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DFT study of the interaction of cytidine and 2′-deoxycytidine with Li^+, Na^+, and K^+: effects of metal cationization on sugar puckering and stability of the *N*-glycosidic bond

Zahra Aliakbar Tehrani, Alireza Fattahi *, Ali Pourjavadi *

Department of Chemistry, Sharif University of Technology, PO Box 11365-9516, Tehran, Iran

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ABSTRACT

Density functional theory (DFT) calculations were performed at the B3LYP level with a 6-311++G(d,p) basis set to systematically explore the geometrical multiplicity and binding strength for complexes formed by Li^+, Na^+, and K^+ with cytidine and 2′-deoxycytidine. All computational studies indicate that the metal ion affinity (MIA) decreases from Li^+ to Na^+ and K^+ for cytosine nucleosides. For example, for cytidine the affinity for the above metal ions are 79.5, 55.2, and 41.8 and for 2′-deoxycytidine, 82.8, 57.4, and 42.2 kcal/mol, respectively. It is also interesting to mention that linear correlations between calculated MIA values and the atomic numbers (Z) of the above metal ions were found. The influence of metal cationization on the coordination modes and the strength of the *N*-glycosidic bond in cytosine nucleosides have been studied. In all cases, the N1–C1′ bond distance changes upon introducing a positive charge in the nucleosides. It has been found that metal binding significantly changes the values of the phase angle of pseudorotation P in the sugar unit of these nucleosides. With respect to the sugar ring, metal binding changes the values of the glycosyl torsion angle and sugar ring conformation. The present calculations in the gas phase provide the first clues on the intrinsic chemistry of these systems and may be of value for studies of the influence of metal cations on the conformational behavior and function of nucleic acids.

1. Introduction

2′-Deoxynucleosides are building blocks of DNA and consist of a nucleobase and a 2-deoxyribose residue linked by an *N*-glycosidic bond. Cleavage of this bond is a process of great importance for the stability of DNA because it implies the release of a nucleobase, which may lead to the loss of genetic information and, thus, cause mutations in the DNA sequence. Moreover, the cleavage of this bond is involved in the so-called base excision repair (BER) pathways. The enzymes responsible of BER pathways are DNA glycosylases, which recognize damaged bases and excise them from DNA by hydrolyzing the *N*-glycosidic bond between the nucleobase and the sugar moiety,[1] leading to apurinic or apyrimidinic sites (AP sites). Depurination and depyrimidation can also occur spontaneously or be induced by chemical damage to DNA.[2]

Due to the biological relevance of the *N*-glycosidic bond, many reports have dealt with the enzymatic processes carried out by glycosylases,[3–8] placing special emphasis on the mechanistic aspects by using kinetic isotopic effects, and on the role of catalytic residues. In addition, in the last 30 years many experimental non-enzymatic studies have analyzed the intrinsic chemical properties of nucleosides glycosidic bond hydrolysis and evaluated the influence of environmental and chemical agents such as pH, metal cations, and alkylating compounds.[9–16] On another hand, sugar ring puckering has also been put forward as influencing the hydrolysis of the *N*-glycosidic bond.[17] The first principles of calculation can help in understanding the fundamental properties of the *N*-glycosidic bond as well as the changes induced by metal cation binding. However, theoretical papers on this subject are scarce,[18–21] even though there seems to be an increasing interest in this area.

It is well known that metal ions play an important role in various biochemical processes.[22–24] Interaction of metal cations with nucleic acids is of topical interest in the bio-inorganic field because synthesis, replication, and cleavage of DNA and RNA as well as their structural integrity are affected by the presence of these ionized metals in the cell nucleus. The conformational behavior and function of DNA are often influenced by the presence of metal ions.[25–29] For example, by means of PCR (polymerase chain reaction), DNA replication was promoted by the presence of Mg^{2+}, but not by Mn^{2+}.[30–33] Metal cations can interact with many sites in DNA and RNA[25–27,34,35] because ribonucleic and deoxyribonucleic acids contain a large number of oxygen and nitrogen donors

* Corresponding authors.
E-mail addresses: fattahi@sharif.edu (A. Fattahi), purjavad@sharif.edu (A. Pourjavadi).

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with different properties. Additionally, alkali metal ions have an inhibitory effect on the chain initiation process by RNA polymerases which may in turn alter the extent and fidelity of RNA synthesis.[36]

Metal–nucleoside interactions play a crucial role in the resulting structure and function of nucleic acids. Therefore, knowledge of their intrinsic properties, particularly the influence of metal ions on their structure and conformational stability, is of importance for understanding both the structural organization of DNA and RNA and the biological activity.

Theoretical calculations on model systems can provide fundamental informations on the intrinsic properties of these systems, which may be useful to understand more complex situations. Furthermore, because experimental studies on this subject are often performed in the framework of mass spectrometry (i.e., a kinetic method), the theoretical determination of metal affinities in the gas phase can find confirmation immediately and in turn be used as a guideline to interpret the measured values.[35,37–39]

In the present density functional theory study, we have considered the gas phase interactions between 2′-deoxycytidine, cytidine (Chart 1) and Li^+, Na^+, and K^+ as typical monovalent cations for investigation of metal complexation. The gas phase is an ideal environment in which complexation mechanisms, binding energies, enthalpies, and reactivity of the metal ions can be obtained in the absence of any complicating solvent effect. Vacuum approximation (isolated molecule) used in this work seems to be reasonable, because active centers of most enzymes are rather hydrophobic and characterized by low dielectric constants.[40] An earlier suggestion was made that, when nucleosides enter the active site of enzymes, water is displaced.[41,42] For example, in a replicative polymerase active site, DNA is in a dehydrated A form.[43]

We have probed the effect of the metal cation on the coordination modes of cytidine and 2′-deoxycytidine and clarified how the strength of the *N*-glycosidic bond in these molecules is influenced by the effect of cationic species. Furthermore, conformation changes due to metalation were considered.

