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Cation-Binding to Biomolecules

II. An *ab initio* Study on the Interaction of Mg^{2+} with Uracil

David Perahia, Alberte Pullman and Bernard Pullman

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au C.N.R.S., 13 rue P. et M. Curie, F-75005 Paris, France

Ab initio SCF computations indicate that Mg^{2+} should bind essentially to the oxygen atoms of uracil, the remaining part of the base being rather repulsive towards such an interaction. The Coulombic component predominates in the interaction, the essential feature of which may thus be deduced from the study of the molecular electrostatic potential of uracil. These *ab initio* results contradict an earlier CNDO prediction that the binding of uracil and Mg^{2+} should occur preferentially at the $C_5{=}C_6$ double bond of the base. It is shown that the CNDO result is an artifact due to an exaggeration by this method of the charge transfer between the ligand and the cation. The small amount of available experimental data seem in favor of the *ab initio* results.

Key words: Uracil, interaction of Mg^{2+} with ~

1. Introduction

The first paper of this series [1] has reported an *ab initio* SCF investigation of the binding of Na^+ and Mg^{2+} to the dimethylphosphate anion, representing the phosphodiester linkage of nucleic acids and of the phospholipid components of membranes. As a continuation of the study on cation binding to nucleic acids and their constituents we now present an investigation on the binding of Mg^{2+} to uracil. The choice of this particular system was dictated by the existence of a similar study performed by the CNDO/2 method [2] the results of which seemed to us *a priori* extremely surprising and representing possibly, we felt, a methodological artifact. The CNDO/2 procedure predicts that the preferred binding site should be in the vicinity of the $C_5{=}C_6$ double bond of the uracil ring. Although there is no direct experimental evidence on the system investigated, this result seemed sufficiently at variance with the predictions on the attraction for a positive charge obtained through the construction of the electrostatic molecular potential for the related thymine ring system [3] and with the available information about the binding of cations to the biological pyrimidine bases (*vide infra*) to warrant a deeper exploration of the subject.

2. Procedure

The computations have been performed within the SCF LCAO *ab initio* procedure using Gaussian basis functions. The Gaussian orbitals adopted for the uracil moiety consist of a (*7s*, *3p*/*3s*) basis contracted to a minimal set with the exponents and contraction coefficients utilized by Clementi *et al.* [4] in their computation of the DNA bases. This choice has the obvious advantage of making comparisons possible. The basis set used for Mg^{2+}

was derived [5] from the STO-3G basis given for the magnesium atom [6] by reoptimization of the 1*s*, 2*sp* and 3*sp* exponents with respect to the energy of the decay, and suppression of the 3*p* empty orbitals. It has been shown elsewhere by a detailed analysis of the components of the binding energies of alkali and alkaline-earth ions to water [5] that the use of this type of basis for the cation together with the (7*s*, 3*p*/3*s*) set on the ligand yields reasonable values of both the binding energies and the distances of approach: thus, the interaction energy between water and Mg^{2+} so obtained is –76.1 kcal/mole for an equilibrium $O \ldots Mg^{2+}$ distance of 1.87–1.88 Å on the bisectrix of the HOH angle, with a flat energy region between 1.85 and 1.90 Å. No experimental value is available for comparison, but the fact that the result compares very favorably with that of Kollman *et al.* [7] who obtained an interaction energy of –80 kcal/mole for a distance of 1.95 Å using a much more elaborate basis set is an indication of its validity for the kind of investigation that we wish to perform here.

In order to facilitate the comparison with the CNDO results the geometry adopted here for uracil is that used by Nanda *et al.* [2].

3. Results and Discussion

3.1. Interaction Energies

Fig. 1 indicates the *ab initio* binding energies of Mg^{2+} to uracil in a series of significant positions numbered 1 to 7. (More detailed information on the binding energies is given in Table 1 which will be discussed later.) The most stable binding site, indicated by 1, is situated in the vicinity of O_4 on an axis making an angle of about 10° with the C_4–O_4 bond, in the plane of the base on the side of C_5. Three distances between O_4 and Mg^{2+} have been explored in this direction, namely 1.75, 1.85 and 2 Å; the most stable arrange-

