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Theoretical Study of the Interactions between Isolated *DNA* Bases and Various Groups IA and IIA Metal Ions by *Ab Initio* Calculations

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Summary. Interactions of the *DNA* bases adenine (*A*), guanine (*G*), cytosine (*C*), and thymine (*T*) with various metal ions (*M*) of groups IA and IIA of the periodic table of the elements were studied at the *HF*, *MP*2, and *DFT* levels of theory. The structures and thermodynamic stabilities of these species were studied at the gas phase. The calculations uphold that there exist two active sites in *G* and one in *A*, *C*, and *T*. The calculations also show that the O^2 atom in *T* is a more active site for metal ion bindings than that in *C*. The stability energies for $G \ldots M$ complexes are larger than those for $A \ldots M$ complexes and the stability energies for $T \ldots M$ complexes are larger than those for $C \ldots M$ complexes. As z/r ratio for the metal ion increases, the interaction energy for the complex increases systematically. Thermodynamic quantities such as ΔH, ΔG, ΔS, and $\ln K$ were determined for each complexation reaction, $[\text{Base} + \text{M}^{n+} \rightarrow (\text{Base} \ldots M)^{n+}]$. *A*, *G*, and *C* complexation reactions except for $C \ldots \text{Rb}^+$ are exothermic. The situation is quite different for *T* complexation reactions and all except for $T \ldots \text{Be}^{2+}$ and $T \ldots \text{Mg}^{2+}$ are endothermic.

Keywords. Nucleic acid bases; *Ab initio* calculations; Metal complexes.

Introduction

Living organisms contain a set of instructions that specifies every step required for the organism to construct a replica of itself. This information is stored in deoxyribonucleic acid (*DNA*) and thus this molecule is one of the most important molecules in our life. The origin of nucleic acid chemistry is generally attributed to *Friedrich Miescher* who started to isolate what was called nuclein from human pus cells in 1860s [1]. Nucleic acids are strong acids so they can react with cations such as metal ions or protonated amines [2]. *Barnet Rosenberg*'s seminal discovery was that cis-Platin (*cis*-$[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$) is a potent antitumor agent and that Pt-*DNA* binding is responsible for killing triggering tumor cells [3]. Synthesis, replication, and cleavage of *DNA* as well as its structural integrity are affected by the presence of ionized metals in the cell nucleus. These ions can interact with many sites in *DNA*: phosphate groups and the sugar moiety, as well as the *DNA* bases. Despite the fact that the metal cations usually interact with the phosphate groups and, to a lesser extent, with the bases, cation-base interactions are expected to be involved in many important biophysical processes. Alkali metal ions, and other ''hard'' metal ions, have a low tendency to form covalent bonds and are therefore relatively non-specific binders. Their primary influence is to neutralize the negative charges on the backbone phosphate groups, thereby stabilizing the double helix structure of *DNA*. When metal ions bind to the bases instead of the phosphate groups, they also neutralize negative charges on the phosphate groups in a zwitterions effect. In either event, such stabilization is accompanied by an increase in the ''melting temperature of *DNA*'' [4–7]. Because each metal ion bound to *DNA* neutralizes some of

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the charge on it, additional metal ion bindings becomes increasingly weaker, *i.e.* the binding is anticooperative [8]. If enough metal ions are added to neutralize the negative charges in the backbone of the nucleotide, additional metal ions destabilize the *DNA* double helix structure [9]. From the historical review carried out by *Bernhard Lippert* on the multiplicity of metal ion binding patterns to nucleobases [10], it is clear that in single-stranded *DNA* and likewise with isolated bases (model bases, nucleosides, and nucleotides) virtually any site in the heterocyclic base could be metalated, including the C atoms.

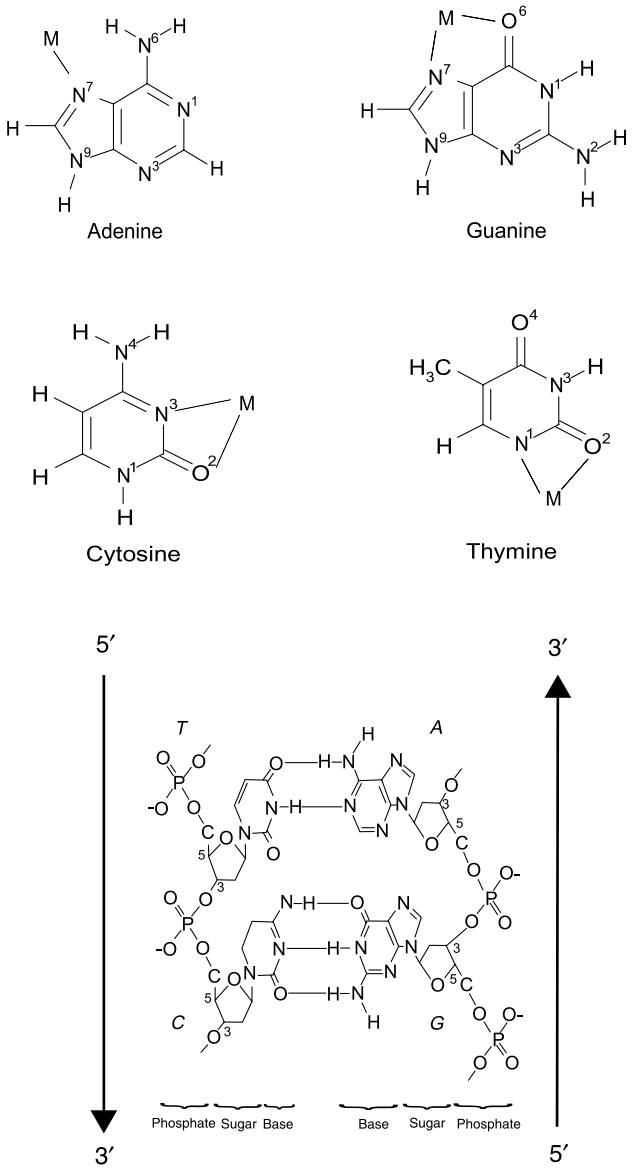

Fig. 1. Structures of the Adenine . . . *M*, Guanine . . . *M*, Thymine . . . *M*, and Cytosine . . . *M* complexes. Positions of the *DNA* bases in the structure of *DNA*