Regarding the coordinating properties of these molecules, at least four questions arise (Chart 1): (i) Does N-3 show the properties of a cytosine-like binding site? (ii) Does the neighboring amino group (N-4) at C-4 affect the stability of M (ligand)$^+$ complexes? (iii) What is the role of the carbonyl oxygen atom at C-2 in cytidine complexes? (iv) What is the role of the sugar unit on the coordination modes of cytidine and 2′-deoxycytidine with alkali metal cations?

As expected, this study has provided useful informations since these changes may not only influence the hydrolysis mechanism of the *N*-glycosidic bond but can also affect other properties such as the N1–C1′ shielding tensors, as recently shown.[44]

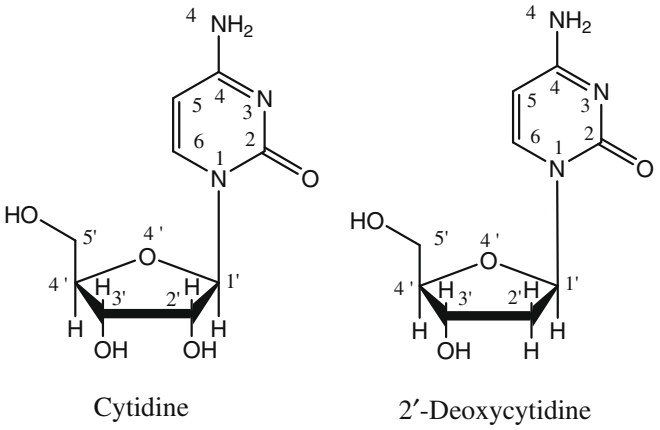

Chart 1. Structures of cytidine and 2′-deoxycytidine.

2. Computational method

Calculations were performed for cytidine and 2′-deoxycytidine complexes corresponding to the positions known as the active sites for the interaction of metal ions. These initial structures could be divided into two groups. One is the cation heteroatom complex in which M^+, lying in the same plane as the ring structure of cytosine, interacts directly by flanking the heteroatom. The other one is the cation complex whereby M^+ is located near the sugar ring of cytidine and 2′-deoxycytidine is supposed to interact with all ring atoms of the sugar ring.

Initial search of minima on the potential energy surface for cytidine and 2′-deoxycytidine metal complexes at the relative energy range of 10 kcal were carried out using the MMFF in the SPARTAN software.[45] The most stable conformers were optimized by the density functional theory (DFT) method using Becke3 (B3) exchange[46] and Lee, Yang, and Parr (LYP) correlation[47] potentials, in connection with the 6-311++G** orbital basis set as implemented in the SPARTAN software. This basis set was selected for all calculations as it contains both polarized basis set and diffuse functions. The choice of basis set represents a good compromise between computational costs and reliability of the results as previously demonstrated by Zhu et al.[48] Results from these authors demonstrated that the basis set 6-311++G** is large enough to generally reduce the basis set superposition error (BSSE) to ~1 kcal/mol. Energy minimizations followed by harmonic vibrational calculations were performed at this level of theory. The absence of imaginary frequencies proved that energy-minimized structures correspond well to the local minima of the energy landscape (local minima were verified by establishing that the matrix of energy second derivatives has only positive eigenvalues). No degree of freedom (bond lengths, valence angles, or torsion angles) have been frozen in the course of the geometry optimization of given systems. Zero point vibrational energies were computed in order to correct all the calculations to 298.15 K. Metal ion affinity (MIA) was assumed to be negative for the enthalpy variation (ΔH), namely the dissociation energy of the B—M^+ bond for the process:

$$L + M^+ \rightarrow [L{-}M]^+ \quad -\Delta H^{\circ}_{rxn} = MIA$$

where L represents cytidine or 2′-deoxycytidne and M^+ is the metal ion. Using the standard thermodynamic scheme, we can write:

$$\Delta H^{298} = \Delta U^{298} + \Delta(pv) = \Delta U^{298} + \Delta n_g RT = \Delta U^{298} - RT$$

$$MIA(L) = -\Delta H^{298} = -\Delta U + RT = -U(L{-}M)^+ + U(L) + U(M^+) + RT$$

The five-membered furanose ring is generally nonplanar. It can be puckered in an envelope form with four atoms in a plane and the fifth atom out of the plane. The geometry of the sugar unit is very flexible and depends on the furanose ring conformation. With respect to the deoxyribose or ribose ring, the geometrical parameters that undergo some changes are the ν_0, ν_1, ν_2, ν_3, and ν_4 angles, which correspond to endocyclic torsion angles about O4′–C1′(ν_0),

Table 1
Notation for torsion angles in nucleosides

Torsion angle	Notation
O4′–C1′–N1–C2 (pyrimidine)	χ
C4′–O4′–C1′–C2′	ν_0
O4′–C1′–C2′–C3′	ν_1
C1′–C2′–C3′–C4′	ν_2
C2′–C3′–C4′–O4′	ν_3
O3′–C4′–O4′–C1′	ν_4

C1′–C2′(ν_1), C2′–C3′(ν_2), etc. in a clockwise manner[34] (see Table 1 for more details). Many conformers are possible depending on the values of these angles.