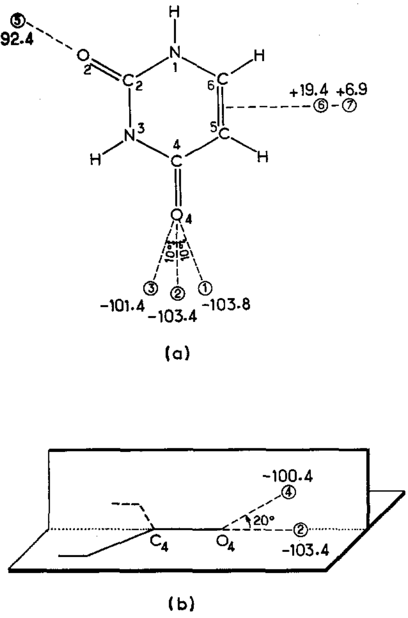

Fig. 1. *Ab initio* interaction energies (kcal/mole) of Mg^{2+} with uracil. The positions labelled 1 to 5 in the circles correspond to a distance of 2.25 and 2.60 Å respectively from Mg^{2+} to the middle of the C_5=C_6 bond

Table 1. Binding energies of Mg^{2+} to uracil

Sites of Cation Binding[a]	Ligand-Cation Distance (Å)	Energy of cation binding (kcal/mole)					
			Ab initio			CNDO	
		Total	Coulombic	Exchange	Polarization + Charge Transfer	Without *d* Qrbitals on Mg^{2+}	With *d* Orbitals on Mg^{2+}
1	O_4–Mg^{2+} = 2.00	− 95.51	−63.63	8.69	−40.57	−147.95	−181.34
	O_4–Mg^{2+} = 1.85	−102.63	−73.98	20.20	−48.85		
	O_4–Mg^{2+} = 1.75	−103.81	−82.98	34.41	−55.24		
2	O_4–Mg^{2+} = 1.75	−103.39	−82.30	34.12	−55.21		
3	O_4–Mg^{2+} = 1.75	−101.44	−81.19	34.42	−54.67		
4	O_4–Mg^{2+} = 1.75	−100.45	−78.76	33.68	−55.37		
5	O_2–Mg^{2+} = 1.75	− 92.41	−75.91	33.95	−50.45		
6	M–Mg^{2+} = 2.25	+ 19.44	32.10	28.67	−41.23	−209.91	−221.59
7	M–Mg^{2+} = 2.60	+ 6.91	29.10	7.80	−29.99		

[a] The numbering is indicated in Fig. 1. M is the middle of C_5–C_6.

ment corresponding approximately to the distance 1.75 Å, this same distance has been adopted for the computation of other positions around O_4 and O_2 [1].

The region around O_4 seems altogether to exert the strongest attraction towards the cation. The binding energy decreases however when the cation turns around O_4 towards N_3. An out-of-plane position of the cation in this neighborhood has also been explored which is symbolized by 4 (Fig. 1b). In this position the cation is situated in the plane passing through the C_4-O_4 bond and perpendicular to the plane of the base, with the direction Mg^{2+}-O_4 making an angle of 160° with C_4-O_4. The binding energy in this position is 3.4 kcal/mole less than in position **1**. Thus, with a single uracil molecule the out-of-plane positioning of Mg^{2+} is somewhat less favored than the inplane positioning; however, the energy difference is small enough to allow the occurrence of an out-of-plane binding in favorable circumstances, for instance if other cation-binding groups are available in the proximity as it may occur in the helical structures of nucleic acids and their constituents.

The binding of Mg^{2+} to O_2 (site **5**) is less favorable than the binding to O_4 by 11.4 kcal/mole.

Finally, two sites along the axis bisecting the $C_5{=}C_6$ bond have been explored (sites **6** and **7**). At a distance of 2.25 Å from the middle point M of $C_5{=}C_6$ the total energy of the complex is 123.2 kcal/mole *above* that at most stable site 1 and corresponds, in fact, as seen in Table 1 and Fig. 1, to a repulsive interaction between the base and the cation. The value changes to 110.7 kcal/mole for a distance of 2.6 Å. The positions in the proximity of the $C_5{=}C_6$ double bond are thus unfavorable for the binding of cations.

We have indicated in Table 1 the values of the binding energies of Mg^{2+} to uracil for all the positions studied together with their components (Coulombic, exchange, polarization + charge transfer) computed by the procedure of Dreyfus and Pullman [8] in order to obtain further insight into the main features of the interaction. A further separation of the delocalization energy into its polarization and charge transfer components was not performed in this case since it is well established that the contribution of charge transfer is relatively small for the alkali [9–12] and alkaline-earth [5] ions (we shall come back to this point in section 3.3). Thus the delocalization energy of Table 1 represents essentially the polarization contribution which appears to be appreciable. However, the dominant part of the binding component of the energy is the Coulomb attraction, the variation of which directs the variations in the interaction energies. Similarly it is the (repulsive) Coulomb energy which is responsible for the instability of site **6**. In this site, the attractive delocalization component is unable to overcome the joint destabilizing effect of the Coulomb and exchange contributions.