Among these, the N^7 positions in the purine bases adenine and guanine, which are well accessible in the major groove of duplex *DNA* and single-stranded *DNA* and are major binding sites, either experimentally [10, 11] or theoretically [12–14], are considered in the present study (see Fig. 1). Various metal bindings to N and O atoms in the pyrimidine bases thymine and cytosine are also possible. With the isolated cytosine base, binding to either N^3 or O^2 is favored [10] and these were chosen as the binding sites in this work (Fig. 1). The floor of the minor groove of double-stranded *DNA* also presents metal binding sites. Among these, the combination of metal ions with the O^2 atom in thymine is particularly efficient [10]. In most of the theoretical and experimental works [11], the O^4 atom in thymine has been considered as the most active site but regarding the *DNA* double helix structure, this site is blocked through hydrogen bonding. However, an alternative site for metal ion binding in the *A*:*T* base pairing is the O^2 site in thymine, in which case nonhydrogen bonds are needed to be broken. Thus, in the *A*:*T* base pairing the O^2 site in thymine is likely to be the thermodynamically preferred binding site. On the contrary to the other theoretical works, in the current investigation we considered the O^2 and N^1 atoms in thymine as binding sites (Fig. 1) due to having nonbonding electron pairs, being accessible positions, and having less steric hindrances in the *DNA* structure. The existence of these binding sites has been widely confirmed by several spectroscopic investigations including NMR, *Raman*, and X-ray structural studies carried out on metal ion-nucleotide complexes [10].

Ab initio quantum chemical calculations carried out at the *Hartree-Fock* (*HF*) theoretical level with a minimal basis set predict polarization effects of the metal cations on the base caused by N^7 coordination [15]. *Ab initio* calculations [16] (*HF*/minimal basis set) demonstrate that interactions of various cations with the O^4 and O^2 atoms in thymine lead to destabilization of the *A*:*T* base pairing. The approach of the cation to other available sites (thymine, O^2; adenine, N^1, N^3) leads, on the other hand, to the stabilization of the pair.

Evidently, metal cations play an important role in stabilizing, as well as destabilizing, *DNA* bases, base pairings, and the *DNA* double helix structure. Knowledge about the fundamental modes of metal ions bindings to the basic structures in *DNA* (nucleo-

bases, nucleoside, *etc.*) would greatly improve our understanding on how metal ions interact with the more complex *DNA* structure. To understand the role of cations in the biophysics of *DNA*, it is necessary first to carry out a detailed study on the interactions of the cations with isolated bases. This is the aim of the present study.

Results and Discussion

Investigation of the Active Sites

Net atomic partial charges on all atoms in each base were calculated at the *HF* and DFT levels. The results are listed in Table 1. The active site in each *DNA* base was determined by investigating the net atomic charge on the isolated base, the position of each base in the *DNA* double helix structure [1], the steric hindrance for each site, and the empirical evidence for its proton ionization [10].

The net partial charge on each atom in the structure of adenine is tabulated in Table 1. The N^7 atom in adenine is the most active site because of a low steric hindrance and being accessible in the double helix structure of *DNA*. After carrying out geometry optimization calculations at the *HF* and DFT levels and determining the net partial charge on each atom in the structure of guanine, the results were tabulated in Table 1. The N^7 atom in guanine is considered to be the most active site among the nitrogen atoms in its structure because of the absence of steric hindrances, not taking part in hydrogen bonding, and most importantly, accessibility of this site in the double helix structure of *DNA*. The O^6 atom in guanine is considered to be among the most active sites because of having two lone pairs of electrons. In most cases, the O^6 atom is more active than the N^7 atom, and the lower X–O^6 bond length relative to the X–N^7 bond length confirms this. The net partial charge on each atom in the structure of cytosine is tabulated in Table 1. The N^3 atom in cytosine, being blocked *via* hydrogen bonding in the double helix structure of *DNA*, is the most active site among nitrogen atoms. From the experimental review [10], N^3 atom in cytosine is the most basic one, hence has the highest pK_a value and the highest affinity for H^+. X-Ray results also indicate that this site is the most active site among the nitrogen atoms in cytosine [10]. The O^2 atom in cytosine is considered to be an active site for metal ion bindings. Studying the bond lengths indicates that the O^2 atom in cytosine in most of its complexes is more active than the N^3 atom; shorter M–O^2 bond length compared with the M–N^2 bond length confirms this. The N^1 atom in thymine is blocked through sugar binding in the double helix structure of *DNA* and the N^3 atom is blocked by hydrogen bonding. The O^2 atom in thymine is considered to be an active site for metal ion bindings because of having two lone pairs of electrons. Shorter M–O^2 bond length relative to the M–N^1

Table 1. Net atomic partial charges for the *DNA* bases

	Adenine			Guanine			Cytosine			Thymine	
	HF	DFT		*HF*	DFT		*HF*	DFT		*HF*	DFT
N^6	−1.0145	−0.8547	C^6	0.7952	0.5521	C^4	0.5875	0.4987	C^4	0.6233	0.4994
C^6	0.5848	0.4517	N^1	−0.9399	−0.7338	N^3	−0.6271	−0.5006	N^3	−0.8234	−0.7252
H^6	0.4689	0.4206	C^5	−0.151	−0.0908	C^5	−0.2101	−0.0433	C^5	0.4922	0.7698
H^6	0.4634	0.4139	O^6	−0.6227	−0.549	N^4	−0.9843	−0.8397	O^4	−0.5942	−0.511
N^1	−0.483	−0.3888	C^4	0.3165	0.1179	C^6	−0.0695	−0.219	C^2	0.9816	0.7123
C^5	−0.1894	−0.0145	C^2	0.9383	0.772	C^2	0.8004	0.5617	C^6	−0.2353	−0.4464
C^4	0.3857	0.2306	N^7	−0.3702	−0.3602	H^5	0.2513	0.186	C^7	−0.7857	−0.9415
C^2	0.1768	0.0734	H^1	0.4948	0.4239	H^4	0.4689	0.4193	H^3	0.5231	0.4526
N^7	−0.4204	−0.413	N^3	−0.6003	−0.5067	H^4	0.4449	0.3984	N^1	−0.9509	−0.595
N^3	−0.4287	−0.3263	C^8	0.284	0.2033	N^1	−0.7776	−0.5679	O^2	−0.6412	−0.5362
C^8	0.2979	0.2039	N^9	−0.7708	−0.5928	O^2	−0.6375	−0.5299	H^6	0.2559	0.2054
N^9	−0.7924	−0.6011	N^2	−1.0256	−0.8559	H^6	0.2545	0.2044	H^7	0.2262	0.2368
H^2	0.2182	0.1787	H^8	0.236	0.1951	H^9	0.4984	0.4319	H^7		
H^8	0.2401	0.1977	H^9	0.488	0.4171				H^7		
H^9	0.4925	0.4221	H^2	0.475	0.4233				H^1		
			H^2	0.453	0.4026						

