The Influence of Mg^{2+} Ion on the Hydrogen Bonds of the Adenine–Thymine Base Pair

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The influence of Mg^{2+} ion on the hydrogen bonds of the A–T base pair was studied using ab initio *calculations with minimal GLO basis set combined with the Boys–Bernardi counterpoise procedure to eliminate the basis set superposition error. Mg^{2+} binding to the O(2) atom of thymine gives rise to considerable hydrogen bond stabilization of the A–T pair. A possible reason for the 'mispairing' of poly-(I,U) and poly(A) at high Mg^{2+} concentrations is proposed.*

Introduction

Complex formation between metal ions and nucleic acids has been studied intensively. These studies were motivated by the experimental finding that many physiological and biochemical processes are only occurring in the presence of certain monovalent and/or divalent metal ions. These investigations concentrate mainly on four topics, the stoichiometry and energy balance of the reaction, the changes of conformation and structure, the kinetics of the reaction process and the nature of the binding site of the ions. Several reviews pertaining to these topics have been published recently [1–3].

Nucleic acids contain three reactive sites to which metal ions can bind, namely phosphate groups, ribose hydroxyls and heterocyclic bases: adenine, thymine, guanine, cytosine *etc.* Metal ions binding to these sites influence strongly the structure and reactivity of the nucleic acids.

In a previous paper of this series [4] we investigated the influence of Li^+ ion on the hydrogen bonds of the Watson–Crick DNA base pairs, adenine–thymine (A–T) and guanine–cytosine (G–C). The results of these *ab initio* calculations have indicated that the cation can both strengthen and weaken the donor–acceptor interaction of the base pair hydrogen bonds depending mainly on the metal ion binding position. Li^+ binding to N_3 or (N_7··O_6) (see Fig. 1) of guanine leads to a hydrogen bond stabilization

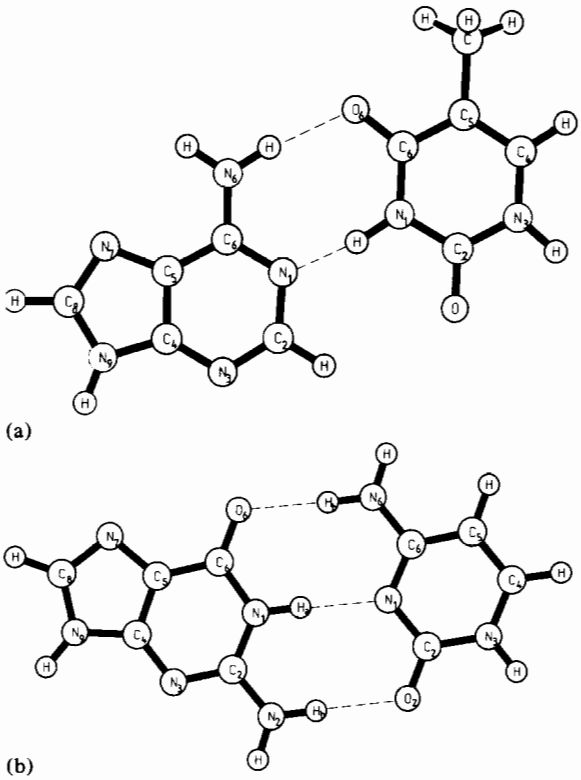

Fig. 1. The Watson–Crick DNA base pairs. a) adenine–thymine; b) guanine–cytosine.

of the G–C pair whereas binding to N_3 or N_7 of adenine decreases the A–T hydrogen bond energy by about 6 or 3 kcal/mol, respectively. By such energies however, rupture of the A–T pair seems to be hardly possible since stacking of successive aromatic bases and backbone conformation of the main chains still play an important role in stabilizing the DNA structure [5].

In continuation of that study, we have investigated the effect of Mg^{2+} ion, a very important divalent metal ion in the biosynthesis of nucleic acids.

