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Theoretical Study of Guanine from Gas Phase to Aqueous Solution: Role of Tautomerism and Its Implications in Absorption and Emission Spectra

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Correlated ab initio methods have been exploited to study geometries and electronic structures of ground and lowest singlet excited states of isolated and solvated keto−amino tautomers of guanine. Nuclear structures have been obtained at the CIS level, whereas calculations of electronic structures have been performed using a multireference perturbed CI method. In all calculations, the solvent (water) has been described within the integral equation formalism continuum model. The results have been successfully compared to experimental absorption and emission spectra. In particular, the analysis of the two computed spectra has shown an opposite role of the different tautomers (being absorption mostly because of 9NH form and the emission induced by 7NH) which has lead us to investigate possible mechanisms giving origin to the keto−9NH and keto−7NH tautomerism in the ground or in the electronically excited states.

1. Introduction

This contribution is the second paper in a series of publications aimed at the description of the photophysics of nucleic acid bases in solution through ab initio methods. The study of the effect of the environment is a rather unexplored field, as most of the theoretical papers that have appeared so far in the literature focus exclusively, or almost exclusively, on isolated molecules. On the contrary, the largest amount of experimental data refers to solvated systems and, in particular, to aqueous solutions.[1]

The first paper[2] in this series was a study on isolated and solvated adenine, its electronic absorption and emission spectra, the importance of tautomerism, the individuation of possible deactivation mechanisms leading to its very low emission, and the comparison with its highly fluorescent isomer, 2-aminopurine. Here, we shall present a parallel study on guanine in the gas phase and in water. Like adenine, also guanine can occur in more than one tautomeric form; in principle, all possible forms should be included in the study; however, previous calculations[3] have shown that, both in vacuo and in solution, two keto−amino forms (N9H and N7H) have a similar stability which is sufficiently higher than those of all of the other possible forms. Following such findings, in the present study, only the two keto tautomers will be considered; attention will be also focused on possible mechanisms leading to their tautomerism, either in the ground or in an electronically excited state.

Both the previous study on adenine and the present one on guanine follow the same basic idea and the related computational strategy. These have been formulated on the basis of very general considerations: solvent effects on structures, energies, and properties of molecular solutes are impossible to be exactly modeled by computational methods and some, even strong, approximations have thus to be introduced. Many alternative models have been proposed, each accounting only for some aspects of the whole effect; among them, large success has been obtained by the so-called continuum approach. According to this approach, solute−solvent interactions are represented in terms of mean forces exerted by an infinite macroscopic continuum medium characterized by suitable properties. The molecular, or microscopic, description is thus limited to the solute subsystem which can be either a single molecule (the dissolved molecule under examination) or the same molecule plus few solvent molecules (i.e., a supermolecule). In the second case, the additional microscopic solvent molecules are exploited to introduce in the calculations more specific effects (generally H bonds) not, or not completely, taken into account in terms of an external continuum effect only.

To study nucleic acid bases in water solution, it is very reasonable to guess that specific (in particular H bonds) effects are important and that continuum models are likely not sufficient if not applied in their extended formulation, i.e., that including a supermolecular solute (here the nuclei acid basis plus some H-bonded water molecules). As a consequence, many previous studies on solvated bases have introduced mono- and dihydrated complexes (obtained through ab initio geometry optimization procedures), eventually surrounded by an external continuum model. Such studies have more often considered electronically ground states only, and focused on structural and energetic aspects of the problem (see ref 4 just to quote the most recent one focusing on guanine).

The correct analysis of the role of H bonding in the study of solvent effects is indeed a very difficult task; our previous experience, however, seems to suggest that H bonding cannot be described in terms of a single unifying definition, but on the contrary, its effects significantly change passing from one aspect to another, still remaining focused on the same system and/or the same phenomenon. In particular, in previous studies[5,6] on the photophysics of solvated molecules, we have found that such effects are not compulsory to get a proper description of solvent-induced modifications on UV−vis spectra and that the limits shown by some continuum models have to be imputed more to their strongly approximated formulation than to the neglect of specific effects as those related to H bonds. Following this idea, we have began a series of studies on the photophysical properties of the bases of nucleic acids when immersed in water solution

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by exploiting a method which couples correlated ab initio methods to describe solute electronic states and an accurate continuum solvation model to take into account solvent effects.

Correlated quantum chemical calculations will be used to investigate both the geometry of ground and excited electronic states (DFT and CIS) and the spectroscopic properties (perturbation-MRCI) of the two tautomers in gas phase and in water solution; for the latter, the revised version of the polarizable continuum model (PCM)[7] which is known with the acronym IEF (integral equation formalism)[8] will be exploited.

2. Computational Details

Solvation. In the IEF solvation model,[8] the solute is assumed inside a molecular cavity (with proper shape and dimension) immersed in a continuum dielectric (the solvent) which can be polarized. Solvent polarization induces an electric field (the "reaction field") which interacts with the solute modifying its state. The solute−solvent interactions included in the standard IEF version are of electrostatic nature only, and they are represented in terms of an apparent charge density spreading on the cavity surface which is then discretized in terms of pointlike charges placed on small patches covering the whole surface. These charges give rise to specific operators to be added to the Hamiltonian of the isolated system to obtain the final effective Hamiltonian and the related Schrödinger equation. Solvent terms depend both on the molecular cavity and on the solvent dielectric constant (ϵ) but also on the solute wave function they contribute to modify, and thus, the problem requires the solution of a proper self-consistent scheme.

