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Investigations of Zn(II) Complexes with DNA/RNA Bases by Means of Quantum Chemical Calculations

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Abstract

The interaction of Zn^{2+} ions with the five purine and pyrimidine bases of DNA and RNA, *i.e.*, adenine (Ade), cytosine (Cyt), guanine (Gua), thymine (Thy) and uracil (Ura) has been studied using *ab initio* SCF-HF computations with minimal basis set. Energy optimized structures of the possible complexes of the metal ion with the bases and the corresponding interaction energies have been determined. Zn^{2+} coordinating simultaneously to the carbonyl oxygen and to one nitrogen in guanine or cytosine gives rise to the highest values of binding energy, followed by the nitrogen sites of adenine and guanine. The least favoured complexes are those involving binding of Zn^{2+} to oxygen sites of thymine and uracil. One exception in this series of relative interaction energies is the position O4 in thymine. Most of the resulting geometrical features are similar to those found in previous studies on complexes of the bases with alkali and alkaline earth metal ions.

The electronic structure of the coordinated ligands was analysed to obtain a picture of the H-bonding ability between the molecules in the Watson–Crick base pairs A=T and G=C in the presence of zinc ions. The results show that for Zn^{2+} bound to guanine via N3 and N7, the H-bonds in G=C are stabilized and also when Zn^{2+} is coordinated to O2 of thymine (uracil) the H-bonds in A=T(U) are stabilized, whereas for N3 coordination in adenine the A=T(U) base pair is destabilized. For N7 coordination in adenine, it has been assumed that the coordination does not significantly change the interaction between Ade and Thy (Ura).

Introduction

The binding of cations to biomolecules such as nucleic acid constituents has been studied intensively, in solution and by means of X-ray crystallography, since the importance of various metal ions in biochemical processes such as DNA replication, transcription and translation is well known.

In 1968 Shin and Eichhorn [1] showed that in the presence of zinc ions it is possible to unwind and rewind double-helical DNA reversibly by heating and cooling respectively, and they postulated that this metal ion possesses a balanced affinity to both the bases and to the oxygen of the phosphodiester group during this process. Numerous reports on solution studies [2–7] and crystal structures [8–14] of zinc complexes with DNA constituents have been published so far. They have clarified several aspects of the coordination mode of the metal ion, but a detailed understanding of the ion base interactions involved in the rewinding/unwinding process and the ion binding sites on these bases is not yet available [8]. Nevertheless, models for this reversible 'melting' have been proposed [8, 11].

We therefore have extended our previous calculations on cation/base interactions for Li^{1+}, Na^{1+}, Mg^{2+} and Ca^{2+} [15–17] to the zinc ion. This cation is also very important in enzymes of nucleic acid metabolism, including replication [8, 18]. The aim of this work was first to find out the relative stabilities of the various possible zinc complexes of the most common purine and pyrimidine bases when there are no distortions and perturbations by other ligands and solvent molecules present. Furthermore, we wanted to examine the electronic rearrangements in the bases due to zinc binding so as to obtain a picture of the resulting H-bonding abilities between the Watson–Crick base pairs A=T and G=C in the presence of the transition metal ion.

Only a few *ab initio* calculations on DNA bases interacting with metal ions have been published besides our previous investigations [19–21], and only one of them [19] deals with zinc(II), using a pseudopotential basis set. Not all DNA and RNA bases and not all possible binding sites have been considered in that work, so we decided to study the zinc complexes and to use, for methodical comparison, an all electron basis set.

Method

The chemical systems treated here are considered to be large from the view-point of *ab initio* SCF-HF methods. Therefore we had to use a minimal basis set

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[22–24] which has been shown to be applicable to an evaluation of intermolecular parameters and relative energy effects [25], so long as basis set superposition error corrections [26] are employed. The standard geometries used for the bases were the same as in ref. 15; for uracil the geometry was taken from ref. 27. The molecular structures were kept constant throughout the calculations, as it is known that the geometries of the bases do not change significantly while interacting with the metal ions [13].