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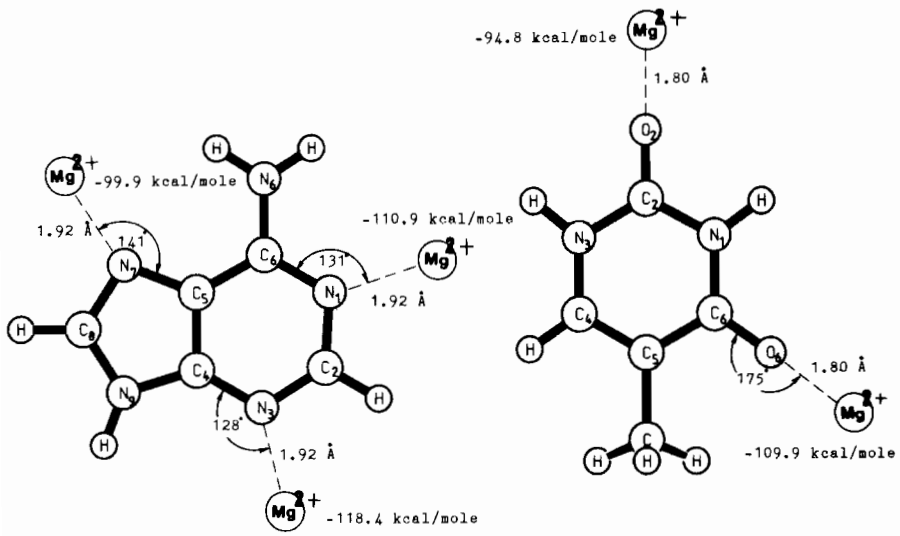

Fig. 2. Molecular structures of adenine and thymine, energy optimized Mg^{2+} coordination geometries and corresponding binding energies obtained by *ab initio* calculations including the counterpoise correction.

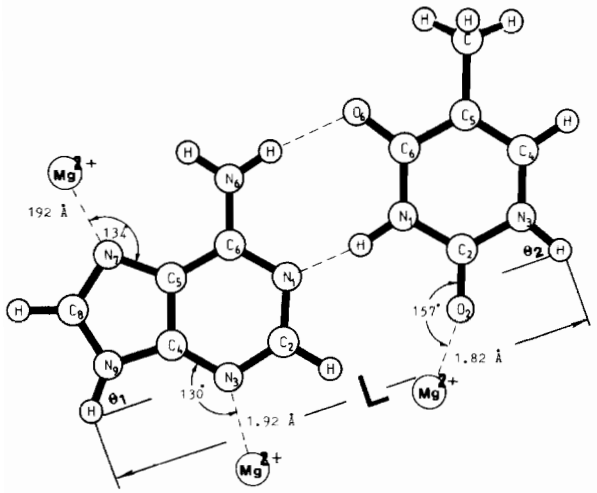

Fig. 3. A–T pair and optimized Mg^{2+} coordination centers.

Method

Ab initio calculations were performed with a minimal GLO basis set using the exponents as given in refs. 6 and 7. This basis set has been used successfully in previous investigations on similar systems [4, 8, 9], and comparison with the results obtained with larger basis sets has shown that relative changes are reflected correctly in all cases.

In order to improve the interaction energy computed with the minimal GLO basis set, the Boys–Bernardi counterpoise procedure [10] was employed in every final energy evaluation. In this procedure, each molecule is computed in the presence of the 'empty' basis set functions of its partner in the system.

The experimental geometries of adenine (A) and thymine (T) were taken from ref. 11 and kept constant throughout the calculations. The molecular structures and numbering scheme used are illustrated in Figs. 1 and 2.

The planar orientation of the A–T pair is described by distance L (see Fig. 3) and by the angles θ_1 and θ_2. These parameters were optimized starting from the experimental data of ref. 12.

All possible in-plane orientations of the cation around A, T and A–T pair were investigated according to the electrostatic molecular potential maps for nucleic acid bases presented by Bonaccorsi *et al.* [13].

All calculations were performed on the CDC Cyber 170-720 computer of the Interuniversity Computer Center at the Technical University of Vienna. The program used is discussed in detail in ref. 14.

