostatic components are strengthened, although apparently the change in the covalent term is more important than that of the electrostatic one. In general, it is found that to explain the effect of ionization or metal interaction, changes in the covalent component of the H-bonds are more important than variations in the electrostatic part of the interaction.

[†]It is worth noting that according to the natural population analysis (NPA) of Weinhold and co-workers [56], the results are somewhat different and both Ca²⁺–GC and Cu⁺–GC increase their negative charge [17].

Just to conclude this electron-pair analysis, it must be mentioned that all previous DIs have been split into their σ and π components. This decomposition has been possible because of the C_s symmetry of the GC base pair [30]. Nevertheless, no noticeable additional information is achieved from it, as the π contribution is never found to be larger than 0.006 *e* for the non-bonded interaction between the proton and the proton–acceptor atoms. Accordingly the corresponding values have not been included in table 2.

3.3. Aromaticity analysis

In this last section we discuss the local aromaticity of the 5- and 6-MRs present in the Watson–Crick pair GC and how it is modified by the interaction of GC with the different metals or by ionization. To this end, NICS, HOMA and PDI values have been calculated for the 6-MRs of guanine and cytosine. The aromaticity of the 5-MR of guanine has been assessed by means of NICS and HOMA indicators of aromaticity, since by definition the PDI index cannot be applied to 5-MRs.

In a recent work Cyrański *et al.* [20] have calculated the NICS and HOMA values for a series of nucleobases constituting DNA and RNA, also including base pairs. Even though we have measured HOMA and NICS for the GC system at another level of theory, results from both studies are very close, following exactly the same tendency. It is worth noting that the work by Cyrański *et al.* [20] considered neither the effect of ionization nor the interaction of metal cations with the GC base pair.

Aromaticity results for the different systems studied can be found in table 3. First, focusing on the 6-MR of guanine in the GC system, it is seen how all three aromaticity criteria attribute to it an intermediate aromatic character[‡]. In addition, it must also be noticed that all three measures coincide in the tendencies followed when GC is interacting with a metal. Thus, according to all three criteria of aromaticity, the introduction of Ca²⁺ and Cu⁺ slightly increase the aromaticity of the guanine's 6-MR, whereas the Cu²⁺ cation causes a noticeable decrease. The increase of aromaticity of the 6-MR of guanine in Ca²⁺–GC and Cu⁺–GC is consistent with the enhancement of the H-bond interactions, which stabilizes the charge separation resonance structure **2** in scheme 2, required for increasing the aromaticity of the guanine 6-MR [21]. This effect is stronger for the divalent Ca²⁺ metal cation than for the monovalent Cu⁺. The H-bond formation in

[‡]As a reference, for benzene the HF/6-31+G**/B3LYP/6-31G* NICS, B3LYP/6-31G* HOMA, and HF/6-31G**/B3LYP/6-31G* PDI are –9.7 ppm, 0.981 and 0.101 *e*, respectively [40].

Table 3. NICS, PDI and HOMA aromaticity measures of the five- and six-membered rings of guanine (G) and the six-membered ring of cytosine (C). NICS in ppm and PDI in electrons.

System	NICS			PDI		HOMA			
	G-5	G-6	C-6	G-6	C-6	G-5	G-6	C-6	
GC	-11.94	-4.10	-1.86	0.036	0.040	0.848	0.795	0.703	
[GC] ⁺	-5.41	-0.31	-2.49	0.023	0.042	0.829	0.550	0.773	
Ca ²⁺	GC	-10.67	-4.76	-2.53	0.044	0.045	0.843	0.886	0.797
	GC ^{wm}	-11.66	-3.50	-2.06	0.035	0.041	0.843	0.886	0.797
Cu ⁺	GC	-10.64	-4.59	-2.25	0.040	0.043	0.869	0.898	0.761
	GC ^{wm}	-11.98	-3.56	-1.98	0.035	0.041	0.869	0.898	0.761
Cu ²⁺	GC	-7.37	-2.00	-3.07	0.022	0.040	0.915	0.760	0.822
	GC ^{wm}	-12.12	-3.23	-2.30	0.033	0.041	0.915	0.760	0.822