The positions of the Zn^{2+} ion in the fields of the five bases were selected only in the molecular plane according to the molecular potential maps for the purine and pyrimidine bases [28]. The coordination geometries were optimized taking the binding sites that had resulted from earlier computations [15]: N1, N3 and N7 of adenine; N3, N7 and O6 of guanine; N3 and O2 of cytosine; and O2 and O4 of thymine or uracil (see Fig. 1). The distances of zinc from the bases and the angles between the metal and the ligand were optimized with a step width of 0.01 Å and 1° respectively, and for the resulting geometry the interaction energy was corrected by the counterpoise method (C.P.) [26].

Finally the differences in partial charge (zinc coordinated ligand minus free ligand) of each atom involved in the base pairs as an H-donor or H-acceptor were calculated using the population analysis reported by Mulliken [29]. As these partial charges are proportional to the coulombic term of the H-bond energy [30], which is known to be the important part of the total H-bond interaction [31], estimates of the effect of Zn^{2+} coordination on the A=T and G=C pairs can be performed easily.

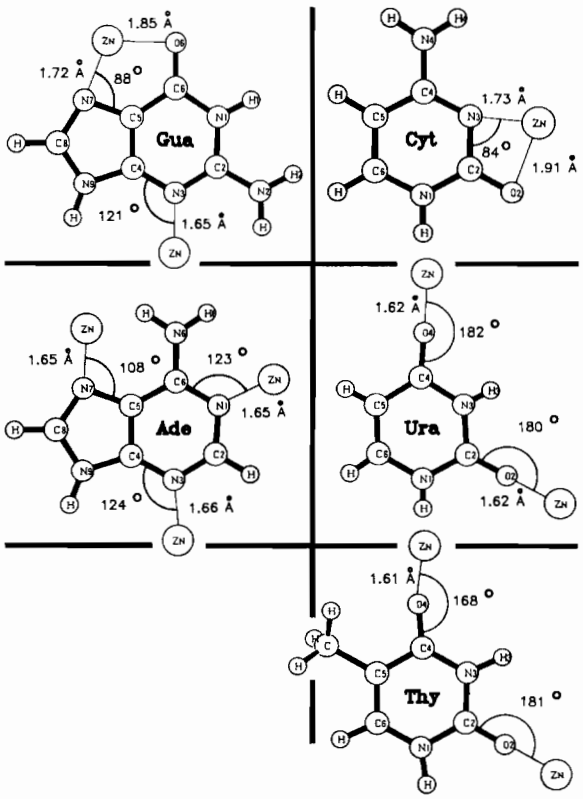

Fig. 1. Structures and numbering schemes of the Zn^{2+}–nucleic acid base complexes.

The calculations were performed partly on the CDC CYBER 170-120 and 170-130 of the Interuniversity Computer Center at the Technical University of Vienna, and partly on the CDC CYBER 180-830 of the University of Innsbruck, using the program of Ahlrichs, Lischka and Staemmler [32]. The calculation of one geometry point of the zinc–adenine complex took an average of 7500 s of CPU time on a CDC 180-830. In total, about 130 points had to be evaluated for this work.

Results and Discussion

The energy optimized Zn^{2+} binding positions at the various reactive sites of Gua, Ade, Cyt, Ura and Thy are shown in Fig. 1. The corresponding interaction energies are reported in Table I.

It is obvious that all resulting distances between Zn^{2+} and the electron donor atoms of the bases in the range between 1.6 and 1.9 Å are unusually small, since the normal bond lengths found in crystal structures are around 2.1 Å. A few test calculations elucidated the role of the basis set used on this result. One error source could be removed by using the C.P. correction [26] throughout the whole optimization of bond lengths and not only for the final configuration. Optimization of the distance at the Thy O2 and Ade N1 sites in this modified way is shown in Table I. An elongation of the bond of about 0.1 Å for both types of coordination results. Another reason for the distances being too short is the fact that free cations, not surrounded by solvent molecules, are being considered. By this the metal–ligand bond is shortened by about 0.2 Å because of the low coordination number (*cf.* Zn^{2+}–O distances calculated by using the basis set of this work: Zn^{2+}–(H_2O) = 1.67 Å, Zn^{2+}–$(H_2O)_6$ = 1.85 Å (ref. 33)). In order to avoid unreasonable consumption of computing time, the small distances were not corrected, as the mentioned effects should be quite constant and only relative binding energies of the complexes were of interest.