Table 2. Dipole moments/D of the isolated bases and their complexes with metal ions

	Adenine		Guanine		Cytosine		Thymine	
	DFT	Exp.	DFT	Exp.	DFT	Exp.	DFT	Exp.
Base	2.468	2.54	7.106	>7	6.914	>6	4.625	4.98
Base-Li	8.249		2.273		3.750		7.692	
Base-Na	9.648		3.958		4.762		10.945	
Base-K	10.575		5.143		5.385		13.550	
Base-Rb	9.075		3.683		3.226		14.620	
Base-Be	9.930		4.469		9.243		9.808	
Base-Mg	14.335		8.677		10.754		15.386	
Base-Ca	17.134		12.149		11.135		19.935	
Base-Sr	14.400		9.450		8.33		19.303	

bond length indicates that the O^2 atom in thymine is a more active site. Activity of the O^4 atom in thymine is approximately similar to that for the O^2 atom but one should take this point into consideration that the O^4 atom in thymine is blocked through hydrogen bonding in the *DNA* double helix structure. By considering all aspects, the O^2 atom in thymine is an active site in the present study. In general, thymine does not have a lot of active sites in its interactions with metal ions. We investigated this by studying the thermochemistry results obtained from studying the reaction:

$$T + M^{n+} \rightarrow [T - M]^{n+}.$$

From the investigations carried out on the complexes of thymine with metal ions we determined the O^2 atom to be an active site because of having a higher amount of partial charge relative to other nitrogen and oxygen atoms.

Dipole Moment

Dipole moments of each isolated base and its complexes are listed in Table 2. Calculated dipole moments for isolated bases show a good agreement with available experimental data [19, 20]. The following order holds for the nucleic acid bases, either experimentally or theoretically: Adenine < Thymine < Cytosine < Guanine. Dipole moments for complexes with bivalent ions are larger than those with monovalent ions. In general, dipole moment increases as the ion atomic number increases. Finally, an interesting observation from Table 2 is the same dipole moment variations for the bases which participate in conjugated base formations.

Geometry Parameters

Structural parameters were determined for guanine, adenine, cytosine and thymine by the *B3LYP* method, and the 6−31+G* and Lanl2DZ basis sets were used for the bases and metals. From the theoretical works [21] it is clear that the introduction of a first diffuse function, as well as the polarization function, notably improves the value obtained by the simpler 6−31G basis set. In fact, the 6−31+G(2df,2p) basis set gives the results closest to the most recent experimental counterparts for both sodium and potassium ions. Guanine and adenine, having double ring structures, were compared with each other, likewise cytosine and thymine with single ring structures. Finally, the results obtained for all bases were compared with one another.

Adenine and Guanine

The M–N^7 and M–O^6 distances are reported in Table 3a. The M–N^7 intermolecular distances for adenine complexes are shorter than the corresponding distances in guanine. This is due to the existence of two active sites in guanine, the O^6 and N^7 atoms. As could be seen in guanine complexes, the M–O^6 distance is shorter than the M–N^7 distance; this indicates a stronger bonding of metal ions with the O^6 site. The M–N^7 and M–O^6 intermolecular distances in complexes of adenine and guanine increase monotonically with increasing atomic numbers of alkaline and alkali earth metals.

Geometries of the isolated bases and bases in the complexes differ considerably. We only analyzed bond lengths and bond angles because only these geometric parameters could be determined by X-

Table 3. Optimized intermolecular parameters (in Å and deg) for the adenine and guanine complexes

(a)

	Adenine				Guanine			
	X–N^7	X–N^7–C^4	X–N^7–C^5	X–N^6	X–N^7	X–N^7–C^2	X–O^6	X–N^7–C^5
Base-Li (DFT)	1.99	131.848	97.2785	2.1007	2.063	100.425	1.9392	92.4428
Base-Na (DFT)	2.408	133.074	100.938	2.5821	2.454	106.668	2.3080	97.54
Base-K (DFT)	2.853	128.357	100.551	2.9632	2.877	111.315	2.6783	101.642
Base-Rb (DFT)	3.086	125.686	100.018	3.1921	3.09	113.101	2.8656	103.285
Base-Be (DFT)	1.568	93.1641	70.3949	3.3195	1.654	96.4995	1.3207	91.817
Base-Mg (DFT)	2.011	133.38	98.849	2.1449	2.062	103.111	1.9509	95.8088
Base-Ca (DFT)	2.414	138.983	104.056	2.5431	2.472	108.63	1.2728	100.07
Base-Sr (DFT)	2.592	141.049	106.052	2.7109	2.659	110.231	2.4754	101.358

(b)

	Adenine					
	C^8–N^9	N^7–C^8	N^7–C^5	N^3–C^4	C^4–N^9	C^5–C^6–N^6
Base (DFT)	1.3821	1.3120	1.3857	1.3396	1.3781	122.362
Base-Li (DFT)	1.58649	1.36692	1.32386	1.38919	1.32693	177.596
Base-Na (DFT)	1.36769	1.32257	1.39128	1.3287	1.38066	120.169
Base-K (DFT)	1.37022	1.32087	1.39255	1.33136	1.3798	121.472
Base-Rb (DFT)	1.37032	1.32022	1.39121	1.33159	1.37947	121.763
Base-Be (DFT)	1.33547	1.34665	1.50208	1.28526	1.40213	125.827
Base-Mg (DFT)	1.34988	1.34169	1.39764	1.32147	1.39268	116.686
Base-Ca (DFT)	1.35263	1.33601	1.39992	1.32311	1.38567	119.686
Base-Sr (DFT)	1.3548	1.33418	1.39957	1.32204	1.38449	120.464
Base (exp.)	1.354	1.311	1.379	1.338	1.359	125.7
Base (StoBe)	1.387	1.323	1.387	1.350	1.387	–