Results and Discussion

The energy optimized Mg^{2+} binding positions at the various reactive sites of A, T and the A–T pair are shown in Figs. 2 and 3. All energies reported in Table I are the final results of the *ab initio* calculations, including the Boys–Bernardi counterpoise procedure. The optimized values of L, θ_1 and θ_2 and the hydrogen bond energies of the A–T pair and the Mg^{2+}/A–T complex are given in Table II.

TABLE I. *Ab initio* Energy Values Calculated Including the Counterpoise Procedure. $\hat{C}=\hat{A}$, $\hat{B}$ denotes the corresponding subsystem. SCF($\hat{C}$) denotes the SCF energy of subsystem $\hat{C}$ calculated with the basis set of the whole system, in hartrees. SCF† SCF energy of the system, in hartrees. ΔE interaction energy obtained after the counterpoise correction, in kcal/mol.

System	$\hat{A}$	$\hat{B}$	SCF($\hat{A}$)	SCF($\hat{B}$)	SCF†	ΔE
Mg^{2+}/A:						
Mg^{2+} at N_1	Mg^{2+}	A	−185.6072	−394.1551	−579.9390	−110.9
Mg^{2+} at N_3	Mg^{2+}	A	−185.6072	−394.1548	−579.9507	−118.4
Mg^{2+} at N_7	Mg^{2+}	A	−185.6073	−394.1551	−579.9216	−99.9
Mg^{2+}/T:						
Mg^{2+} at O_2	Mg^{2+}	T	−185.6073	−383.1992	−568.9575	−94.8
Mg^{2+} at O_6	Mg^{2+}	T	−185.6073	−383.1993	−568.9818	−109.9
$Mg^{2+}/A-T$:						
Mg^{2+} at N_3	Mg^{2+}–A	T	−579.9759	−383.2168	−963.2040	−7.1
Mg^{2+} at N_7	Mg^{2+}–A	T	−579.9462	−383.2168	−963.1840	−13.2
Mg^{2+} at O_2	Mg^{2+}–T	A	−568.9766	−394.1676	−963.2045	−37.8

TABLE II. Energy Optimized Values of L, θ_1, θ_2, and Hydrogen Bond Energies of A–T Pair and Mg^{2+}/A–T Complexes. NSE = Net Stabilization Energy. † = data taken from ref. 4.

System	L (Å)	θ_1 (degree)	θ_2 (degree)	Hydrogen Bond Energy (kcal/mol)	NSE (kcal/mol)
A–T†:	10.10	51.0	59.2	15.9	–
$Mg^{2+}/A-T$:					
Mg^{2+} at N_3(A)	9.95	51.0	59.2	7.1	−8.8
Mg^{2+} at N_7(A)	9.95	51.0	59.2	13.2	−2.8
Mg^{2+} at O_2(T)	9.93	51.0	61.2	37.8	+21.9

In order to specify the metal ion influence on the hydrogen bonded system, a 'Net Stabilization Energy' (NSE), which is defined as the difference between the hydrogen bond energies after and before complexing a metal ion, was introduced. The computed NSE values are listed in Table II.

The Magnesium Complexes with Adenine and Thymine

The magnesium–adenine complex

All optimized Mg^{2+}–N bond lengths are equal (1.92 Å). The most favourable Mg^{2+}/adenine binding site is located at N_3 with a binding energy of 118.4 kcal/mol. The binding energies for N_1 and N_7 are 110.9 kcal/mol and 99.9 kcal/mol.

Proton magnetic resonance studies of metal complexation of nucleosides in dimethyl sulfoxide have been reported [15]. It was pointed out in this reference that the binding site of adenosine, which is a nucleoside consisting of adenine base and ribose sugar, is N_7 for group IIa elements. In this case, however, it is likely that N_1 is unavailable because of the hydrogen bond formation, and the existence of ribose sugar at N_9 leads to a steric hindrance to N_3. Apparently, the N_7 position, which is predicted to be the weakest binding site of adenine in ref. 13 and in our study, is dominating.