Adenine Complexes

The optimized bond distances were found to be almost identical for all three possible positions, *i.e.*, 1.65 Å for N1 and N7 and 1.66 Å for the N3 site. For the binding angle at N7 a remarkable value of 108° was found, while in our previous computations on alkali and earth alkaline ions this angle was always

TABLE I. Energy Optimized Zinc Ion Coordination Geometries and Corresponding Interaction Energies of Zn^{2+}–Nucleic Acid Base Complexes

Position at base	Distance (Å)	Angle (deg)	C.P.-corrected interaction energy[a] (kJ/mol)
Adenine			
N(1)	1.65	123	−837
C.P.corr.[b]	1.79	123	−894
N(3)	1.66	124	−899
N(7)	1.65	108	−744
N(7)/N(6) chelate	1.75 N(7) 1.80 N(6)	89	−605
Guanine			
N(3)	1.65	121	−805
N(7)/O(6) chelate	1.72 N(7) 1.85 O(6)	88	−1095
Uracil			
O(2)	1.62	180	−643
O(4)	1.62	182	−703
Thymine			
O(2)	1.61	181	−712
C.P.corr.[b]	1.72	180	−753
O(4)	1.61	168	−790
Cytosine			
N(3)/O(2) chelate	1.73 N(3) 1.91 O(2)	86	−1014

[a]Interaction energy calculated using the counterpoise (C.P.) correction (see text). [b]Bond length calculated using the counterpoise correction during the whole optimization of zinc–base distance.

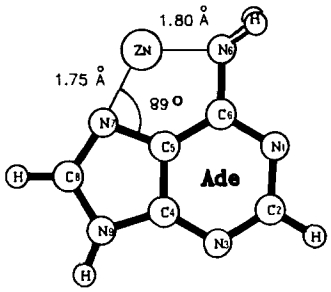

Fig. 2. Structure of the Zn^{2+}–adenine chelate complex with the 6-amino group rotated 90° around the C–N bond in a pyramidal configuration.

near to 140°. In analogy to the work of ref. 19, we tried to optimize further the interaction energy for this position by rotating the 6-amino group by 90°, as shown in Fig. 2. The resulting N7–N6 chelate position that has become possible upon this rotation is (in contrast to the results of ref. 19) less stable (−605 kJ/mol) than the single binding to N7 (−744 kJ/mol). Our calculation for the rotation energy of the amino group (472 kJ/mol) proves that due to the partly double bond character of the C6–N6 bond [5] the loss of energy is too high to be compensated for by the gain through chelate binding of the zinc ion.

The remaining two binding sites in adenine possess higher binding abilities than the N7 position; N1 with an interaction energy of −837 kJ/mol and N3 with the highest energy of all imino centres, namely −899 kJ/mol.

Guanine Complexes

The distance for Zn^{2+}–N3 is the same as those found in the three possible complexes of adenine. There is no special attraction towards the 2-amino group similar to that found for adenine, because the angle is of the same unperturbed magnitude (121°) as observed in the corresponding adenine complex. This different strength of interaction of the zinc(II) ion with the two amino groups in Ade and Gua was also observed in NMR experiments [7].

The binding at the N7 site occurs in form of a chelate complexation because Zn^{2+} is also near to the carbonyl oxygen O6. Therefore the interaction energy of the N7–O6 position is much higher (−1095 kJ/mol) than the energy found for the N3 position (−805 kJ/mol).

Uracil Complexes

The resulting bond lengths for Zn^{2+}–O (1.62 Å) are (as expected) shorter than the ones obtained for nitrogen sites, showing that the relative trends are reflected correctly, even if the absolute values for bond lengths are not very satisfactory.