	Guanine						
	N^1–C^6	C^6–O^6	C^6–C^5	C^5–C^4	N^3–C^4	C^2–N^2	C^5–C^6–O^6
Base (DFT)	1.439	1.2223	1.4382	1.3967	1.3572	1.3637	131.379
Base-Li (DFT)	1.39218	1.25452	1.40841	1.3845	1.34402	1.3475	124.235
Base-Na (DFT)	1.40338	1.24567	1.41974	1.39094	1.34475	1.3497	127.013
Base-K (DFT)	1.40911	1.24201	1.42448	1.39509	1.34605	1.3521	128.147
Base-Rb (DFT)	1.41185	1.23986	1.42623	1.39492	1.34751	1.3576	128.659
Base-Be (DFT)	1.35434	1.32079	1.36818	1.36897	1.32394	1.3269	114.911
Base-Mg (DFT)	1.36832	1.28952	1.38961	1.38341	1.32722	1.3315	120.992
Base-Ca (DFT)	1.37897	1.27287	1.40243	1.39066	1.33136	1.3367	123.719
Base-Sr (DFT)	1.38388	1.26835	1.40537	1.39219	1.33227	1.3379	124.65
Base (exp.)	1.398	1.293	1.405	1.392	1.364	1.333	127.7
Base (StoBe)	1.396	1.231	1.442	1.405	1.396	1.369	–

ray measurements. Experimental geometry parameters of isolated adenine and guanine which have been studied at room temperature by X-ray diffraction [22a–22b] and also recently calculated geometry parameters [23] by use of StoBe [24] are included in Table 3b for the reason of comparison. StoBe is a software designed to analyze electronic structures of molecules, with a focus on the inner-shell energy levels. This approach uses a linear combination of Gaussian-type orbital approach to form self-consistent solutions of the *Kohn-Sham* DFT equations, therefore is comparable with our DFT results. There is a good agreement between the experimental and corresponding calculated data. In complexes of guanine, due to the presence of ions, most of the changes occur in the six-membered ring of guanine and these changes are observed as a decrease in bond lengths in the six membered ring. This decreasing trend is

also observed for the C^2–N^2 bond length. On the other hand, the C^6–O^6 bond length increases due to the presence of the O^6 active site and its binding with the metal ion participating in the structure. Bond angles in guanine are less affected by ions; the most important changes occur in the C^5–C^6–O^6 bond angle due to direct interaction of the O^6 atom with metal ions.

The situation differs considerably in adenine complexes. In these complexes, the most important bond length changes take place in the five-membered ring. Our investigations show that the amino group in adenine complexes changes as a result of the direct repulsion between metal ions and the NH_2 group. The C^5–C^6–N^6 bond angle usually decreases, while the C^6–N^6 distance is almost constant. Correlation

Table 4. Optimized intermolecular parameters (in Å and deg) for the cytosine and thymine complexes

(a)

	Cytosine				Thymine		
	M–N^3	M–N^3–C^4	M–O^2	M–N^4	M–N^1	M–O^2	M–N^1–C^4
Base-Li (DFT)	2.099	160.312	1.88033	3.9851	1.941	1.86643	141.23
Base-Na (DFT)	2.557	157.905	2.2423	4.3488	2.322	2.22645	145.933
Base-K (DFT)	3.074	157.722	2.59839	4.81185	2.704	2.58438	149.351
Base-Rb (DFT)	3.301	156.942	2.7965	5.0032	2.998	2.9045	155.206
Base-Be (DFT)	1.58	91.0912	3.42122	1.73705	1.591	1.55019	138.814
Base-Mg (DFT)	1.995	98.7039	3.59425	2.19122	1.98284	1.91906	21.2196
Base-Ca (DFT)	2.57	156.029	2.282	4.36323	2.37	2.2746	147.82
Base-Sr (DFT)	2.78	156.01	2.426	4.5466	2.534	2.40994	148.703

(b)

	Cytosine					
	N^3–C^4	N^3–C^2	C^4–N^4	C^2–O^2	N^3–C^2–O^2	N^3–C^4–N^4
Base (DFT)	1.322	1.3701	1.3595	1.2338	125.493	116.942
Base-Li (DFT)	1.34461	1.36445	1.34115	1.2526	119.375	118.308
Base-Na (DFT)	1.3426	1.3667	1.3451	1.246	121.231	118.371
Base-K (DFT)	1.34003	1.366	1.34809	1.24208	122.892	117.726
Base-Rb (DFT)	1.378	1.3658	1.3494	1.2419	117.691	123.267
Base-Be (DFT)	1.36525	1.4147	1.4843	1.19154	125.287	103.104
Base-Mg (DFT)	1.34145	1.40453	1.4692	1.2022	123.695	108.646
Base-Ca (DFT)	1.3636	1.368	1.33441	1.2677	118.924	119.13
Base-Sr (DFT)	1.36072	1.3658	1.33611	1.26483	119.686	119.405
Base (exp.)	1.332	1.358	1.336	1.234	122.4	117.4
Base (StoBe)	1.331	1.392	1.365	1.016	–	–

	Thymine				
	N^3–C^4	N^3–C^2	C^4–O^4	C^2–O^2	N^1–C^2–O^2
Base (DFT)	1.408	1.3848	1.2241	1.2207	123.146
Base-Li (DFT)	1.41281	1.36842	1.22991	1.27587	119.194
Base-Na (DFT)	1.40778	1.37798	1.23369	1.26844	121.587
Base-K (DFT)	1.40555	1.38488	1.23597	1.26544	122.756
Base-Rb (DFT)	1.3996	1.38902	1.2369	1.26401	125.859
Base-Be (DFT)	1.46347	1.31586	1.20556	1.33246	109.638
Base-Mg (DFT)	1.438	1.33712	1.21343	1.30626	115.707
Base-Ca (DFT)	1.42509	1.35182	1.21833	1.29208	118.514
Base-Sr (DFT)	1.4213	1.35604	1.2204	1.28892	119.247
Base (exp.)	1.380	1.385	1.223	1.240	123.8
Base (StoBe)	1.397	1.397	1.233	1.233	–

between the bond length changes and stabilization energies was found for both types of complexes; consequently, the bond length changes in guanine are larger than those in adenine (Table 3b).