This is the general approach in which solute electronic and nuclear charge distribution and solvent reaction field can mutually equilibrate; however, in vertical electronic transitions (both absorptions and emissions), the relaxation of the reaction field in the direction of the new solute electronic state may be incomplete in the sense that only the fast part of the solvent reaction which is induced by the polarization of its electrons can immediately modify according to the new solute electronic state, whereas all of the rest remain frozen in the original equilibrium condition. In the framework of IEF−PCM, this scheme is realized introducing two sets of apparent charges representing the electronic (or fast) and the slow contributions of the solvent reaction, respectively (see the next section for more details). In a reasonable approximation, the fast charges can be taken proportional to the dielectric constant at infinite frequency ϵ_∞, where $\epsilon_\infty \approx n^2$ and n is the refractive index of the solvent. For water, the two values used for the dielectric constant are 78.39 and 1.776, for ϵ and ϵ_∞, respectively.

In the IEF version used in this paper, to the solvent electrostatic reaction described above, additional repulsive interactions between solute and solvent are added. For the latter, we have used the model originally formulated by Amovilli and Mennucci.[9]

For both electrostatic and repulsive interactions, we exploit a molecular cavity obtained in terms of interlocking spheres centered on selected nuclei (here the heavy atoms plus the hydrogens bonded to nitrogen). The chosen radii are 1.9 for the aromatic carbons bonded to an hydrogen atom, 1.7 for C bonded to amino nitrogen, 1.6 for all N, 1.5 for oxygen, and 1.2 for hydrogens bonded to N. All of the radii are multiplied by 1.2 in order to take into account the impenetrable core of the solvent molecules.[10]

Geometries. The geometry optimizations for ground and excited states both in vacuo and in water solution were performed with a development version of the Gaussian program package.[11] The ground states were obtained at the density functional theory (DFT) using the hybrid functional which mixes the Lee, Yang, and Parr functional for the correlation part and the Becke's three-parameter functional for the exchange (B3LYP).[12] The basis set used was the correlation consistent valence double-ζ (cc-pVDZ) developed by Dunning and coworkers.[13] To evaluate excited state geometries, a configuration interaction approach including only single excited configurations (CIS), was used. The basis set was still that used for ground state DFT calculation. Also for CIS optimizations, as for the DFT ones, the solvent was analytically included in both the energy and the gradient step of calculation.[14,15]

Spectra and Transition Properties. To describe the ground and excited states energies and properties, the multireference perturbation configuration interaction method, known with the CIPSI acronym,[16] was used with the same basis set exploited to get geometries. In this algorithm, at each molecular geometry, both in solvent and in vacuo, configurations can be selected[17] in such a way to define a variational subspace of electronic configurations giving a balanced description of all of the electronic states of interest. For the isolated system, two iterations are used (the first to get the natural orbitals used in the second one) and each of them is constituted by three perturbative selections. The resulting variational wave functions, expanded over up to about 10 000 selected determinants, were the basis for a diagrammatic quasidegenerate perturbation theory treatment,[16,18] with a Møller−Plesset (MP) partition of the Hamiltonian.

For solvated systems, the CIPSI algorithm is more complex because of the dependency of the solvent-induced terms on the solute wave function. The strategy we have chosen to take into account such dependency is to repeat the CI scheme and the following diagonalization of the modified Hamiltoniian and evaluation of the density matrix, so that at each step, a new set of solvent charges computed from the previous density matrix are calculated and used to evaluate the current matrices. The procedure is repeated until convergency on the final energy is obtained. The molecular orbitals defining the CI basis are renewed at each step of the iteration in terms of the CI natural orbitals; in this way, in fact, solute wave function and solvent charges can completely readjust in a self-consistent scheme. An important feature of this procedure is that all CI matrices, including the off-diagonal elements, are explicitly modified by solute−solvent interactions.

Concerning the perturbative part of the algorithm, the basic equations are still valid also for solvated systems, but they all require a redefinition of the involved quantities. The first difference is in the definition of the unperturbed part of the Hamiltonian which, for solvated systems, includes also solvent operators computed on the final zero-order wave function. The same considerations apply to the Fock operator exploited in the MPB partition.

CIPSI calculations were carried out interfacing the original algorithm, modified as described in ref 5 so as to take into account the solvent, to a development version of GAMESS package.[19]

3. Results and Discussions

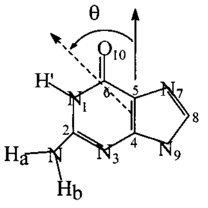
3.1. Ground State Geometries and Properties. The most important geometrical parameters for the ground state of the two keto tautomers of guanine in vacuo and in water are collected in Table 1 where the numbers used to label atoms are

TABLE 1: B3LYP/cc-pVDZ Geometrical Parameters of 9NH and 7NH Tautomers of Guanine in Vacuo and in Solution[a]