Finally, a noteworthy feature of the results is the fact that binding of Mg^{2+} to uracil (both to O_4 and O_2) appears more favorable than its binding to water, thus indicating the possibility of at least partial dehydration of the cation for binding with the oxygens of the base.

There does not seem to be any direct experimental verification on the binding scheme of Mg^{2+} to uracil. In fact very few experimental results are available about the interactions of alkali or alkaline-earth cations with uracil or with other bases of the nucleic acids. The majority of the experimental results refer to more complex nucleotide or polynucleotide systems in which the interaction of such cations with the phosphate group is the dominant

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[1] In fact the equilibrium distance is 1.77–1.78 Å, roughly 0.1 Å shorter than in Mg^{2+}–H_2O. A further refinement in the neighborhood of the minimum at O_2 (position 5) yielded the values −91.0, −92.4 and −92.5 kcal/mole for 1.70, 1.75 and 1.80 Å respectively.

one. However, some information may be found in the available X-ray crystal structure data on compounds containing uracil (or thymine) complexed with different cations. Thus, the crystal structure of the dinucleoside monophosphate ApU complexed with Na^+ indicates that one of the two cations in the unit cell is bound to two O_2 oxygens belonging to different uracils [13]. The O_4 oxygens are engaged in hydrogen bonds with the adenine moieties and their binding capacity towards cations is thus probably reduced. The crystal structure of the dinucleotide pTpT complexed with Na^+ [14] indicates that this cation is bound to O_2 of thymine. In this crystal the O_4 carbonyl oxygens are engaged in contact with water and with the free 3'-OH group. The crystal structures of complexes of uracil-mercuric chloride [15] and of 5'UMP-Ba^{2+} [16] show that the cations are bound respectively to O_2 and O_4 of the base. There are no experimental indications of any binding of cations to the $C_5{=}C_6$ double bond.

Under these circumstances, and in view of the *ab initio* results indicating a repulsion for Mg^{2+} in the $C_5{=}C_6$ region, we have recomputed the CNDO binding energies in this region using the parameters of Ref. [2], both with and without the inclusion of *d* functions on the ion. The results for positions 1 and 6 are given in Table 1 for the distances indicated. In sharp contrast with those of the *ab initio* computation, they indicate a large stabilization energy for the fixation of Mg^{2+} in the $C_5{=}C_6$ region. This is clearly due to an artifact of CNDO/2 which will be discussed in the following sections.

3.2. The Electrostatic Potential of Uracil

The predominance of the Coulombic component in the interaction energies between uracil and Mg^{2+} suggests that the examination of the electrostatic isopotential map of uracil should provide sufficient indication for the preference of this molecule towards the binding of the cation.

The *ab initio* electrostatic isopotential map of uracil in the plane of the base is given in Fig. 2. This map is very similar to that reported for thymine [3]. Two regions of attraction for a positive charge are situated around the oxygen atoms, the rest of the molecule being repulsive. The minimum potential energy area near O_4 is deeper than that near O_2; the global minimum around O_4 is displaced toward C_5.

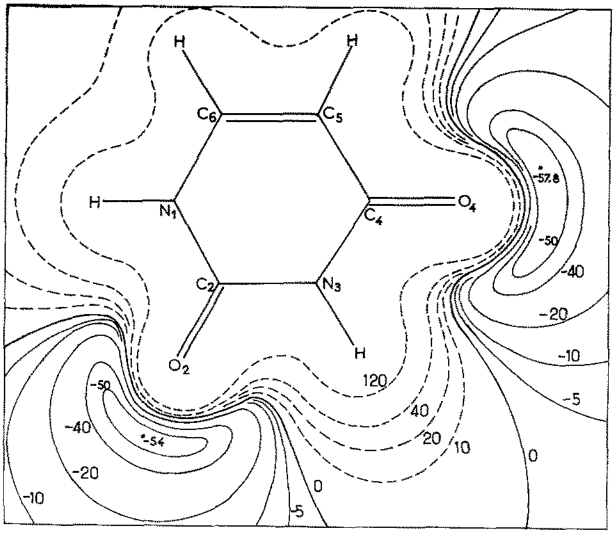

Fig. 2. *Ab initio* electrostatic molecular potential for uracil in the plane of the ring