No significant deviation from the ideal C4–O4–Zn^{2+} angle of 180° could be found in this work, as can be seen by inspection of Table II. In ref. 19 a value of 190° resulted, because the authors used too large a scale of step width (10°) for the optimization of the angle. Our results therefore show that it is quite important to refine the coordination geometry

TABLE II. Various Zinc Ion Coordination Geometries and Corresponding Interaction Energies of Uracil Complexes with Zn^{2+} Bound to Oxygen O4[a]

Distance (Å)	Angle (deg)	Interaction energy (kJ/mol)
1.62	179	−984.7
1.62	182	−985.0
1.62	185	−984.7
1.62	187	−983.8
1.62	190	−983.4
1.62	193	−982.1

[a]Energies not C.P. corrected.

fairly exactly, so as not to obtain misleading values of the coordination angle.

The calculated binding energies lead to the weakest interaction found between Zn^{2+} and a nucleic acid base: −703 kJ/mol for the O4 and 643 kJ/mol for the O2 position. This result fits well with NMR experiments [7] in solution where, in contrast to other nucleosides, no complexes of Zn^{2+} with uridine have been observed.

Thymine Complexes

The bond lengths for Zn^{2+}–O are almost identical to that in the uracil complexes. A difference lies in the repulsion of the Zn^{2+} at O4 by the 5-methyl group of thymine with a resulting Zn^{2+}–O4–C4 angle of 168° instead of 182°.

For the binding energies, somewhat higher values than in uracil are found and position O2 is slightly less stable (−712 kJ/mol) than the O4 site (−790 kJ/mol).

Cytosine Complex

The final geometry of the cytosine complex shows a bridge position for the cation between N3 and O2, similar to the situation at N7–O6 in guanine. The distance for Zn^{2+}–O2 is 1.91 Å and for Zn^{2+}–N3 1.73 Å, in contrast to the findings of ref. 19 where the shorter bond is reported for Zn^{2+}–O. As far as a large bond distance can be associated with a weaker interaction, the resulting longer Zn^{2+}–O bond reflects correctly the weaker coordination of zinc to O2 also found in crystal structures of complexes reviewed recently [9, 34].

In the hard–soft acid–base (HSAB) context of Pearson it is well known that Zn^{2+} is a soft Lewis acid on the borderline between typical hard and typical soft metal ions and therefore it prefers binding to nitrogen rather than binding to oxygen. We assume that the result of ref. 19 could be due to the use of a pseudopotential for the core and consideration of valence electrons only for Zn^{2+}, as similar discrepancies are well known for ion–ligand geometries in semi-empirical methods, which neglect the inner electrons.

The stabilization energy for this chelate position is quite high (−1014 kJ/mol) and comparable to the energy for the N7–O6 site in guanine.

In a comparison of all ten binding sites it is possible to assign a relative stability order of the complexes (see also Fig. 3):

Gua (N7–O6) > Cyt (N3–O2) > Ade (N3)
> Ade (N1) > Gua (N3) > Thy (O4) > Ade (N7)
> Thy (O2) > Ura (O4) > Ura (O2)
> Ade (N7–N6)

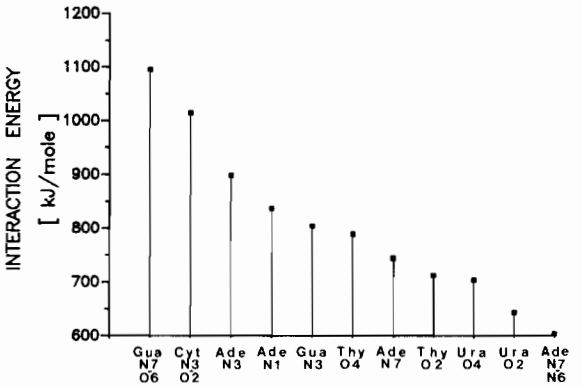

Fig. 3. Relative interaction energies of the ten possible Zn^{2+}–nucleic acid base complexes.

For comparison we also give the relative order of ref. 19:

Gua (N7–O6) > Cyt (N3–O2) > Ade (N7–N6)
> Ura (O4) > Ade (N1) > Ura (O2) > Ade (N7)

The especially high interaction energies of zinc at the chelate positions in guanine and cytosine are common to both series. The possible reason for the discrepancy in the energy for the Ade (N7–N6) position has been discussed previously. The N3 position of adenine and guanine and the two thymine positions have not been considered in Pullman's study [19] and are therefore not given. The reason for the exchange of the Ade N1 and Ura O4 positions in the series of ref. 19 may be also a result of the pseudopotential basis set overestimating the zinc/oxygen compared to the zinc/nitrogen interaction, as also observed in the zinc–cytosine complex.