Depending on the relationship between these angles, a great number of conformations are possible for a given five-membered ring. The concept of pseudorotation *P* as described in Ref. 49 has been used to establish the conformation in our systems. This value was calculated from the following equation (note that when ν_2 is negative one should add 180° to the calculated value of *P*):

$$\tan(P) = \frac{(\nu_4 + \nu_1) - (\nu_3 + \nu_0)}{2\nu_2(\sin(36) + \sin(72))}$$

Atoms displaced from four-atom planes and on the same side as C5′ are called endo. Those on the opposite side are called exo. For example, according to this equation, values of *P* between 0° and 36° correspond to a C3′-endo conformation, while those between 114° and 180° correspond to a C2′-endo conformation (Chart 2).

All conformers of cytidine and 2′-deoxycytidine complexes are denoted in accordance with orientation of the base and the conformation of the furanose ring. For example, an *anti*/C2′-endo conformer is the one where the base has an *anti*-orientation with respect to the furanose ring and the conformation of the sugar belongs to the C2′-endo region.

3. Results and discussion

3.1. Structural and conformational analysis of free molecules

The first step was to identify all the minima on the conformational potential energy surface for cytidine and 2′-deoxycytidine starting form several low-lying conformations with the relative energy in the range of 10 kcal by using the SPARTAN program.[45] Conformational search for these molecules was performed with the HF method using the 6-31G orbital basis set. In the range of 10 kcal, 11 conformers for each nucleoside were then optimized by using B3LYP/6-311++G**. The optimized structures of the most stable conformer of cytidine and 2′-deoxycytidine are given in Figure 1 to better clarify their structures. The optimized structures of all 11 conformers of each nucleoside are also given in Figures 3S and 4S (see Supplementary data). These structures consist of three (1.771, 2.050, and 2.400 Å for cytidine) and two (1.834 and 2.782 Å for 2′-deoxycytidine) intramolecular hydrogen bonds.

The most stable conformers of cytidine and 2′-deoxycytidine are associated with the *anti*-orientation and *syn*-orientation of the base and the C2′-endo conformation of the furanose ring, respectively (see Table 2 for more details). In the most stable conformer of 2′-deoxycytidine, the *syn*-orientation of the base unit with respect to the sugar unit is strongly stabilized by the formation of an intramolecular hydrogen bond involving the C-5′ OH group.

Geometrical changes in these molecules due to metal cationization are different for each component (i.e., cytosine and sugar unit). In the former, variations basically occur on bond distances, whereas in the latter the endocyclic torsion angles are the main modified parameters. We will then discuss separately both kinds of geometrical changes. Variations with respect to neutral molecules (cytidine and 2′-deoxycytidine) upon metal cationization

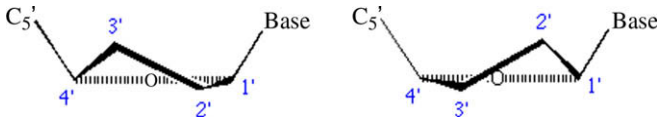

Chart 2. C3′-endo (left) and C2′-endo (right) conformations in a five-membered ring.

are attributed mainly to electrostatic effects due to the presence of a positive charge, which produce changes in the σ and in the more polarizable π density. Furthermore, the geometry of different conformers of cytidine and 2′-deoxyxytidine complexes have been analyzed by considering, on one hand, the sugar conformation and, on the other hand, the *anti*- and *syn*-orientation of the base with respect to the sugar.

3.2. Geometry of the cytosine moiety

A comparison of the values of bond lengths within the base unit (cytosine moiety) of the nucleoside complexes and the free nucleosides, calculated at the same level of theory, demonstrates significant differences during complexation.

In metal complexes of cytidine and 2′-deoxycytidine, the C2–O2 bond becomes longer in comparison with the corresponding parameter free cytosine nucleosides ($\Delta d_{\text{elongation}}$ = 0.012–0.015 Å and $\Delta d_{\text{elongation}}$ = 0.018–0.027 Å for cytidine and 2′-deoxycytidine, respectively) since the presence of the M^+ atom polarizes the carbonyl bond in such a manner that O2 transfers a certain amount of electron density; consequently, a loss of double bond character is produced. This behavior can be interpreted by means of resonance structures, as shown in Chart 3. As a result of this lengthening, the contiguous bonds (C5–C6 and C6–N1) become shorter and C2–N1 becomes longer. It is also noticeable that M^+ binding induces a shortening of the C2–N2 bond length because the electron-donating N2 atom tends to compensate the electron deficiency in the rings.