GC implies a certain loss of π charge on N₁ and a gain on O₆, respectively, thus increasing the relevance of the resonance structure **2** which favours the intensification of the aromatic character of the guanine 6-MR. In addition, this increase in aromaticity must be attributed to the polarization of the charge density by the metal and not due to changes in geometry as can be deduced from the fact that the NICS and PDI values indicate a lower aromaticity for GC^{wm} as compared to M⁺ⁿ-GC. Indeed, there is a decrease of local aromaticity of the guanine's 6-MR when going from GC to GC^{wm} and then a clear increase from GC^{wm} to M⁺ⁿ-GC (M⁺ⁿ = Ca²⁺, Cu⁺). It is worth noting that this latter effect is not indicated by HOMA because geometries of GC^{wm} and M⁺ⁿ-GC are exactly the same and therefore a geometry-based index like HOMA gives the same value for the local aromaticities of GC^{wm} and M⁺ⁿ-GC. In contrast, the strong decrease of aromaticity caused by Cu²⁺ is consistent with the fact that the interaction of Cu²⁺ with GC causes the removal of an electron from guanine [17], thus disrupting its π structure. This hypothesis is supported by (a) the values for Cu²⁺-GC^{wm}, which are very close to those found for the GC species and (b) the fact that for the radical cation [GC]⁺ species a similar (somewhat larger) decrease of aromaticity is found.

On the other hand, for the 6-MR of cytosine in the GC system, NICS and HOMA attribute to it a lower aromatic character than that of guanine, whereas PDI considers it slightly more aromatic. To find indicators of aromaticity that afford different orderings for relatively similar 6-MRs such as those of cytosine and guanine is not completely unexpected [57]. In this case, it is not possible to reach a definite conclusion on the relative local aromatic character of the 6-MRs in guanine and cytosine [57]. When adding a metal, NICS, PDI and HOMA show the same trend, with all three metal cations and ionization causing an increase of aromaticity with respect to the GC system. This result is another indication that the radical character is concentrated on

the guanine nucleobase and that the cytosine fragment is not much affected by the radical formation.

For the 5-MR of guanine, both NICS and HOMA consider it more aromatic than the 6-MRs of guanine and cytosine. It is worth mentioning that the π -system of this 5-MR has six π electrons, thus matching the Hückel's $4n + 2$ rule. Absolute NICS values for this 5-MR are especially high, being about more than two times those for the 6-MRs. These large negative values of NICS for the 5-MR are in line with the general observation for this index that the magnitude of NICS is usually larger in absolute value for the smallest rings [39, 58]. In fact, the differences for aromaticity between 5- and 6-MRs are not so substantial if one considers the HOMA results. According to the NICS values, the introduction of a metal, as well as the radical formation, cause a decrease of aromaticity when compared to the GC system, especially noticeable for Cu²⁺ and GC⁺ systems for the reasons stated above. As before, this reduction of aromaticity is not caused by relaxation of geometry but because of charge polarization, as can be seen by comparing the GC, M⁺ⁿ-GC and GC^{wm} results. Because relaxation of geometry is not a key factor in the change of aromaticity detected by NICS, the variation of HOMA values is, in contrast, much lower, and even an increase of aromaticity is observed for Cu⁺ and Cu²⁺.

Finally, it must be noticed that the behaviour of the SPT1 system parallels that of the corresponding GC species (values not included), with the only exception of PDI values for the cytosine 6-MR which are almost unaffected by metal interaction or ionization.

4. Conclusions

This work discusses the influence of ionization and metal cations coordinated to the N₇ of guanine on the hydrogen bonding and aromaticity of the guanine-

cytosine base pair. Using delocalization indices derived from the AIM theory, we have demonstrated that the increase of strength in the $N_1 \cdots N_3$ and $N_2 \cdots O_2$ hydrogen bonds and the weakening of the $O_6 \cdots N_4$ hydrogen bond due to ionization or interaction with metal cations is mainly due to a change in the donor–acceptor (covalent) interactions and not to a significant variation of electrostatic interactions. The interaction with Cu^+ and Ca^{2+} metal cations stabilizes the guanine–cytosine base pair by increasing the hydrogen bond interactions, which, in turn, favours the charge separation resonance structure that increases the aromaticity of the six-membered ring of guanine. On the other hand, the reduction of aromaticity of the five- and six-membered ring of guanine observed in Cu^{2+} –GC is attributed to the disruption of the π electronic structure produced by the oxidation process taking place in guanine.

In summary, we have shown that interaction of a base pair with metal cations changes the strength of the H-bonds involved in the base pair formation. In turn, these alterations may have a significant effect on the aromaticity of the five- and six-membered rings of implicated nucleobases which is expected to have appreciable effects on the π -stacking interactions that stabilize the double helix. More research is underway in our laboratory concerning this particular issue.

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