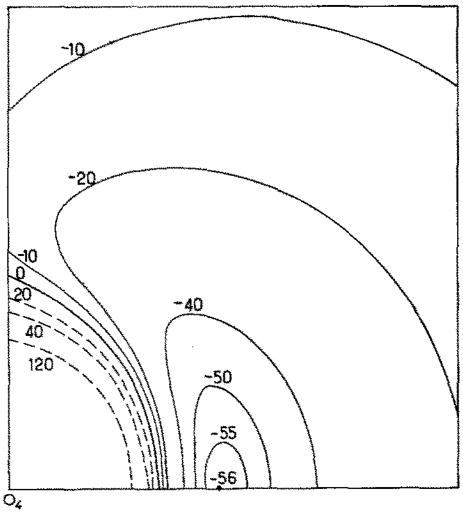

Fig. 3. *Ab initio* electrostatic molecular potential of uracil in the plane perpendicular to the ring and passing through C_4–O_4

The isopotential curves in the perpendicular plane passing through C_4–O_4 is given in Fig. 3. This map, in conjunction with that of Fig. 2, indicates that the minimum of the potential is situated in the plane of the ring. It may also be seen, by comparing these two maps, that the electrostatic potential energy decreases more rapidly in the perpendicular plane than in the plane of the ring. The potential is positive (repulsive) in the vicinity of the C_5=C_6 double bond.

The shape of these isopotential maps, as well as the magnitudes of the potentials, reflect very well the results found in the preceding section concerning the binding preferences of Mg^{2+}.

In order to trace the origin of the artifact inherent in the CNDO result for Mg^{2+} binding to uracil, the CNDO electrostatic isopotential curves of uracil in the plane of the ring have also been evaluated and are given in Fig. 4. (In order to be significant in the energy de-

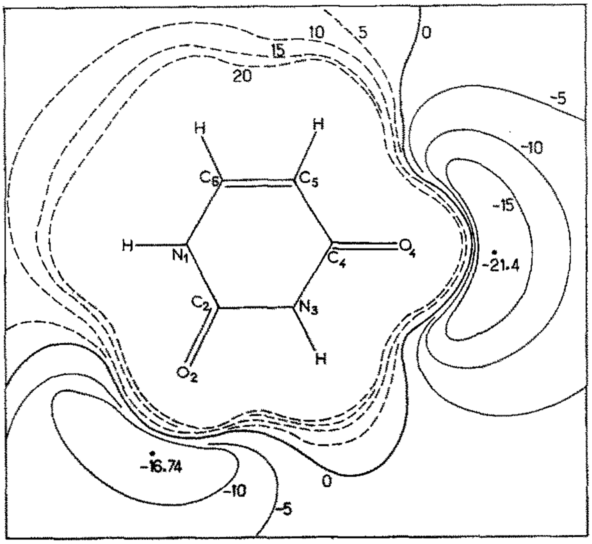

Fig. 4. CNDO electrostatic molecular potential of uracil in the plane of the ring

Table 2. Atomic net charges of the uracil . . . Mg^{2+} adduct for different positions of the cation[a] (in electron units)

	Separated ligands		Uracil complexed with Mg^{2+} placed in the different positions[b]							
			Ab initio				CNDO[c]			
Atoms	*Ab initio*	CNDO	1	3	5	6	1	6	1	6
N1	−0.473	−0.188	−0.456	−0.457	−0.458	−0.442	−0.162	−0.160	−0.161	−0.160
H1	0.369	0.137	0.433	0.433	0.387	0.435	0.203	0.211	0.205	0.215
C2	0.498	0.449	0.513	0.513	0.572	0.510	0.455	0.452	0.455	0.452
O2	−0.398	−0.377	−0.317	−0.320	−0.750	−0.299	−0.274	−0.244	−0.271	−0.240
N3	−0.483	−0.236	−0.466	−0.468	−0.463	−0.478	−0.206	−0.229	−0.203	−0.229
H3	0.367	0.141	0.392	0.373	0.384	0.429	0.177	0.218	0.180	0.220
C4	0.357	0.369	0.434	0.435	0.364	0.387	0.462	0.376	0.463	0.374
O4	−0.360	−0.353	−0.737	−0.736	−0.279	−0.271	−0.389	−0.219	−0.359	−0.212
C5	−0.293	−0.157	−0.334	−0.327	−0.251	−0.284	−0.189	−0.094	−0.187	−0.077
H5	0.226	0.038	0.230	0.249	0.296	0.019	0.067	0.078	0.070	0.082
C6	−0.056	0.174	0.013	0.010	−0.073	−0.041	0.256	0.225	0.258	0.234
H6	0.245	0.002	0.322	0.323	0.299	0.034	0.067	0.034	0.069	0.038
Mg^{++}	2.000	2.000	1.972	1.972	1.973	2.000	1.532	1.349	1.480	1.304

[a] The distances considered are O–Mg^{2+} = 1.75 Å (*ab initio*) and 2 Å (CNDO), and M–Mg^{2+} = 2.25 Å.