3.3. Sugar bond length and valence angles

The values of bond lengths and angles within the furanose ring strongly depend on the ring conformation. The results demonstrate that the geometry of the sugar unit is very flexible and that it depends on the furanose ring conformation. The values of the C–C bond lengths are not equal within the furanose ring of complexed and free molecules. They essentially depend on the conformation of the sugar unit. In general, the values of the C–C bonds oscillate around the mean magnitude for tetrahydrofuran obtained experimentally based on X-ray diffraction.[50–52] It should be noted that the calculated C–C bond lengths are longer as compared with the crystal data. Shorter values of the C–C bonds in the crystal phase, probably, are caused by the thermal motion of the atoms. It is known[53] that such motions lead to a shortening of the bond lengths obtained from the X-ray diffraction data. The results of the calculations reveal a strong correlation between the pseudorotational phase and the bond angles within the furanose ring. In general, values of the endocyclic bond angles in the nucleosides retain the same order as in tetrahydrofuran, that is, C–O–C > C–C–O > C–C–C.

In the cytidine complexes with Li^+ and Na^+, it can be seen from Table 2 that the values of *P* become higher as the positive charge introduced in the system increases. In the case of the cytidine–K^+ complex, this value becomes smaller in comparison with free cytidine (see Table 2 for more details).

For 2′-deoxycytidine–M^+ complexes, in all cases the five-membered ring of the sugar remains in the C2′-endo conformation, since the corresponding *P* values lies within the $140° \leqslant P \leqslant 180°$ range. The main variations in endocyclic torsion angles are those corresponding to C3′–C4′–O4′–C1′ (ν_4, from −1.0° to 8.8°) and C2′–C3′–C4′–O4′ (ν_3, from 22.6° to 17.4°), which increase and decrease, respectively, as the positive charge is introduced in 2′-deoxycytidine. Furthermore, on the basis of DFT calculations, in all cytidine complexes *anti* χ angles (which characterizes the orientation of the base with respect to the sugar unit) are located in the 170 ± 5° range, whereas at *syn* arrangement of the base unit in

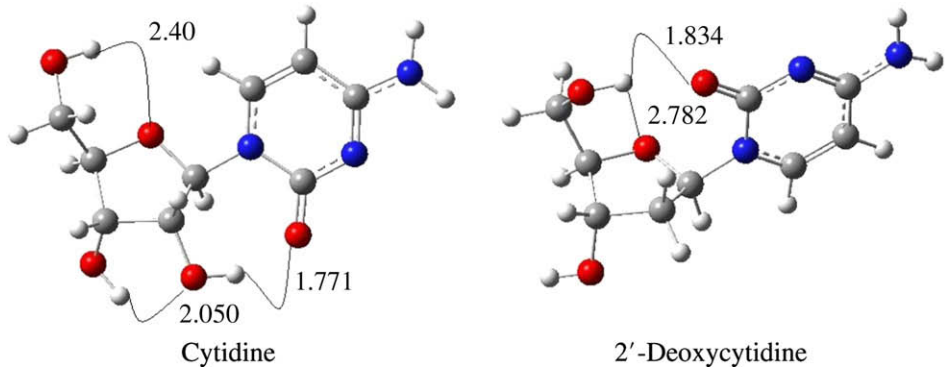

Figure 1. Optimized structures of the most stable conformers of cytidine and 2′-deoxycytidine calculated at B3LYP/6-311++G**. Distances are in Å.

cytidine complexes (i.e., cytidine + Li^+ complex and 2′-deoxycytidine complexes with Li^+, Na^+, and K^+ ions), the value of χ for 2′-deoxycytidine–K^+ is larger than in other complexes.

The results demonstrate that the length of the glycosyl bond depends on the orientation of the base unit and sugar unit and the conformation of the furanose ring. For example, the transition of the furanose ring from the C2′-endo in free cytidine to the C3′-exo conformer in its complex with Na^+ results in elongation of the *N*-glycosidic bond.

In the case of the cytidine complex with Na^+ (N1–C1′ = 1.500 Å) and 2′-deoxycytidine–K^+complex (N1–C1′ = 1.476 Å), the N1–C1′ bond distance increases upon cationization, the observed variations correlating with the positive amount of charge M^+ introduced in the system. Thus, these results seem to indicate that the presence of a positive charge in these systems generated through metal cationization weakens the *N*-glycosidic bond. In cytidine–Li^+, cytidine–K^+, 2′-deoxycytidine–Li^+, and 2′-deoxycytidine–Na^+ complexes, the *N*-glycosidic bond decreases upon cationization.

In general, the analysis of the nucleosides geometry reveals strong correlation between the puckering degree of the furanose ring in nucleoside complexes and the glycosyl bond length. The longest C1′–N1 bond corresponds to the most flattened furanose ring.

3.4. Comparison of the three metal ions Li^+, Na^+, and K^+ in complexation with cytosine nucleosides

Based on our results, electrostatic and polarization interactions between cations sand neutral molecules determine the possible attachment sites and the geometry of adducts. As seen in Figure 1, in principle, Li^+, Na^+ and K^+ cations can coordinate with cytosine nucleosides in different positions: (i) on amine nitrogen and the carbonyl oxygen of the cytosine moiety; (ii) on oxygen atoms of the sugar ring (hydroxyl groups and O4′ hetero-atom); (iii) above the nucleobase moiety (via a cation-π complex);and (iv) upon combination of 1, 2, and 3 situations as a tri-coordinated or bi-coordinated ligand. To form a tri or bi-coordinated complex, the intramolecular hydrogen bonding in free cytosine nucleosides should be broken and rearrangement of functional groups is needed. However, there is no direct relationship between the global minima of the nucleoside free molecule and those of nucleoside–M^+ complexes.