Upon base pairing of adenine with thymine (uracil) and guanine with cytosine, the metal ion positions Ade (N1), Thy (O4), Ura (O4), Ade (N7–N6) and Cyt (N3–O2) are occupied by hydrogen bonds (Fig. 4). In the case where no H-bond is broken these five positions are excluded from metal coordination, and the following stability order will be significant:

Gua (N7–O6) > Ade (N3) > Gua (N3) > Ade (N7)
> Thy (O2) > Ura (O2)

The H-Bonds of the Watson–Crick Base Pairs

As pointed out before, the formation of the two base pairs (Fig. 4) excludes four zinc binding sites that are possible with the free bases. Because we were interested in estimates for the H-bond energy of the intact base pairs, we did not include these four sites in the following considerations. Therefore cytosine is not discussed because there is no favoured position for zinc left when it is paired with guanine.

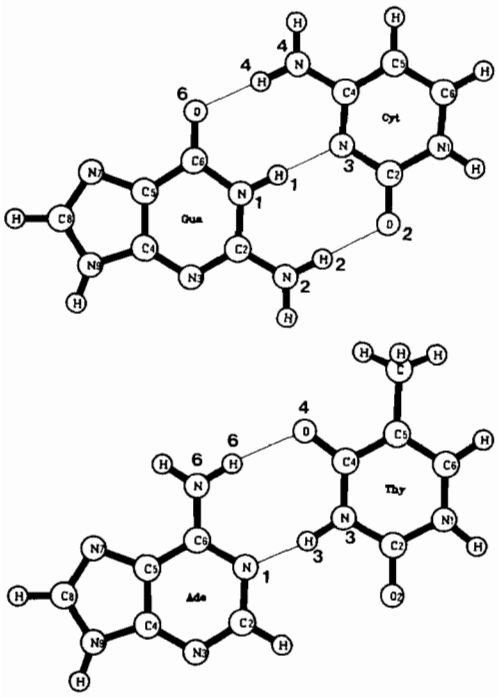

Fig. 4. Structure of the Watson–Crick base pairs (thymine and uracil are exchangeable bases).

TABLE III. Differences in Partial Charge of Atoms Involved in Hydrogen Bonds in the Base Pairs A=T(U) and G=C

Nucleic acid base	Position of zinc ion	H-Bonding atom in base	Partial charge change (me)[a]
Guanine	N(3)	H(1)	−85
		N(1)	−43
		H(2)	−112
		N(2)	−27
		O(6)	−167
	N(7)/O(6) chelate	H(1)	−77
		N(1)	−41
		H(2)	−59
		N(2)	−50
		O(6)	+97
Adenine	N(3)	N(1)	−145
		H(6)	−66
		N(6)	−85
	N(7)	N(1)	−122
		H(6)	−131
		N(6)	−106
Thymine	O(2)	H(3)	−27
		N(3)	−60
		O(4)	−149
Uracil	O(2)	H(3)	−27
		N(3)	−61
		O(4)	−153

[a]Values given in millielectrons, [10^{-3} e].

Table III shows the differences in the partial charges between the coordinated and the free ligands in electron charge units (10^{-3} e). A negative sign corresponds to a loss of electron density and therefore the atom is more positive in the complex than in the free ligand. For increasing the strength of an H-bond it is desirable to have the proton and the H-donor more positive and the H-acceptor more negatively charged. The results of Table III can be summarized as follows.

Zn^{2+} at N3 of guanine increases the donor ability of both N2–H2 and N1–H1, but on the other hand decreases the acceptor ability of O6, which will weaken the O6··H4–N4 hydrogen bond. Since only one of the three existing H-bonds in G=C is disfavoured, an overall stabilization of the base pair is likely to occur.