[b] The numbering at the head of each column corresponds to that of Fig. 1.

[c] First two columns: *d* orbitals not included; last two columns: *d* orbitals included.

composition the γ_{ss} approximation has been used [17-18]). The shape of these isopotential curves is similar to that given by the *ab initio* computations, although the magnitude of the CNDO potentials is much lower than that of the *ab initio* ones due to the γ_{ss} approximation of the nuclear attraction integrals [18]. It is observed that the CNDO potential in the region of the $C_5{=}C_6$ double bond is positive. The Coulombic energy component of the interaction energy of Mg^{2+} with uracil in the proximity of $C_5{=}C_6$ must therefore also be positive in the CNDO computations. The strong attraction for Mg^{2+} found in the CNDO computation must accordingly be due to a highly negative value of the delocalization energy. A further insight into this problem may be obtained by the analysis of the electronic charge distribution.

3.3. Electronic Charges

The electronic charges of uracil alone and of the uracil-Mg^{2+} adduct for different positions of the cation as obtained by the *ab initio* and the CNDO calculations are presented in Table 2. The charges obtained in the *ab initio* computations indicate that a small charge transfer of about 28 millielectrons occurs towards Mg^{2+} when the cation is bound to an oxygen atom (positions **1**, **3** and **5**). There is no charge transfer towards Mg^{2+} when the cation is situated in front of the $C_5{=}C_6$ double bond (position **6**). These results are in contrast with those obtained by the CNDO computations which indicate a very large charge transfer from uracil towards Mg^{2+} for any position of the cation. Moreover, the charge transfer is larger in this method when the cation is facing the $C_5{=}C_6$ double bond (position **6**) than when it is bound to an oxygen atom (position **1**) contrary to the *ab initio* result. This occurs independently of whether the empty *d* orbitals are or are not included in the basis set of Mg^{2+}, although the transfer is larger when the *d* orbitals are included in the computations, as might be expected.

It is thus clear that the difference between the *ab initio* and CNDO predictions concerning the most stable position of Mg^{+} comes from the incorrect weighting of the electrostatic, polarization, and charge-transfer components of the binding energy intrinsic in the CNDO approximation [10, 19], and particularly to the overweighting of the charge-transfer effect. In the case of alkali and alkaline-earth ions where this effect is in fact very small, this may result in inversions of relative stabilities in different positions. For instance, an out-of-plane position is erroneously found as the most stable for binding alkali ions to ester and amide carbonyls [11]; the present case is another example of such an inversion.

It may be interesting to note, in Table 2, the nature of the polarization effect of Mg^{2+} on uracil. The *ab initio* electronic charges of the oxygens to which the cation is bound (O_4 for the positions **1** and **3**, O_2 for position **5**) become highly negative compared to the charges of the respective atoms in uracil alone. On the other hand, all the hydrogens of the base become more positive upon Mg^{2+}-binding. Thus hydrogens behave as donors of electrons in the overall polarization process of uracil under the influence of Mg^{2+}. So also do the heavy atoms situated away from Mg^{2+}.

4. Conclusions

The main conclusions which can be drawn from this study consist of the following observations: (1) The *ab initio* computations show that Mg^{2+} binds essentially to the oxygen atoms of uracil, the remaining portions of the ring being highly repulsive. (2) These results can be derived from the examination of the electrostatic isopotential map of the base, a situation which is due to the predominance of the Coulombic energy component in the interaction energy between uracil and Mg^{2+}. (3) The analysis of the *ab initio* electronic charge distributions in uracil and in the uracil-Mg^{2+} adducts shows that there is a very small charge transfer from uracil towards Mg^{2+} whereas the polarization of the ligand by the cation is important. (4) The comparison of results obtained by the CNDO/2 and *ab initio* computations indicates once more that the former method is inappropriate for the study of ligand-cation interaction. The CNDO/2 method erroneously indicates that the preferred binding site for Mg^{2+} is facing the $C_5{=}C_6$ double bond of uracil. This failure appears to be due to a very strong overestimation of the charge transfer from the ligand to the cation in the CNDO procedure.

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