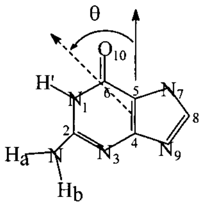
	9NH		7NH	
	vac	water	vac	water
N1−C2	1.371	1.376	1.382	1.381
N1−C6	1.440	1.420	1.420	1.412
C2−N3	1.314	1.323	1.306	1.316
N3−C4	1.360	1.355	1.369	1.366
C4−C5	1.398	1.399	1.398	1.399
C5−C6	1.444	1.436	1.430	1.428
C5−N7	1.383	1.387	1.379	1.381
N7−C8	1.310	1.315	1.365	1.358
C8−N9	1.386	1.381	1.323	1.330
C4−N9	1.371	1.370	1.375	1.376
C6−O	1.217	1.230	1.225	1.232
C2−N	1.380	1.363	1.385	1.369
N1−C2−N3	123.7	123.3	124.6	123.7
C2−N3−C4	112.3	112.7	114.1	114.1
N3−C4−C5	129.4	128.7	124.3	124.9
C4−C5−C6	118.6	118.6	122.9	122.0
C5−C6−O	131.5	129.7	130.0	129.5
C4−C5−N7	111.0	110.9	105.6	105.6
C5−C4−N9	104.9	104.8	110.4	110.2
C5−N7−C8	104.5	104.3	106.1	106.3
N7−C8−N9	112.8	112.9	113.5	113.3
H_aNC2N1	36.2	25.8	43.1	31.2
H_bNC2N3	−14.3	−16.2	−12.5	−16.8

[a] Bond lengths are in angstroms, and angles are in degrees.

reported in the following scheme. All of the geometries have been obtained at the B3LYP/cc-pVDZ level of calculation without imposing any symmetry.



9NH



7NH

Because of the lack of experimental data on geometries of guanine either in gas phase or in solution, the only possible comparison on the geometrical parameters reported in Table 1 is with previous calculations. However, also in this limit, the available data refer only to isolated systems. To the best of our knowledge, there are no previous calculations including solvent effects in the geometry of any of the tautomers; all of the studies taking into some account the environment in fact completely neglect this aspect both for the ground and the electronically excited states.

Remaining in the case of isolated molecules, the agreement with previous calculations exploiting different approaches (we mainly refer to MP2 data[20]) is sufficiently good. The important aspect to note is that our calculations correctly reproduce both the nonplanarity of the amino group, because of the partial sp^3 hybridization of the related nitrogen, and the high asymmetry of such pyramidalization, because of the repulsion between the H′ ring hydrogen atom and the adjacent amino group hydrogen atom, H_a (see scheme above).[21] These two aspects are quantified in Table 1 in terms of the two dihedral angles H_aNC2N1 and H_bNC2N3.

For both molecules, the inclusion of solvent effects leads to some differences in the value of all bond distances and bond angles, with the main changes found in the pyramidalization of amino group. For both tautomers, including solvent effects leads to a net decrease (around 10°) of the H_aNC2N1 dihedral angle and a small increase of the H_bNC2N3 angle. The reduction of the first angle seems to show that solvent reduces repulsion effects acting between H′ and H_a hydrogen atoms: this is a reasonable result if we consider that the solvent can induce a shielding effect which partially reduces the direct repulsion interaction between the two atoms.

TABLE 2: Dipole Moments (D) and Relative Energy Difference (kcal/mol) between 9NH and 7NH Tautomers of Guanine in Vacuo and in Water Solution

	vac	water
	9NH	
μ	6.448	8.820
	7NH	
Δ	−1.60	+0.67
μ	2.154	2.923

On these geometries, we have computed CIPSI dipole moments and relative energies for the two tautomers in vacuo and in solution; these are reported in Table 2.

As shown in Table 2 the dipole moments of both solvated tautomers increase with respect to the gas phase by ∼2 and ∼1 D on 9NH and 7NH, respectively. These different effects of solvation on the two tautomers is reflected in the change in their relative energies passing from gas-phase to solution: 7NH tautomer is energetically favored in vacuo, whereas the opposite happens in water; in both phases, however, the computed differences are rather small, and quantitative analyses are thus not possible.

No experimental data are available for dipole moments (either in vacuo or in solution), whereas from photoelectron spectral investigations, it seems that 7NH is the predominant form in gas-phase,[22] on the contrary, in the polar solutions, guanine is mainly found as 9NH.[23] Our computed results agree with both these experimental findings. A further confirmation of the larger stability of the 9NH form in water solution is given by calculations on mono- and dihydrated complexes of the two forms of guanine;[24] this agreement also supports our original assumption on the validity of our continuum solvation model to describe this kind of system.

3.2. Absorption. The extension of the IEF solvation model to electronically excited states requires some refinements. In treating solutes in their ground states, one can always reduce the analysis to a completely equilibrated solute−solvent system. On the contrary, when the attention is shifted toward dynamical phenomena as those involved in electronically transitions (absorptions and/or emissions), one has to introduce new models, in which solute and solvent have proper response times which have not to be coherent or at least not before very long times.

It is convenient to introduce a partition of the sources of the dynamical behavior of the medium into three main components.[25] One is represented by the internal molecular motions inside the solvent (rotations and translations with time scales of the order of $10^{-9}-10^{-11}$ s), the second collects the internal geometrical variations of the single solvent molecules, i.e., their vibrations, described by specific shorter time scales ($10^{-14}-10^{-12}$ s), and the third, which can be considered as complementary to the first two (also collectively indicated as ‘nuclear’), is that induced by motions of the electrons inside each solvent molecule; these motions are extremely fast (of the order of $10^{-16}-10^{-15}$ s), and they represent the electronic polarization of the solvent. These “nuclear” and electronic components, because of their different dynamic behavior, will give rise to different effects. In particular, the electronic motions can be considered as instantaneous, and thus, the part of the solvent response they originate is always equilibrated to any change, even if fast, in the charge distribution of the solute. On the contrary, solvent nuclear motions, by far slower, can be delayed with respect to fast changes, and thus, they can give origin to solute−solvent systems not completely equilibrated in the time interval interested in the phenomenon under study. This condition of nonequilibrium will successively evolve toward a more stable and completely equilibrated state in a time interval which will depend on the specific system under scrutiny.