To probe all possible binding sites for complexation, different initial structures were designed for geometrical optimization. Starting from the MM level provides many conformers. In the range of 10 kcal, some conformers of each metal-complexed nucleoside were then optimized by using B3LYP/6-311++G**. The optimized structures of the most stable conformer of the metal-complexed species of cytidine and 2′-deoxycytidine are given in Figure 2. The optimized structures of all metal-complexed conformers for each nucleoside are also given in Figures 1S and 2S (see Supplementary data). In Figure 2, coordination modes of lithium, sodium, and potassium ions with both cytosine nucleosides are shown. The most significant geometrical parameters of the most stable M^+–L (M^+: Li^+, Na^+, K^+ and L: cytidine and 2′-deoxycytidine molecules) complexes obtained by B3LYP/6-311++G (d,p) computations are also reported.

For the most stable complex of cytidine with Li^+ (Fig. 2), this cation appears to be tri-coordinated. In this structure, the cation interacts with the carbonyl oxygen and O5′ and O4′ atoms of the sugar ring in a C2′-endo conformation. In fact, to favor this coordination mode, the cytosine base unit should turn around the glycosyl linkage to make formation of this complexation mode possible. In this structure, geometry optimization leads to formation of an O3′H···O2′ hydrogen bond with the length 2.316 Å between two hydroxyl groups on the two adjacent C2′ and C3′ carbon atoms.

Unlike tri-coordinated η^3(O,O,O) complexes of cytidine with lithium, sodium, and potassium, complexes with cytidine show a similar behavior. These structures including η^2(O,O) coordination modes are stabilized by the attractive electrostatic interactions between the cations and the oxygen atoms on the carbonyl oxygen and the 2′-hydroxyl oxygen (see Fig. 2 for more details). The geometrical parameters reported in Figure 2 underline that the sodium cation establishes shorter bonds with cytidine oxygen

Table 2
Endocyclic torsion angles ν_0–ν_4, phase angle of pseudorotation *P*, and glycosyl torsion angle χ (in deg) for different systems calculated at B3LYP/6-311++G**

System	ν_0	ν_1	ν_2	ν_3	ν_4	*P*	χ
Cytidine	−27.5	−27.5	−30.1	15.2	7.7	149.1	−171.6
Cytidine + Li^+	−28.8	40.9	−37.2	21.4	4.4	155.2	47.8
Cytidine + Na^+	6.9	10.2	−22.1	26.4	−21.4	213.4	−175.4
Cytidine + K^+	−26.1	1.3	21.5	−37.5	40.3	57.7	−170.2
2′-Deoxycytidine	−21.3	34.4	−33.7	22.6	−1.0	162.8	63.3
Deoxycytidine + Li^+	−28.6	41.1	−37.5	21.7	4.1	155.7	49.3
Deoxycytidine + Na^+	−32.1	41.7	−35.1	17.4	8.8	161.9	56.3
Deoxycytidine + K^+	−24.5	34.6	−30.9	17.8	4.05	154.5	63.3

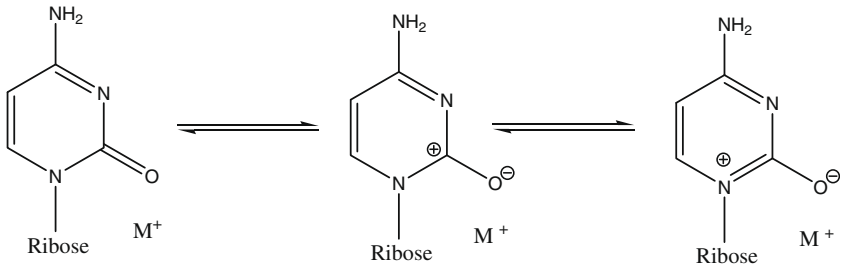

Chart 3. Resonance structures involving the carbonyl bond.

atoms than potassium. This depends essentially on the size of the metal ion because the nature of the interaction seems to be very similar for the two cations.

Furthermore, the cytidine–Na^+ complex is stabilized with two hydrogen bonds: one between two hydroxyl groups present on the two adjacent C2′ and C3′ carbon atoms. The other one, a particular intermolecular H-bond, that is, C6–H6···O5′ exists between the base unit and the sugar unit. In fact, to favor such particular hydrogen bond in this system, the cytosine base should turn around the glycosyl linkage to make possible the formation of the intramolecular C–H···O hydrogen bond. It should be mentioned that two particular types of intermolecular H-bonds, that