The geometry parameters indicate that the M–O^6 interaction in guanine is stronger than the X–N^7 interaction. The internuclear M–O^6 distances were found to be mostly shorter than the M–N^7 distances. This can be explained by the fact that the O^6 atom in guanine has two non-bonding electron pairs and a considerable partial charge. X-Ray results show that the N^7 atom in adenine and the N^7 and O^6 atoms in guanine are active sites for metal ion bindings [25, 26]. The favored orientation of charge and dipole in guanine complexes probably also play a role [11, 12].

Thymine and Cytosine

All the M–N^1 intermolecular distances in thymine complexes are shorter than the M–N^3 bond lengths in corresponding complexes of cytosine because M–O^2 bindings in complexes of thymine are stronger. The results tabulated in Table 4a show that in both complexes of thymine and cytosine the O^2 atom is an active site but the M–O^2 bond lengths in thymine complexes are shorter than those in cytosine complexes.

The geometries of isolated thymine and cytosine and these bases in the complexes differ considerably. For thymine and cytosine the situation is slightly different because these are single ringed bases and therefore changes are concentrated on one ring, thus the changing trend becomes slightly more complicated (see Table 4b). Experimental geometry parameters for isolated cytosine and thymine have been studied by X-ray and neutron diffraction, respectively [27a, b] and the results calculated with StoBe [23] are also included. A reasonable agreement between theory and experiment is observed in these cases.

In spite of binding of metal ions to the N^3 atom in cytosine in the initial structures of optimization calculations, studying geometry parameters of the final structures at the *HF* and DFT levels show that binding with the O^2 position in cytosine is more stable. The M–O^2 intermolecular distance in these complexes is shorter than the M–N^3 intermolecular distance, which is indicative of the fact that the O^2 site in cytosine is more active than the N^3 site. The only exceptions to this are for Be^{2+} and Mg^{2+}, due to very small radii and large z/r ratios for these ions. In complexes of $C\ldots Be^{2+}$ and $C\ldots Mg^{2+}$, the metal ions bind to the N^3 and N^4 atoms and as a result of these bindings stable complexes are formed. Bond length changes in complexes of cytosine in which metal ions bind to the O^2 atom are similar and they are similar in complexes in which metal ions bind to the N^3 and N^4 atoms. In complexes of cytosine in which metal ions bind to the O^2 atom, bond lengths in the six-membered ring increase and decrease alternately. In these complexes, bond lengths of the bonds outside the ring show a decreasing trend for C^4–N^4 and an increasing trend for C^2–O^2; the reason can be found in the binding of metal ions to the O^2 atom. Change in bond lengths in cytosine ring in complexes of $C\ldots Be^{2+}$ and $C\ldots Mg^{2+}$ is quite contrary to the above trend. Bond lengths of the out of the ring C^4–N^4 bonds show an increasing trend due to binding of metal ions to the N^4 atom. Regarding the cytosine bond angles, the highest change is observed in the N^3–C^2–O^2 bond angle due to the direct interaction of the O^2 atom with metal ions. For the two exceptions Be^{2+} and Mg^{2+}, the highest change occurs in the N^3–C^4–N^4 bond angle due to interactions of these metal ions with the N^4 atom.

Investigations show that the N^3–C^2–O^2 bond angle in cytosine changes due to direct interaction of the O^2 atom with metal ions. But in $C\ldots Mg^{2+}$ and $C\ldots Be^{2+}$ most of the changes were observed in the N^3–C^4–N^4 bond angle due to direct interaction of the N^4 atom with metal ions. The N^3–C^4–N^4 bond angle changes in all complexes containing cytosine due to direct repulsion between metal ions and the NH_2 group in cytosine.

Bond length variations in thymine complexes are more considerable than those in isolated thymine. Bond length variations for complexes with bivalent metal ions are larger than those with monovalent ones. The N^1–C^2 intermolecular distances for thymine complexes are shorter, in comparison with the corresponding distances in isolated thymine. This can be explained by the comparable activities of the O^2 and N^1 atoms. In fact, thymine has two active sites, nitrogen and O^2 (and in some cases, a third active site, O^4). The M–O^2 intermolecular distances are shorter than the M–N^1 bond lengths. Consequently, thymine has two active sites for metal ion bindings. The N^1–C^2–O^2 bond angle changes in thymine due to direct interaction of the O^2 atom with metal ions. But in $T\ldots Rb^+$ complexes, Rb^+ binds to the O^4 atom due to larger ionic radius of Rb^+ and smaller z/r ratio as compared with the other metal ions.

Table 5. Interaction energies/kJ · mol^{-1} for complexes of the *DNA* bases with metal ions

	Adenine			Guanine			Cytosine		Thymine	
	HF	DFT	*MP2*[a]	*HF*	DFT	*MP2*[a]	*HF*	DFT	*HF*	DFT
Base-Li	−200.96	−180.03	−169.15	−294.88	−273.44	−327.8	−283.57	−259.66	777.86	773.89
Base-Na	−129.08	−97.30	−107.18	−227.85	−194.52	−237.8	−213.86	−181.37	879.64	886.76
Base-K	−75.70	−54.30	−61.13	−168.06	−144.65	−170.4	−155.04	−133.43	969.07	961.54
Base-Rb	−61.13	−39.35	–	−148.80	−124.05	–	912.01	845.69	1070.73	990.05
Base-Be	−863.36	−892.92	–	−1244.1	−1270.48	–	−1043.73	−1074.37	−558.94	−605.08
Base-Mg	−642.13	−614.24	−512.05	802.19	−767.31	−862.5	−615.96	−595.40	−46.51	−53.50
Base-Ca	−381.71	−371.62	−279.68	−540.01	−532.06	−573.6	−492.53	−471.01	278.09	264.94
Base-Sr	−313.42	−301.66	−222.74	−470.84	−452.84	−488.2	−375.38	−406.203	366.93	353.53