When we apply this scheme to vertical electronic transitions (absorptions and emissions), we can safely assume a Franck−Condon like response of the solvent, exactly as for the solute molecule; the nuclear motions inside and among the solvent molecules will not be able to immediately follow the fast changes in the solute electronic charge distribution, and thus, the corresponding part of the response will remain frozen in the state immediately previous to the transition.

In the framework of the PCM solvation model, this will lead to a partition of the apparent charges in two separate sets,[26] one related to the instantaneous electronic response, q_{fast}, and the other to the slower response connected to the nuclear motions, q_{slow} (their sum is just the already defined total apparent charge q). According to what said before about the origin of the apparent charges, the electronic components will depend on the instantaneous wave function of the solute, on the optical dielectric constant (ϵ_∞), and on the complementary set of slower apparent charges. The latter, on the contrary, will still depend on the solute wave function of the initial state. Explicit expressions of the involved quantities within the IEF formalism can be found in ref 27.

The main consequence of the scheme outlined above when applied to the CIPSI algorithm is that each electronic state (ground and excited) requires a separated calculation involving an iteration procedure optimized on the specific state of interest; in each iteration, the natural orbitals defining the CI basis and the density matrix used to evaluate the solvent-induced component of the effective Hamiltonian are those corresponding to the specific state under scrutiny so that in the following step both the solute wave function and the solvent charges better describe the real system. In particular, for the nonequilibrium scheme described above, a two-step calculations is required; first we have to perform a ground state calculation from which the slow apparent charges are obtained and saved in a file for the successive calculation on the excited state. The latter is performed exactly in the same way as it is for the ground state but with an interaction potential composed of three components: the fixed slow charges of the previous calculation, the current nuclear charges, and the wave function dependent electron-induced fast charges.

In Table 3, we summarize the computed and the experimental, where available, excitation energies, oscillator strengths, and transition moment directions for the two tautomers of guanine in gas-phase and in solution (for the definition of the angle θ identifying transition moment directions see scheme above where we have used the Devoe−Tinoco convention). All of the computed results have been obtained using the B3LYP/cc-pVDZ geometries reported in Table 1.

Experimentally, two excited states are observed at low energies for the solvated system; they are clearly separated and positioned at 4.5 and 5.0 eV,[28] respectively. To the best of our knowledge, no clear data are available for the isolated system. From data of oscillator strengths, a $\pi \rightarrow \pi^*$ character has been assigned to both bands. Concerning polarization assignments based on experiments on 9-ethylguanine crystal, no ambiguity exists for the long-axis (−75°) assignment for the second band, whereas different data have been given for the first band: these results lead to the conclusion that the band is polarized at −4° or +35°.[1]

TABLE 3: Summary of Experimental and Present Theoretical Values (CIPSI) for the Excitation Energies (eV) and Transition Properties of Guanine Isomers in Vacuo and in Water

	CIPSI		exp
	vac	water	
		9NH	
$\pi\pi^*$	4.76	4.41	4.51[a]
f	0.135	0.164	0.16[b]
θ	−17	−30	−4[b]/+35
$\pi\pi^*$	5.64	4.94	4.96/5.04[a]
f	0.184	0.349	0.25[b]
θ	−74	−75	−75[b]
		7NH	
$\pi\pi^*$	4.94	4.36	
f	0.155	0.166	
θ	2	−2	
$\pi\pi^*$	5.55	4.99	
f	0.157	0.115	
θ	−84	−99	

[a] In water solution: ref 28. [b] 9-Ethylguanine: ref 32.

When we compare these experimental data with computed results, it comes out clear that inclusion of solvent effects largely improves the agreement between calculations and measurements. For both bands, solvent effects significantly reduce the corresponding transition energies (of 0.3 and 0.6 eV, respectively), and this leads to an important improvement in the agreement with observed values (reducing the differences always less than 0.1 eV). No significant differences are found for the two tautomers, which show very similar transition energies; some discrepancies appear, on the contrary, in oscillator strength values and in the directions of transition moments. Globally, the computed results for the solvated system show that the 9NH form better agrees with observed data confirming what previously predicted through energetic considerations on the preferential stabilization of this form when the molecule is immersed in water solution.

All of these results show an important aspect: previous calculations including solvent effects found very small solvent-induced shifts.[29,30] The reasons for these discrepancies with respect to our calculations can be different; in some cases, the exploited solvation model is too approximated, for example, it uses a simple spherical cavity and, even more importantly, it accounts for solute−solvent dipole-like interactions only (here, in fact, ground and excited states present very similar dipole moments, see next section, and thus, no preferential stabilization of one with respect to the other, with following shift in the transition energy, can be found in this dipole approximation). In other, more recent, calculations,[31] on the contrary, more accurate solvation models have been introduced and, in fact, more appreciable solvent-induced modifications are found; however, they are still significantly smaller than ours. In these cases, the most probable explanation is that solvent effects are included in the calculations only in a incomplete way (for example, they have been neglected for the ground state geometry); in addition, it is not completely clear if nonequilibrium effects, as those described at the beginning of the section, have been included in the calculation of excitation energies.