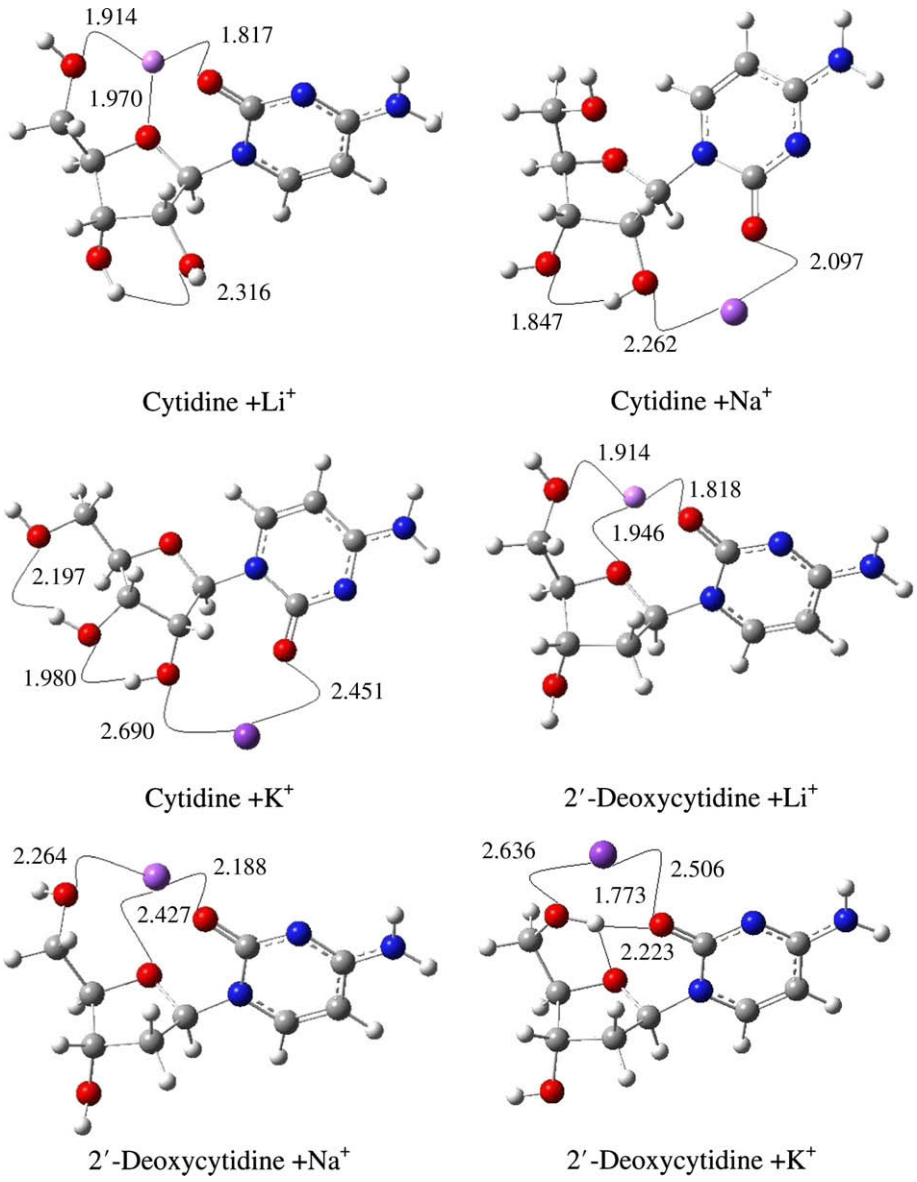

Figure 2. Structures and main geometrical parameters of the complexes obtained by metalation of cytidine and 2′-deoxycytidine with M^+ (M^+: Li^+, Na^+, and K^+) calculated at B3LYP/6-311++G**. Distances are in Å.

is, C6–H6···O5′ and C8–H8···O5′ in pyrimidine and purine nucleosides, respectively, have been experimentally confirmed,[54] as well in nucleic acid chains.[55,56] Of course, the C–H···O interaction is weaker than the other H-bonds arising for instance from the O–H···O-type interactions. Moreover, theoretical analysis[57] has shown that the C···O distance in the C–H···O hydrogen bonding is located in a range between 3.3 and 3.6 Å, depending on the strength of this particular interaction. The result of calculation shows that the C···O distance in the cytidine–Na^+ complex is well located within this range, confirming the existence of the intermolecular C–H···O hydrogen bond.

In the case of the potassium cation, cytidine acts as a bidendate ligand and the K^+–O2 and K^+–O2′ distances are 2.451 and 2.690 Å, respectively. This complex is stabilized by two hydrogen bonds: one between two hydroxyl groups that are on the two adjacent C2′ and C3′ carbon atoms and the other between O3′H in the sugar ring and the lone pair of O5′ atom. The latter hydrogen bond formation is correlated with changes in the O5′–C5′–C4′–C3′ and C5′–C4′–C3′–C2′ torsion angles (see Fig. 2 for more details). A closer look at the geometrical parameters in Figure 2 shows that the O2′H···O3′ hydrogen bond becomes longer upon the interaction of cytidine with potassium and shorter when sodium is involved. This can be explained by the fact that the O2′ atom in the cytidine–Na^+ complex has more negative charge than that in cytidine–K^+, and this fact results from the donor nature of the oxygen atom in hydrogen bonding (see Table 4 for more details).

In general, as illustrated in Figure 2, the most favorable coordination sites for Li^+ involve the carbonyl oxygen and O4′ and O5′ atoms of the sugar unit. The η^2(O,O) coordination with the oxygen atom of the carbonyl group and 2′-hydroxyl oxygen is also possible for Li^+. But the results show that the cytidine–Li^+ tri-coordination mode is more stable than the corresponding bi-coordination mode by 15.3 kcal/mol. The Cartesian coordination of all optimized structures for free cytosine nucleosides and their complexes with metal cations are given in Supplementary data (Table 4S).