Zn^{2+} at N7–O6 of guanine increases the positive charge at N2, H2, N1 and H1, as well as the negative charge at O6 and consequently an enhanced H-bond energy will be the result.

Zn^{2+} at N3 of adenine induces a positive charge at atoms N6 and H6 but also at N1 (which is an H-acceptor site). Therefore it is likely that it destabilizes the H-bond according to earlier studies, where the leading role of effects along the N1··H3–N3 bond has been pointed out [16, 17].

Zn^{2+} at N7 of adenine induces a more positive charge at the positions N6 and H6, but actually withdraws not as much negative charge from N1 as in the N3 case. So it has to be assumed that the H-bond energy is not decreased as before but remains about the same as in the free base pair.

For Zn^{2+} binding to O2 of thymine (the following also applies to uracil), the H-donor N3 together with H3 is becoming more positive as well as the O4 acceptor atom. The fact that the values for the N3, H3 and O4 atoms are comparable to the N6, H6 and N1 values in the Zn^{2+}–N3 case of adenine would predict a destabilization of the hydrogen bonds. Studies on the Li^+ and Mg^{2+} complexes of the A=T pair have shown that a stabilization of the H-bonds in A=T results [16, 17], although these metal ions produce relative charge differences on N3, H3 and O4 quite similar to the Zn^{2+} ion [35]. So for this complex the simple electrostatic considerations of hydrogen bonding seem to fail in predicting the interaction energy of A=T correctly. One possible explanation for this limitation seems to be the previously mentioned relative importance of the N3–H3··N1 bond in relation to the O4··H6–N6 bond. As the H3 atom of the pyrimidine ring in thymine is more acidic (charge deficiency of −475 me [millielectron]) than the exocyclic amino proton H6 in adenine (charge deficiency of −409 me) and

the hydrogen acceptor N1 possesses more excess of negative charge (+650 me) than the carbonyl O4 does (+237 me), differences in electron density along the N3–H3··N1 hydrogen bond experienced by a metal ion should play a more important role in changing the total interaction energy in A=T pairs (values in parenthesis taken from ref. 35). Together with this additional assumption, it is possible to give an explanation within simple coulombic considerations, that destabilization of the A=T pair occurs upon metal ion binding to Ade N3, and that stabilization upon metal ion binding to Thy O2 occurs. It is probable therefore that in the case of zinc coordination to the O2 site of thymine or uracil the A=T(U) pair is stabilized.

Because of the limitations in the discussion of H-bonding ability in terms of simple electrostatic considerations, a study of the complexes of zinc with the Watson–Crick base pairs is in preparation, which should allow an examination of the applicability of these simple models [36].

The estimates of the last section can be summarized qualitatively: in the G=C base pairs an increase of the H-bond energy upon zinc coordination should always take place, while in the A=T(U) pairs effects in both directions will occur, depending on the position of the coordinating zinc ion.

Combining the relative stabilities of the complexes with the findings for the effects of zinc ions on the energy of the hydrogen bonds in A=T(U) and G=C, the following model can be postulated. Starting with a small 'concentration' of zinc ions, that preferentially bind to the chelate position of guanine, a stabilization of the G=C bonds will result. By further increasing the amount of metal ion the zinc will bind to Cyt (N3–O2), which is the site with the second highest interaction energy, thus disrupting the H-bonds between Gua and Cyt. For A=T(U) pairs, zinc will bind first to Ade N3 causing a slight destabilization of the pair and, at higher concentrations, to Ade N1, disrupting the A=T(U) hydrogen bonds.

This concentration-dependent stabilization or breaking of H-bonds could provide a possible model for the findings of DNA melting experiments [37], namely that at low concentrations Zn^{2+} stabilizes the double strand structure of DNA, whereas at higher concentrations unwinding of the double strand is facilitated. Besides a stabilization effect because of neutralizing some negatively charged oxygens of the phosphodiester backbone of DNA, the preferential Zn^{2+}-binding to Gua (N7–O6) should increase the interaction by hydrogen bonds for G=C pairs. The observed destabilization at higher concentrations could be explained by zinc binding to Cyt (N3–O2) and Ade N1, which apparently causes disruption of both base pairs.

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