Stabilization Energy (Interaction Energy)

Stabilization energies for $T\ldots M$, $A\ldots M$, $C\ldots M$, and $G\ldots M$ complexes were obtained from the results of geometry optimization. Interaction energies (ΔE) for $A\ldots M$, $T\ldots M$, $C\ldots M$, and $G\ldots M$ complexes were determined as the difference between the optimized energy of the base…metal cation [$E(\text{Base}\ldots M)^{n+}$] system and the sum of the energies of the base [E(Base)] and the metal ion [$E(M^{n+})$]:

$$\Delta E = E(\text{Base}\cdots X^{n+}) - [E(\text{Base}) + E(X^{n+})]$$

Interaction energies for the $A\ldots M$, $T\ldots M$, $C\ldots M$, and $G\ldots M$ complexes are reported in Table 5. Effect of electron correlation in quantum mechanical calculations could be evaluated by comparing ΔE^{HF} with ΔE^{DFT}. The stabilization energies obtained form DFT calculations are lower than those obtained from the *HF* calculations. This can be explained by the fact that in the DFT method parts of the electron correlation energy are considered. ΔE^{MP2} which is the interaction energy evaluated at the *MP*2 level using the function counterpoise method by considering the basis set superposition error (BSSE) [12] is also included.

Interaction energies for guanine complexes are larger than those for adenine complexes. The increase in the ion atomic number or z/r ratio leads to a decrease in the interaction energy (from top to bottom in both alkaline and alkali earth metal groups). The increase in the ion atomic number in both alkaline and alkali earth metal groups leads to a decrease in the interaction energies for cytosine complexes; even in the case of $C\ldots Rb^+$ complex, the interaction energy becomes positive. This can be explained by the larger ionic radius or the smaller z/r ratio in Rb^+. The situation differs considerably in thymine complexes. All the interaction energies except for $T\ldots Mg^{2+}$ and $T\ldots Be^{2+}$ are positive. Interaction energies (ΔE) for thymine complexes are mostly positive, contrary to the other three bases. This is due to steric hindrances of the two C=O groups which are in resonance with the ring. The only exceptions are complexes of Be^{2+} and Mg^{2+} that have smaller ionic radii and bigger z/r ratios relative to other metal ions. The deformation energies of bases reflect deformability of the base in the field of a metal ion. Deformation energies for complexes with bivalent metal ions are larger than those with

Table 6. C_T/D values for complexes of the *DNA* bases and metal ions

	Adenine		Guanine		Cytosine		Thymine	
	HF	DFT	*HF*	DFT	*HF*	DFT	*HF*	DFT
Base-Li	0.3272	0.3852	0.2948	0.3729	0.2566	0.3219	0.3472	0.4109
Base-Na	0.127	0.1448	0.1163	0.146	0.1022	0.1324	0.1622	0.2049
Base-K	0.0069	0.0108	0.0156	0.0293	0.0171	0.0349	0.0329	0.057
Base-Rb	0.0128	0.0287	0.0206	0.0441	0.0189	0.0379	0.089	0.0515
Base-Be	1.6668	1.8256	1.5502	1.7769	1.7874	1.9463	1.7223	1.9085
Base-Mg	0.4728	0.5097	0.3691	0.4409	0.3769	0.4454	0.4	0.5074
Base-Ca	0.1005	0.1243	0.083	0.1147	0.092	0.142	0.1053	0.1589
Base-Sr	0.0343	0.0398	0.0352	0.0496	0.0623	0.0734	0.0559	0.0912

monovalent ones. In all of the M^+L systems the measured binding strength which is indirectly related to the interaction energies varies with the metal ion such that Li^+ binds more strongly than Na^+, which in turn binds more strongly than K^+ and so on. Because these complexes are largely electrostatic in nature, this is easily understood on the basis of the size or, equivalently, the charge density on the metal ion. The smaller the ion, the greater will be the strength of the ion-dipole and ion-induced dipole interactions in these systems. In this simplistic point of view, the strength of the interactions of the metal ions with the nucleic acid bases appears to be driven principally by the ion-induced dipole interaction. In general, the relative bond strengths are inversely related to the dipole moments (See Table 2). To some extent, this is because the metal

Table 7. Enthalpy, entropy, free energy variations and stabilization constants for formation processes of the guanine, adenine, cytosine and thymine complexes