The bi-coordination to cytidine, in which the cation interacts with the carbonyl oxygen and oxygen atom at O2′, becomes slightly more favorable in the case of sodium and potassium, which is a behavior different from the Li^+ cation. In bi-coordinated complexes of Na^+ and K^+, the distance between cations and oxygen donor atoms is dramatically increasing from Na^+ through K^+. This behavior is evident for alkali metal cations because they belong to the same group of the periodic table.

The results of calculations have shown that the cytidine sugar conformation during metalation is changed significantly (i.e., C3′-exo and C4′-exo for Na^+ and K^+ complexes with cytidine, respectively). It should be emphasized that these conformers for the sugar unit have rarely been observed in standard pyrimidine nucleosides.[34] Furthermore, the intramolecular hydrogen bonding in free cytidine molecule should be broken and rearrangement of functional groups is needed for metal complexation.

On the other hand, the direct binding of a cation to nucleobases and nucleosides can significantly influence the strength of base pairs in DNA. For example, the strength (base pair interaction energy) of an isolated reverse Hoogsteen GC base pair is ca. −18 kcal/mol when a divalent metal cation binds to N7 of guanine. The energy which is necessary to disrupt the base pair is twice as large with the cation as compared to the absence of cation.[58]

As can be seen from Figure 2, the tri-coordinated complex, η^3(O,O,O), in which the cation interacts with the carbonyl oxygen and oxygen atoms at O2′ and O4′ in the sugar unit of 2′-deoxycytidine is the most stable structure for both lithium and sodium cation complexes of 2′-deoxycytidine. The interaction distances in the 2′-deoxycytidine–Na^+ complex are longer by ~0.35 Å than those in the corresponding 2′-deoxycytidine–Li^+ complex. In these complexes, the above-mentioned OH···O hydrogen bonds between two hydroxyl groups (on the two adjacent C2′ and C3′ carbons) cannot exist due to the replacement of the 2′- hydroxyl group by a hydrogen atom.

Unlike the two previous monovalent cations, Li^+ and Na^+ (see Fig. 2), the most stable structure in the case of potassium corresponds to that in which the metal cation interacts with both oxygen atoms of 2′-deoxycytidine, that is, involving the η^2(O,O) coordination mode. Here, 2′-deoxycytidine acts as a bidendate ligand, and the K^+–O2 and K^+–O5′ distances are 2.506 and 2.636 Å, respectively. Two hydrogen bonds exist, O5′H···O=C and O5′H···O4′ with the length 1.773 and 2.223 Å, in this complex.

3.5. Metal ion affinity

It is obvious that the metal ion affinity is strongly dependent on the coordination mode of the cation to the ligand and on the charge-to-size ratio of the cation. B3LYP absolute affinity values, dipole moments, orientation of the base unit with respect to the sugar unit and sugar unit conformation for cytidine and 2′-deoxycytidine complexes with Li^+, Na^+, and K^+ are given in Table 3. The 0.79–6.65 D dipole moment range evidences variable polarity of nucleoside conformers for free molecules and their complexes with metal cations. In the simplistic point of view, the strength of the interactions of metal ions with the cytosine nucleosides appears to be driven principally by the ion-induced dipole interaction. The dipole moment trend accounts for this situation giving the smallest values for lithium complexes and the greatest values for the potassium ones. In general, the binding energies of 2′-deoxycytidine complexes are systematically greater than those of cytidine complexes.

The results of calculation indicate that, on the average, in all of the L+M^+ systems, the measured binding strength varies with the metal ion such that Li^+ binds 70% more strongly than Na^+, which in turn binds 55% more strongly than K^+. Because these complexes are largely electrostatic in nature, this is easily understood on the basis of the size or, equivalently, the charge density on metal. The greater the charge density of the smaller metal results in, greater the strength of the cation–ligand interaction in the system.

It is also interesting to mention that linear correlation between calculated MIA values and the atomic numbers (Z) of the metal ions of Li^+, Na^+, and K^+ were found (as previously described[59]). For instance, using the data given in Table 3, we obtained linear plots (not shown: these are available upon request). Such a linear correlation between measured MIA and Z for metal complexes of many biomolecules with Li^+, Na^+, and K^+ could be found using the data given in literature.[36]

3.6. Charge transfer and atomic charge on the L–M^+ complexes

Each complex formed by a cation with a nucleoside was divided into two-part components. A careful Mulliken charge analysis of

Table 3
B3LYP/6-311++G** metal ion affinity at 298 K, dipole moments and conformation in various systems

System	MIA[a]	μ[b]	Conformation in system
Cytidine	—	6.41	*anti*/C2′-endo
Cytidine + Li^+	79.5	1.43	*syn*/C2′-endo
Cytidine + Na^+	55.2	3.94	*anti*/C3′-exo
Cytidine + K^+	41.8	3.08	*anti*/C4′-exo
2′-Deoxycytidine	—	6.25	*syn*/C2′-endo
Deoxycytidine + Li^+	82.8	0.79	*syn*/C2′-endo
Deoxycytidine + Na^+	57.4	2.14	*syn*/C2′-endo
Deoxycytidine + K^+	42.2	6.65	*syn*/C2′-endo

All structures are stable on the potential energy surface.
[a] Metal ion affinity (MIA) in kcal/mol.
[b] Dipole moments (μ) in Debye.

the results for all the complexes shows that charge transfer takes place during the complexation reaction. The amount of charge transfer (CT) between a nucleoside and the cation is easily determined as the difference between the charge of the isolated ion and the net atomic charge on the metal.