The magnesium–thymine complex

All computed Mg^{2+}–O distances are identical (1.80 Å). The Mg^{2+}–O_6 and Mg^{2+}–O_2 binding energies are 109.9 and 94.8 kcal/mol, respectively.

D. Perahia *et al.* [16] have studied the interaction of Mg^{2+} ion with uracil (U), which is also a pyrimidine-like structure, using SCF LCAO *ab initio* calculations. The Gaussian orbitals used in this study for U consist of a (7s, 3p/3s) basis contracted to a minimal

basis set and the STO–3G set was employed for the magnesium atom. The energy optimized Mg^{2+} binding positions are similar to ours, the Mg^{2+}–O distance is insignificantly shorter (0.05 Å).

Comparing the results in this section with those of the Li^+/adenine and Li^+/thymine complexes [4], the relative interaction energy orders for Li^+ and Mg^{2+} binding to various reactive sites of A and T are the same. The series found are also in agreement with that predicted by the electrostatic molecular potential-energy maps in the molecular plane for A and T as given in ref. 13. The binding energies of Mg^{2+}/adenine and Mg^{2+}/thymine complexes are about 65 kcal/mol higher than those of Li^+/adenine and Li^+/thymine complexes, respectively.

The Magnesium Complexes with the A–T Pair

In the A–T pair, possible binding positions suitable for Mg^{2+} are N_3 and N_7 of adenine and O_2 of thymine. The optimized Mg^{2+} positions in this case are slightly shifted compared with the Mg^{2+}/adenine and Mg^{2+}/thymine complexes (see Figs. 2 and 3).

Considering the effect of Mg^{2+} ion on the hydrogen bonds, $N_1 \cdot\cdot H–N_1$ seems to experience a more pronounced effect than $N_6–H \cdot\cdot O_6$ for all metal ion binding positions. Thus Mg^{2+} binding to N_3 and N_7 leads to a net destabilization effect (negative NSE value) since the donor–acceptor interaction of the $N_1 \cdot\cdot H–N_1$ hydrogen bond is weakened according to an inductive effect caused by the cation. Apparently, the electron donor or proton acceptor ability of the N_1 atom of A is decreased compared to the free A–T pair. The destabilization effect caused by Mg^{2+} binding at N_3 is more pronounced than that at N_7, since the N_3 atom is closer to the $N_1 \cdot\cdot H–N_1$ bond than N_7. On the other hand, Mg^{2+} binding at O_2 of thymine leads to a considerably higher positive NSE value since the cation in this case can directly enhance the proton donor ability at the N_1 atom of thymine. This hydrogen bond stabilization effect induced by Mg^{2+} ion is similar to that which occurs in the Li^+/formamide-water complex, where Li^+ ion stabilizes the $N–H \cdot\cdot O$ hydrogen bond through the O=C–N backbone [9].

In all cases, small changes in L, θ_1 and θ_2 compared with the free A–T pair are found (see Table II). These small changes may not be significant in the real DNA double helix molecule since the structure of the main chains should be rigid enough to prevent such small deformations.

It has been pointed out that the ability of divalent metal ions to influence the interaction between polynucleotide strands, as in a double helix, can bring about the mispairing of nucleotide bases [1]. This happens in general at high metal ion concentrations. The mispairing of polynucleotides, such as poly(inosine(I), uridine(U)) and poly(A) at high Mg^{2+} ion concentrations has been demonstrated experimentally [17]. At low Mg^{2+} concentrations, only the complementary A–U pairs are formed, but at high Mg^{2+} concentrations, A–I as well as A–U base pairs are produced. Considering the base of inosine (hypoxanthine or deaminoguanine), possible metal binding positions are N_3, N_7 and O_6. But only N_3 and N_7 are available since O_6 is employed in the hydrogen bond formation. At high Mg^{2+} ion concentrations, Mg^{2+} may bind, besides the phosphate groups, to N_3 or N_7 positions. This interaction should induce N_1 of hypoxanthine to become a better proton donor, thus also permitting A–I pair formation.

Acknowledgement

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