	DFT				Experiment			
	$\Delta_r H$ (298 K) / kJ · mol^{-1}	$\Delta_r G$ (298 K) / kJ · mol^{-1}	$\Delta_r S$ (298 K) / kJ · mol^{-1} · K^{-1}	ln K	$\Delta_r H$ (0 K)[a] / kJ · mol^{-1}	$\Delta_r H$ (0 K)[b] / kJ · mol^{-1}	$\Delta_r G$[c] / kJ · mol^{-1}	$\Delta_r H$[c] / kJ · mol^{-1}
Guanine-Li	(−)275.179	(−)244.487	(−)0.1025	98.62	–	–	245	239
Guanine-Na	(−)196.392	(−)163.039	(−)0.1109	65.76	–	–	190	182
Guanine-K	(−)145.755	(−)114.538	(−)0.1046	46.20	–	–	106	117
Guanine-Rb	(−)124.719	(−)95.411	(−)0.0937	38.48				
Guanine-Be	(−)1275.27	(−)1238.94	(−)0.1218	499.75				
Guanine-Mg	(−)770.313	(−)733.176	(−)0.1243	295.74				
Guanine-Ca	(−)524.857	(−)489.755	(−)0.1176	197.55				
Guanine-Sr	(−)453.93	(−)419.749	(−)0.1143	169.33				
Adenine-Li	(−)182.728	(−)151.29	(−)0.1054	61.02	226.1 (6.1)	210 (20)	234	226
Adenine-Na	(−)98.232	(−)67.577	(−)0.1025	27.26	139.6 (4.2)	160 (25)	183	172
Adenine-K	(−)54.647	(−)25.844	(−)0.0965	10.42	95.1 (3.2)	105 (11)	98	106
Adenine-Rb	(−)39.161	(−)11.444	(−)0.0929	4.61				
Adenine-Be	(−)895.99	(−)861.68	(−)0.1147	388.96				
Adenine-Mg	(−)617.256	(−)580.91	(−)0.1218	234.32				
Adenine-Ca	(−)373.493	(−)340.03	(−)0.1088	137.16				
Adenine-Sr	(−)302.754	(−)270.60	(−)0.1076	109.15				
Cytosine-Li	(−)261.998	(−)229.661	(−)0.1084	92.64	–	–	238	232
Cytosine-Na	(−)181.898	(−)152.024	(−)0.1001	61.32	–	–	190	177
Cytosine-K	(−)133.062	(−)106.188	(−)0.0900	42.83	–	–	102	110
Cytosine-Rb	845.147	869.598	(−)0.0795	−350.78				
Cytosine-Be	(−)1079.1	(−)1041.8	(−)0.1251	420.22				
Cytosine-Mg	(−)598.135	(−)562.739	(−)0.1187	226.99				
Cytosine-Ca	(−)472.681	(−)438.885	(−)0.1130	177.03				
Cytosine-Sr	(−)407.28	(−)374.457	(−)0.1096	151.04				
Thymine-Li	769.646	805.335	(−)0.1193	−324.85	210.1 (7.0)	200 (20)	231	215
Thymine-Na	849.351	892.245	(−)0.1436	−359.90	135.3 (3.8)	136 (25)	161	144
Thymine-K	959.593	992.351	(−)0.1096	−400.28	104.0 (3.8)	97 (12)	100	102
Thymine-Rb	988.541	1020.35	(−)0.1063	−411.57				
Thymine-Be	(−)610.43	(−)571.57	(−)0.1302	230.56				
Thymine-Mg	(−)41.644	(−)3.575	(−)0.1272	1.44				
Thymine-Ca	261.905	298.574	(−)0.1226	−120.43				
Thymine-Sr	352.270	388.295	(−)0.1205	−156.62				

[a] TCID from Ref. [11]

[b] Values from Ref. [28] adjusted as described in Ref. [11]

[c] Values from Ref. [28], all literature values taken from NIST Chemistry WebBook (http://webbook.nist.gov). Please note that in the experimental work, the different sign convention is followed and the $\Delta_r H$ values are taken to be positive. For the reason of clarity, calculated data are also represented in the same sign conversion, but one should take the signs given in parentheses into account for complexation reactions. Uncertainties are reported in parentheses and the big deviation from the experimental data in the case of thymine complexes is discussed in the text

ions are not able to bind in sites that allow alignment with the dipole moment.

Charge Transfer

The amount of charge transfer (C_T) between a base and a metal ion is easily determined as the difference between the charge of the isolated ion and the net atomic charge of the metal ion in the complex. C_T values for complexes with different bases are listed in Table 6. C_T values obtained at the DFT level are mostly larger than those obtained at the *HF* level.

Net partial atomic charges for metal ions in guanine complexes are larger than those in adenine complexes and net partial atomic charges for metal ions in cytosine complexes are larger than those in thymine complexes. The metal ion gets a partial atomic charge closer to the charge on the isolated metal ion in all of the four types of complexes with increasing metal ion atomic number in both alkaline and alkali earth metal groups. Charge retained on the metals in these complexes 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. In these systems, the shorter bond distance and greater charge density allow the metal ion to withdraw electron density from the neutral ligand more effectively, thus reducing the charge retained on the metal.

Investigation of Thermodynamic Functions

Harmonic vibration frequencies were obtained by the analytical second derivatives and employed to compute the variation in zero point energies. Calculated thermodynamic data for complexes of groups I and II metal ions with *DNA* bases are tabulated in Table 7. The results for the considered processes were obtained by a thermochemical analysis at 298 K. Available experimental data are also included, which will be discussed in detail in the next part. In adenine and guanine complexes, ΔH values are negative, which indicates that formation of these complexes is exothermic. The increase in the metal ion atomic number in both alkaline and alkali earth metal groups leads to a decrease in the ΔH value for complexation reactions of adenine and guanine. In fact, the reactions get less exothermic due to increasing ionic radii and the resulting steric hindrances in binding with active sites in adenine and guanine. The variation trend in $\Delta_r G$ values for adenine and guanine complexation reactions is similar to that in their $\Delta_r H^0$ values. It is apparent from the complexation reactions that these reactions are accompanied by decreases in entropy, and the negative $\Delta_r S$ values, obtained from frequency calculations, confirm this. ΔH^0 values for formation of guanine complexes are more negative than those for adenine complexes. This can be attributed to the existence of two active sites in guanine complexes and more stability of these complexes. More negative energies for guanine complexes, compared with those for adenine complexes, confirm that guanine complexes are more stable than adenine complexes.

Contrary to other nucleic acid bases, $\Delta_r H$ values for thymine complexation reactions are mostly positive. The only exceptions are for $T\ldots Be^{2+}$ and $T\ldots Mg^{2+}$ complexes, in which $\Delta_r H$ values are negative. In fact, thymine complexation reactions are endothermic in most cases and this is due to the steric hindrances in the single ringed structure of this base because of the increase in the ionic size. Exothermicity of the $T\ldots Be^{2+}$ and $T\ldots Mg^{2+}$ complexes is due to large z/r ratios and small ionic radii of these two ions which face less steric hindrances in binding with the active sites in thymine.

With increasing atomic number of metal ion and decrease in z/r ratio in alkaline metals from top to bottom, $\Delta_r H$ values show an increasing trend, which indicates increased endothermicity of thymine complexation reactions. In the alkali earth metal group with the exception of Be and Mg the increasing trend of ΔH with increasing atomic number is observed. In thymine complexation reactions, the variation in $\Delta_r G^0$ values is similar to variation in $\Delta_r H^0$ values. Negative $\Delta_r S^0$ values for thymine complexation reactions indicate decreases in entropy. As a result, in thymine complexation reactions except for Be and Mg both factors involved in the reaction progress, enthalpy and entropy, are unfavorable, while in complexation reactions with other *DNA* bases except for the $C\ldots Rb^+$ complex the negative $\Delta_r H$ value, *i.e.* exothemicity of reaction compensates for the unfavorable factor of decrease in entropy (negative ΔS values).