3.3. Excited States: Geometries and Fluorescences. To compute fluorescence energies, a preliminary search of excited-

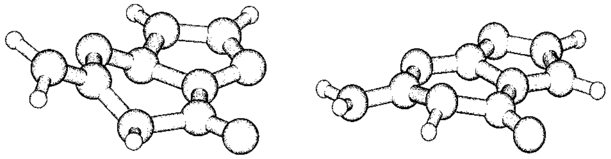
TABLE 4: Selection of Geometrical Parameters for the Lowest Singlet Excited State of 9NH and 7NH Guanine as Predicted with CIS/cc-pVDZ in Vacuo and in Water[a]

	9NH		7NH	
	vac	water	vac	water
N1−C2	1.399	1.388	1.397	1.383
N1−C6	1.432	1.438	1.452	1.438
C2−N3	1.395	1.325	1.335	1.322
N3−C4	1.288	1.324	1.310	1.324
C4−C5	1.432	1.466	1.446	1.461
C5−C6	1.457	1.418	1.418	1.406
C5−N7	1.357	1.355	1.356	1.349
N7−C8	1.296	1.311	1.390	1.405
C8−N9	1.361	1.338	1.282	1.291
C4−N9	1.377	1.405	1.373	1.356
C6−O	1.188	1.216	1.205	1.225
C2−N	1.373	1.338	1.361	1.333
N1−C2−N3	118.4	122.8	123.8	125.3
C2−N3−C4	108.9	114.5	114.3	116.1
N3−C4−C5	125.0	120.4	120.7	121.7
C4−C5−C6	119.3	118.6	122.6	121.9
C5−C6−O	128.4	129.8	129.6	129.4
C4−C5−N7	110.1	110.2	104.5	104.2
C5−C4−N9	104.0	102.4	109.5	109.6
C5−N7−C8	105.1	105.1	106.4	106.9
N7−C8−N9	114.5	115.2	114.2	113.0
N3C4C5C6	18.1	26.9	−14.7	−6.8
N1C6C5C4	−0.5	0.0	−7.5	−5.6

[a] Bond legths are in angstroms, and angles are in degrees.

state geometries is compulsory. In the present work, CIS gradients have been exploited both for the isolated and the solvated systems. A selection of CIS/cc-pVDZ geometrical parameters obtained for the lowest excited state of the two tautomers in vacuo and in water is reported in Table 4.

Contrary to what was found for ground states, excited states geometries are very nonplanar in both phases, with the 9NH tautomer presenting the larger distortions. As shown in the scheme below, in which we present 9NH (left) and 7NH (right) excited state in solution, the molecular geometrical distortion occurs in the six-membered ring of the molecule.



We note that the comparison between these geometries and those computed for the ground states is not formally correct as the two sets of data have been obtained with two different levels of calculation: CIS for excited states and DFT for ground states. However, such, or similar, combinations of different approaches are very common in studies involving electronic states for which it is not possible to find a single accurate method, and they have shown to be very reliable to get sufficiently comparable results.

Solvent effects are quite large in bond parameters (lengths and angles) but mainly in dihedral angles: these results cannot be confirmed by any experimental evidence, as no measurements are available for excited states geometries. Also, comparisons with previous calculations are not possible, as this is the first time that solvated excited state geometries are computed. The only available set of computed data refers to gas-phase systems;[31] the agreement of our data with those is very good as both calculations exploit the same quantum mechanical method (CIS). In the same paper, a comparison with a basis set including diffuse functions is also reported: the minor differences the authors find with respect to the smaller basis set, which are similar to those we find with respect to ours, give us sufficient confidence that the basis set we have used can correctly reproduce all of the main geometrical effects related to this particular transition. Similar conclusions can be predicted also for the following electronic structures; large basis sets possessing diffuse basis functions are in fact fundamental to describe structures, and properties, of particular excited states, generally high and/or Rydberg states, whereas for the lowest singlets we are considering here, also a basis such as cc-pVDZ should be sufficiently flexible to get reliable results when coupled to accurate correlated methods (here CIPSI).

TABLE 5: Summary of Experimental and Present Theoretical Values (CIPSI) of Emission Energies (eV), Dipole Moments (D) and Relative Energies (Δ) for the Two Tautomers of Guanine in Vacuo and in Water[a]

	vac		water		
	9H	7H	9H	7H	exp[b]
$\pi\pi^*$	3.45	3.88	3.29	3.69	3.70
Δ	0.0	+2.73	0.0	−0.48	
μ	4.500	0.638	9.951	3.857	

[a] The relative energies (in kcal/mol) are with respect to the 9NH form. [b] In water solution: ref 28.

As for any other nuclei acid base, also for guanine, the fluorescence quantum yield is very low; however, some experimental data are known, at least for the solvated molecule.[1] In Table 5, we report experimental and calculated fluorescence energies and dipole moments for the lowest excited states of the two tautomers as obtained by using the CIS/cc-pVDZ geometries reported in Table 4. The experimental value refers to water solution. All of the results have been computed at CIPSI level exploiting the CIS geometries optimized for each excited state in vacuo and in solution. For solution, the nonequilibrium scheme described in section 2 has been adopted with a solvent completely equilibrated to the emitting excited state but only partially relaxed in the final vertical state that this time is represented by the ground state.

Results reported in Table 5 lead to two main comments. First, it is clear that solvent effects are important not only for emission energies but also for the nature of the excited states. In particular, in both tautomers, solvation significantly enlarges excited state dipole moments and it also shifts their relative energies, making the 7NH tautomer more stable than 9NH, in opposition with what observed for the ground state. This behavior cannot be confirmed by a direct experimental evidence yet; however, some hints can be derived from data on emission energies, and this leads to the second important aspect to note. By comparing the emission energies computed for the two tautomers in solution with the experimental value, it seems clear that the emission spectrum of guanine is mainly due to its 7NH tautomer. This result becomes very interesting when compared to experimental studies on the one hand and to previous computational results from the other hand.