The data also demonstrate that the CT for 2′-deoxycytidine–M^+ complexes is systematically larger than that for cytidine–M^+ complexes. The transferred positive charge from the metal cation to the nucleoside, *Q*, is in the order of $Q(Li^+) > Q(Na^+) > Q(K^+)$. As already known, the LUMO energies of these cations increase in the above order, therefore, the electrons should be easier to transfer from nucleosides to Li^+ than to K^+. Hence, in these systems, the shorter bond distance and greater charge density allow the metal ion to more effectively withdraw electron density from the neutral ligand, thus reducing the charge retained on the metal. Concordantly, this order correlated well with the binding strength between nucleosides and M^+. Therefore, the transferred charge between nucleosides and M^+ during complexation could be used as an indicator of the binding strength between the nucleosides and the cations.

Natural charge analysis attributes positive charges to the Li^+, Na^+, and K^+ cations that are, respectively, 0.291 |*e*| , 0.926 |*e*|, and 0.980 |*e*| for cytidine complexes and 0.301 |*e*| , 0.724 |*e*|, and 0.955 |*e*| for 2′-deoxycytidine complexes (see Table 4 for more details). Thus, the ligand transfers smaller amount of charge to the Na^+ and K^+ cations than to Li^+ ion. As known, a charge transfer implies the presence of almost an interaction with a covalent contribution. These results confirm the electrostatic nature of the bonding, but also demonstrate that there is some covalency in the metal–ligand interaction, especially in the Li^+ systems. The highest and lowest covalent contribution is found for Li^+ and K^+, respectively. The charge analysis applied to the cytidine–K^+ complex indicates that a small charge transfer occurred between the ligand and the cation. In addition, ionic interaction between K^+ and O atoms is present as evidenced by the net charge values (0.980, −0.448, −0.101, and −0.311 *e* for K^+, the carbonyl oxygen, O4′, and O5′, respectively) given in Table 4. In general, the charge transfer for cytidine complexes is essentially larger than that for 2′-deoxycytidine complexes (with the exception of cytidine–K^+ complex).

4. Conclusion

This paper is the first attempt to study the conformational properties of one of the DNA and RNA constituents during metal complexation (cytosine nucleosides, i.e., cytidine and 2′-deoxycytidine nucleosides) by means of full geometry optimization based on a reliable quantum mechanical method. Structures and energetic aspects of the complexes of Li^+, Na^+, and K^+ cations with cytidine and deoxycytidine were studied at the B3LYP/6-311++G(d,p) density functional level with the aim of evaluating the coordination geometries, electronic features and absolute metal ion affinities for all possible complexation stable products. The important geometrical features related to these systems, such as pseudorotation angle *P*, glycosyl torsion angle χ, intramolecular hydrogen bonds, and the sugar bond lengths and valency angles, are reported by means of theoretical calculations. Cations were allowed to interact with canonical parameters for free nucleosides after a careful selection of several attachment sites. L–M^+ bond energies (where L is cytosine nucleosides, i.e., cytidine and 2′-deoxycytidine) decrease as the size of the metal ion becomes larger. The affinities for cytosine nucleosides increase in the order $Li^+ > Na^+ > K^+$.

The interaction between cytosine nucleosides and alkaline metal cations is multiple, flexible, and strong. In the DNA double helix, cytosine is complementary bonded to guanine via three H-bonds involving the oxygen atom of the carbonyl group, N3 of the pyrimidine ring and N4 of the exocyclic amino group. The results of calculation reveal that hydrogen bonding in a base pair of a nucleic acid could be seriously affected or broken by metal cation interaction, resulting in a change in biological functions of a nucleic acid.

In all cases, the N1–C1′ bond distance changes (upon cationization) depend on the positive amount of charge on M^+ introduced in the system. Thus, these results seem to indicate that the presence of a positive charge in cytosine nucleosides generated through metal cationization weakens the *N*-glycosidic bond. With respect to the sugar ring, it has been also found that metal binding produces significant changes in the values of the phase angle of pseudorotation *P* conformation of the sugar ring.

Therefore, these calculation results might help to better understand the role of various cations in biological systems and to evaluate their effects in biological processing. The revealed geometrical and thermochemical parameters, as well as the calculated total atomic charges, are useful for improving the current force field to reproduce such kinds of interactions that are essential in exploring the role of cations in living systems.

Table 4
Mulliken net charge on the metal ion and oxygen atoms for complexes of cytidine and 2′-deoxycytidine with alkali metal ions (Li^+, Na^+, and K^+) calculated at B3LYP/6-311++G**

System	*q* M	*q* O2′	*q* O4′	*q* O5′	*q* Carbonyl oxygen
Cytidine + Li^+	0.291	−0.239	−0.057	−0.263	−0.193
Cytidine + Na^+	0.926	−0.284	−0.067	−0.288	−0.455
Cytidine + K^+	0.980	−0.251	−0.101	−0.311	−0.448
Deoxycytidine + Li^+	0.301	—	−0.078	−0.262	−0.187
Deoxycytidine + Na^+	0.724	—	−0.159	−0.345	−0.368
Deoxycytidine + K^+	0.955	—	−0.154	−0.400	−0.515

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.02.007.

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