Comparison between Theory and Experiment

Threshold collision-induced dissociation TCID of M^+L ($M^+ = Li^+$, Na^+, and K^+; $L =$ uracil, thymine,

and adenine) with xenon was studied using guided ion beam mass spectroscopy [11]. On the other hand, the same alkali metal ion affinities of the common *DNA* and *RNA* nucleobases are determined at the gas phase by investigating the dissociation of metal ion-bound heterodimers [Nucleobase $+ B]M^+$, in which B represents a reference base of known affinity (kinetic method) [28]. From the experimental point of view, with all the three metal ions studied, the affinities increase in the order uracil < thymine < adenine < cytosine < guanine. Such a trend is observed for the interaction energies in the present study (see Table 5). It is noteworthy that the proton affinities for the *DNA* nucleobases rise in the same direction [29, 30], suggesting that a higher basicity also incurs stronger bonding to alkali metal ions. In the MS/MS spectra of [dinucleotide $+$ Li]$^+$ cations, the relative abundances of the [Nucleobase]Li^+ peaks rose in the order $T \ll A < C < G$, presumably because of the increasing nucleobase basicity in this direction [31]; this confirms our results in this research work. In experiment, the dominant process for all complexes is the loss of the intact neutral ligand in the CID reactions:

$$M^+L + \mathrm{Xe} \longrightarrow M^+ + L + \mathrm{Xe}$$

Metal ion affinity (MIA) was assumed to be the enthalpy variation of the above reaction. What is considered in the current study is the formation energy of the B–M^+ bond which is the negative of the enthalpy variation from the dissociation process. The experimental results [11, 28] of metal ion affinity for some of the complexes are shown in Table 7. By considering the temperature differences, the agreement with experimental data becomes more or less satisfactory. One should take this point into consideration that in the case of thymine complexes, contrary to the experimental results, the O^2 atom is considered as an active site instead of the O^4 atom. These discrepancies, which are not that severe, are not completely understood but appear to be a result of several subtle electronic effects that may require higher level of correlation to predict accurately all the data for these systems.

Conclusion

Regarding the net atomic partial charge on each nucleic acid base, it is clear that the most important factors for determining the binding sites for metal ion bindings are their accessibility for binding with the double strands in *DNA* and having less steric hindrances. Considering geometry parameters, the M–N^7 distance for adenine containing complexes is shorter than that in guanine containing complexes. This is due to the existence of a second active site in guanine containing complexes; the M–O^6 distance is mostly shorter than the corresponding M–N^7 distance. All the M–N^1 intermolecular distances for thymine containing complexes are shorter than the M–N^3 bond lengths for thymine containing complexes. This can be related to the fact that although in both complexes of thymine and cytosine the O^2 atom is an active site for metal binding, but the M–O^2 bond lengths in thymine containing complexes are shorter than those in cytosine containing complexes. Two active sites for metal bindings in cytosine containing complexes are the N^3 and O^2 atoms; the only exception to this is related to complexes of cytosine with Be^{2+} and Mg^{2+}. This is due to smaller radii and larger z/r ratios for these ions compared with other ions. All thymine containing complexes have two binding sites, N^1 and O^2, except for complexes with Rb^+.

Interaction energies suggest that stabilization energies for $G \ldots M$ complexes are systematically larger than those for $A \ldots M$ complexes. Stabilization energies for $T \ldots M$ complexes are larger than those for $C \ldots M$ complexes. Stabilization energies for complexes with bivalent metal ions are larger than those with monovalent ones. Interaction energies for guanine containing complexes are larger than those for adenine containing complexes and an increase in the ion atomic number or z/r ratio leads to a decrease in the interaction energy. The increase in the ion atomic number in both alkaline and alkali earth metal groups leads to a decrease in the interaction energy of cytosine containing complexes; even in the case of $C \ldots Rb^+$ complex, interaction energy becomes positive. This can be explained by the largest ionic radius or the smallest z/r ratio of Rb^+. All interaction energies except for $T \ldots Mg^{2+}$ and $T \ldots Be^{2+}$ are positive. This is due to the steric hindrances of the two C=O groups which have resonance with the six-membered ring. In general, C_T values for these four complexes show a decrease with increase in atomic numbers of metal ions in both groups.

From the point of view of thermodynamic properties, all adenine, guanine, and cytosine complexation reactions except for $C \ldots Rb^+$ are exothermic. The situation is completely different in thymine com-

plexation reactions and all except for $T \ldots Be^{2+}$ and $T \ldots Mg^{2+}$ are endothermic. Consequently, two factors of reaction progress, *i.e.* enthalpy and entropy are unsatisfactory, so their reactions aren't spontaneous. But for other complexes of nucleic acid bases except for $C \ldots Rb^{+}$ the negative $\Delta_r H$ value shows that these are spontaneous, so this favorable factor compensates for the negative $\Delta_r S^0$ value, which is an unfavorable factor.

Computational Details

Gas phase calculations were performed using the *HF*, *MP*2 and Density Functional Theory (DFT) levels of theory at the *B3LYP* level. Geometries of the complexes and monomers were obtained using the 6-31G(d,p) basis set for all atoms in the bases and the LAN2DZ basis set for the metal ions. Structures of the complexes were fully optimized by the analytical gradient method using these basis sets in the framework of the *HF* and DFT levels of theory. Single-point energy calculations were carried out on the final optimized structures at the *MP*2 level of theory. Frequency calculations were carried out on the most stable optimized structures of Base $\ldots M^{n+}$ complexes at the *HF* and DFT levels. The character of stationary points was checked by Hessian calculation within the harmonic approximation for every complex. No imaginary vibration frequencies were found. Frequency calculations were just limited to the complexes of M^{n+} with the N^7 atom in adenine, O^6 and N^7 atoms in guanine, N^1 and O^2 (and in some cases O^4) atoms in thymine, and N^3 and O^2 atoms in cytosine. We used the scaling factor of 0.8970 at the *HF* level and 0.9614 at the DFT level (*B3LYP*) [17]. All calculations were performed using the GAUSSIAN 98 package [18]. The structures and atom numberings of the complexes are depicted in Fig. 1.

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