Concerning experiments, the first room temperature emission spectra of nucleic acid bases exhibited significant deviations from their respective absoprtion spectra; in particular, it was recognized that a minor tautomeric species could dominate the fluorescence, provided it was intrinsically much more fluorescent that the main species. This hypothesis has been successively confirmed for adenine but not for thymine and cytosine, whereas for guanine, no definitive answers are available at room temperature; however, low-temperature behaviors seems to confirm that the same explanations holds for guanine as for adenine,[1] and thus, our computed results could be considered as a further, theoretical, confirmation of this hypothesis.

Passing to previous calculations, the comments to make are the following. If one simply applies well diffused dipole-based models to predict solvent effects on the emission spectrum of guanine, the results one obtains would be completely different as the by far larger dipole moment of 9NH tautomer should imply a larger stabilization of the corresponding state in a polar solution with following greater probability of emission from it. This kind of approximation, on the contrary, is not present in our solvation model which can account for complete solute–solvent electrostatic (and repulsive) interactions (i.e., dependent on the real solute charge distribution and not on its dipole approximation); the computed results seem to show that this more accurate modelization of solute–solvent interactions is indeed very important in this case as leading to a completely different explanation of the phenomenon.

As a further comment on the results of Table 5, we note that the very low emission energy computed for 9NH tautomer is due to its highly distorted structure: the corresponding ground state lying vertically below it is thus largely destabilized (with its optimal geometry being almost planar), and the resulting transition energy becomes small. The same effect happens also for 7NH tautomer but in smaller extent because of its less distorted structure.

The results obtained for both absorption and emission processes in solution seem to suggest an unexpected conclusion.

In its electronic ground state, the molecule is preferentially in an almost planar 9NH form from which electronic absorption mainly takes place; thus, when the 9NH form is excited to its lowest singlet excited state and the excited state geometry undergoes relaxation, the molecule becomes highly nonplanar. This significant distortion leads to a parallel increase in the energy of the corresponding vertical ground state with consequent approaching of the energy surfaces of the two states. The same process when applied to less probable (in the electronic ground state) 7NH tautomer is much less pronounced, both in the distortion of the corresponding excited-state geometry and in the closeness of it and the corresponding vertical ground state.

In a standard scheme, the global fluorescence of guanine should be expected to arise from the lowest excited states of both the forms independently; however, computed results seem to contrast with that indicating the 7NH form as that contributing most (if not exclusively) to the emission spectrum. Two possible processes can be introduced to try to explain these contrasting results.

First, we can assume that the highly nonplanar structure of 9NH would give rise to large amplitude vibrations, particularly those of out-of-plane character, which can vibronically couple with ground state (because of the closeness of the respective energy surfaces) and/or with solvent; in both cases, a nonradiative decay would happen, thus canceling the contribution of this tautomer to the emission spectrum. This first possible mechanism, already proposed by Mishra et al.,[31] is hard to be numerically proved; it would in fact require studies of the dynamics of relaxation of the excited state and of the corresponding ground state, both coupled to the internal dynamics of solvent molecules. Of more direct analysis is, on the contrary, the second possible mechanism involving a transformation from 9NH to 7NH form which can take place in the electronic ground state or in the lowest excited state. Taking into account the protic nature of the solvent (here water) and the sites of easy protonation present in the two tautomers, a possible mechanism of tautomerism is through protonation-deprotonation.

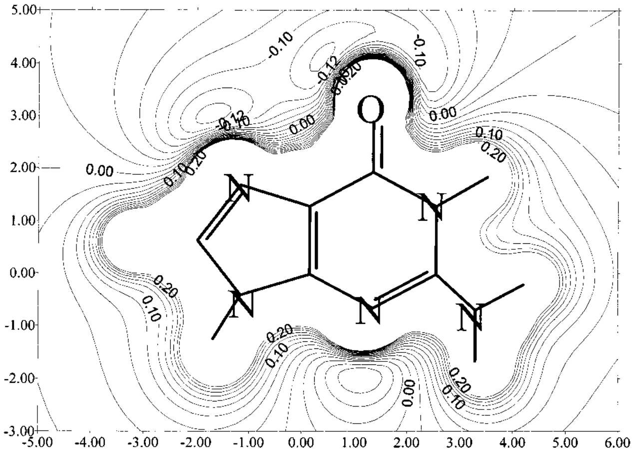


Figure 1. MEP map of the 9NH form of solvated guanine in the ground state. MEP values are given in au of energy.

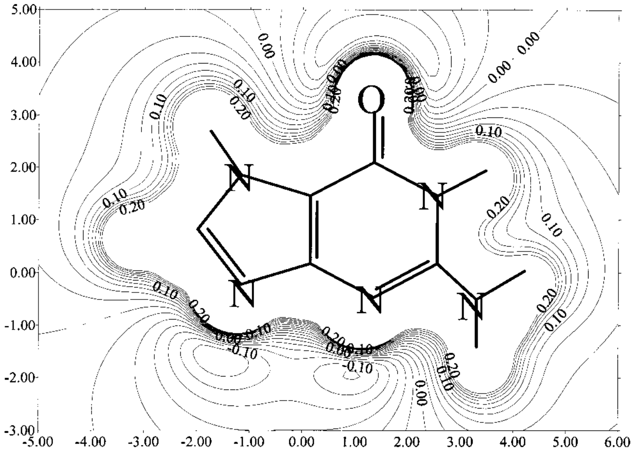


Figure 2. MEP map of the 7NH form of solvated guanine in the ground state. MEP values are given in au of energy.

3.4. Tautomerism and Protonation. To validate the hypothesis of an active mechanism of transformation of 9NH into 7NH via protonation, we have considered both the ground and the lowest excited states of the most probable form of protonated guanine; this study has been limited to the solvated system only because of necessity of an effective source of protons (the protic solvent) for the mechanism to realize.

To individuate the strongest basic sites in guanine, and thus the most probable protonated form, we have computed the maps of molecular electrostatic potential (MEP) of both tautomeric forms in their ground and lowest excited states. Such maps have been obtained by exploiting the CI density matrix produced by CIPSI calculations (to have a detailed analysis of the quality and reliability of CIPSI-MEP maps, we refer to the paper by Luque et al.,[33] in which a comparison with full-CI results is reported). The four maps are reported in Figures 1–4, in which MEP values have been converted in energy values through multiplication by a unit charge.

The four figures show that in the 9NH form of guanine, the MEP minimum is found near the free N7 atom in both the ground and lowest single excited states, whereas in both the ground and lowest excited states of the 7NH form, the MEP minimum is found near the N9 atom. These data confirm the expected result that for both the forms, the free N atom (i.e., that nonbonded to hydrogen) of the five-member ring is the most reactive site for protonation in both the ground and the lowest excited states, and thus, they definitely allow us to neglect a possible protonation on O atom. The MEP maps also support

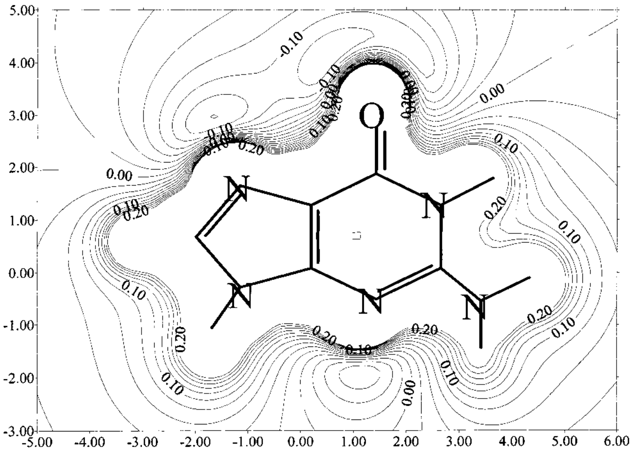


Figure 3. MEP map of the 9NH form of solvated guanine in the lowest singlet ($\pi - \pi^*$) state. MEP values are given in au of energy.

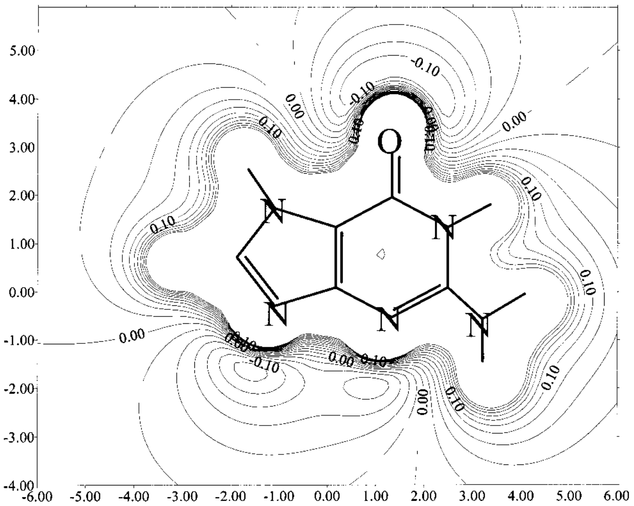


Figure 4. MEP map of the 9NH form of solvated guanine in the lowest singlet ($\pi - \pi^*$) state. MEP values are given in au of energy.

TABLE 6: Energy (Δ) and Minimum of Molecular Electrostatic Potential (V_{max}) (both in kcal/mol) for the Ground and Lowest Singlet Excited States of N9H and 7NH Forms of Guanine in Solution[a]

	9NH	7NH
	GS	
Δ	0	+0.67
V_{max}	−93.0	−86.8
atom	N7	N9
	EXC	
Δ	0	−0.48
V_{max}	−88.4	−81.2
atom	N7	N9

[a] For each electronic state, energies are with respect to the 9NH value. The rows labeled as ‘atom’ indicate the atomic sites near which the minima are located.

the assumption we have made about a larger possibility of a tautomerism between keto-9NH and keto-7NH forms, rather than from the keto to the enol forms.

The lowest MEP values (transformed in kcal/mol) are reported in Table 6 for each tautomer and each electronic state.

By comparing the two sets of MEP minima (the two for ground states and the two for excited states) and the relative energies of neutral and protonated forms, two aspects are to be noted; first, the 9NH form is more reactive to H^+ than 7NH (being its minimum potential more negative of about 7 kcal/mol in both electronic states), and second, the 9NH form is more stable in the ground state, whereas the opposite applies for the excited state. Keeping in mind these two results, we can assume that the two parallel equilibria (one involving ground states and the other for the excited states), namely

$$9NH + H^+ \rightleftarrows GUAH^+ \rightleftarrows 7NH + H^+$$

are shifted toward left (i.e., toward the 9NH form) when involving ground states and toward right (i.e., toward the 7NH form) for excited states. On the bases of these results and of those obtained from absorption and emission calculations, a four-step mechanism can be suggested:

1. absorption of a photon by the 9NH form in its ground state with following transition to the corresponding excited state;
2. protonation of the 9NH excited state;
3. deprotonation toward the 7NH form still in its excited state (more stable than the 9NH analog);
4. emission from the 7NH excited state toward its corresponding ground state.

To have a confirmation of this scheme in terms of protonation barriers, we have computed the N protonated species both in the ground and the lowest excited states (for each state, we have computed the optimal geometry following the same scheme used for neutral analogues, namely, B3LYP/cc-pVDZ for the ground state and CIS/cc-pVDZ for the excited state); we note that in this case both ground and excited states are almost planar.

If we now compare the energy difference between the protonated form in the ground and the lowest excited state and the corresponding states of the neutral 9NH form, we can see that protonation seems more favored in the excited state with the corresponding energy differences, |(GUAH+) − (9NH)|, being smaller by about 1.6 kcal/mol than in the ground state in accord with our original prediction.

The analysis reported above, and the conclusions we have derived from it, are clearly “theoretical”, in the sense that only experimental data of excited state lifetimes could give them a stated confirmation. Only the comparison of such data with the time scales typical of protonation−deprotonation processes can in fact support (or on the contrary invalidate) our results and univocally show the possibility of the scheme we have proposed to explain the fluorescence data indicating a prevalence of emission from the 7NH tautomer. Unfortunately, such comparison is not possible as the required experimental data are, to the best of our knowledge, still missing; the only values we have found in the literature, which refer to fluorescence lifetimes (both 0.5 and 1 ps have been proposed)[1] are in fact not enough to have a complete view of all of the possible processes (fluorescence, and protonation−deprotonation mechanisms but also relaxations) and to indicate the reason one of them is more effective than the others. Also, from the computational point of view, we are not aware of any dynamical study on these molecules (either in gas phase or in solution) that gives an estimate of the required data or at least an indication of which mechanism has to be preferred and for which reasons. For all of these limits in the direct comparison with experimental data or accurate computational studies on the dynamics of guanine excited states, the mechanism we have proposed to explain the tautomerism between 9NH and 7NH forms, for the moment, remains a suggestive possibility; we hope, however, that our results could urge some experimentalists to do studies which could clarify this important aspect.

4. Summary and Conclusions

In this paper, we have presented a study on the photophysics of guanine in gas-phase and in water solution. The analysis has involved correlated ab initio methods both in the calculation of geometries and energies and properties of the various electronic states. For the first time, solvation effects have been included in any step of the study (from geometry optimization to the evaluation of transition properties and energies).

The analysis has involved two tautomeric forms (the two keto 9NH and 7NH) both in the study of ground states and in the evaluation of electronically excited states; for ground states, the computed results have shown the preferential stabilization of the 9NH form in water solution with respect to the gas phase (in complete accord with the experimental evidence). The same result has been indirectly obtained through calculations of absorption energies and related transition properties (oscillator strengths and transition moment directions); the computed data for the solvated system (the only for which a comparison with experiment is possible) show that the absorption spectra of guanine is better reproduced by the 9NH tautomer.

For excited states, on the contrary, the 7NH form becomes the most stable one when the system goes from gas phase to solution. This result together with the better agreement of the corresponding emission energy with experiments seems to show that the fluorescence spectrum of guanine is mainly due to 7NH tautomeric form: once again this is in accord with what predicted from experimental data.

The good qualitative and quantitative agreement with experiments, we have obtained in the reproduction of electronic spectra in water solution (for both absorption and emission energies an accuracy around, or less, 0.1 eV is found), has suggested us to deepen the investigation toward a more detailed understanding of possible mechanisms leading to the 9NH−7NH tautomerism indicated by the reversed relative importance of the two forms in the excitation and emission spectrum of guanine. Because of the basic properties of guanine and the protic nature of the solvent, we have focused on a protonation mechanism acting either in the ground or in the excited state of the solvated system.

To rationalize what experimentalists call the basicity of various sites of the molecule, we have performed molecular electrostatic potential calculations. These have shown that in both forms the most active site is the H-free N atom of the five-member ring (the N7 for the 9NH form and the N9 for the 7NH tautomer) giving, thus, support to our assumption of a preferential tautomerism between the two keto forms with respect to the alternative tautomerism from keto to enol forms (this second mechanism should in fact involve the carbonil oxygen as active basic site). In this framework, a single protonated form has to be to considered (N protonation of 9NH or 7NH leads in fact to the same structure). The analysis of relative energies of neutral and protonated forms, either in their ground or excited states, seem to suggest a four-step mechanism involving first the transition from the ground state of the most stable 9NH form to the corresponding excited state (as confirmed by the comparison of computed and experimental excitation energies and properties). The two following steps involve protonation−deprotonation mechanisms leading to the 7NH form still in its excited state, whereas the fourth and last step coincides with an emission decay of such form to the corresponding vertical ground state in accord with what predicted by experimental observation on the one hand and from our computed emission energies on the other hand.

This study, together with the previous one on adenine, shows the great potentialities of solvation continuum models in the fundamental study of the photophysics of nucleic acid bases. To get such accurate results, however, we have shown that some requirements are necessary: the use of high-level quantum mechanical methods and an accurate formulation of the continuum solvation model including (1) a properly modeled molecular cavity, (2) a complete, i.e., not limited to dipole-only (or also higher but still truncated) expansions, description of electrostatic interactions, and (3) repulsion effects in the description of the modified Hamiltonian. Further improvements are still possible and strongly advisable (suffice it to quote the inclusion of solute−solvent dispersion interactions), but at the same time, this study should show that a detailed description of DNA bases in their natural environment is possible also with computationally feasible approaches, not requiring a high-level quantum-mechanical study of large solute−solvent clusters as usually suggested when a realistic analysis is required.

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