Modeling Protein−RNA Interactions: An Electron-Density Study of the Formamide and Formic Acid Complexes with RNA Bases

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The complexes formed by the double interaction established between the four RNA bases (adenine, cytosine, guanidine, and uracil) and formamide and formic acid as a model for the interacting groups of certain amino acid side chains have been theoretically studied. Density functional theory (B3LYP/6-31+G**) methods have been used for this study. The interaction energies obtained range between 10 and 19 kcal mol^{-1}. The analysis of the electron density and the natural bond orbital analysis show that these complexes are bound by medium strength double hydrogen bonds established between the donor and acceptor groups of formamide and formic acid and those of the RNA bases. Comparisons are made with the results obtained in some experimental studies and the analysis of protein−RNA interactions databases.

Introduction

RNA performs essential and diverse functions within the cell such as gene replication and expression. These processes involve many different RNAs, but a common feature in all of them is the interaction of this macromolecule with proteins. Enormous efforts are being made to exploit RNA as a drug target; therefore, the RNA recognition by peptides has become an extremely relevant issue.

RNAs display very diverse structures, almost as diverse as their functions. They are commonly single-stranded, but sometimes they include short lengths of double helices, hairpin loops, bulges, and pseudoknots. Proteins tend to interact with RNAs where they form complex secondary structures such as stem-loops and bulges. Draper has published a comprehensive review of protein−RNA interactions.[1] Also, different analyses on protein−RNA interactions and strategies for targeting RNA−protein complexes have appeared in the literature.[2−5]

The most important interaction established between the RNA bases and amino acids is through hydrogen bonds (HBs).[6] In particular, it has been pointed out that glutamic acid (Glu) and asparagine (Asn) show a good number of HB contacts with the RNA bases (mostly with guanine and uracil).[2]

In the study published by Hermann,[3] it was shown that RNA bases tend to interact mainly with the side chain of the amino acid residues of proteins, even though the main interaction between proteins and RNA occurs with the phosphate linkages of RNA. These phosphate−amino acid interactions have been thoroughly studied at the theoretical level by Leszczynski et al. in several articles.[7] Also, our group has carried out a theoretical study of the complexes due to hydrogen-bond formation of a protein backbone model and nucleic acid bases.[8]

In the more recent works of Allers et al.[4] and Cheng et al.,[5] the actual HBs between the RNA bases and some groups representing proteins (functional groups of side chain amino acids and the peptide bond) are analyzed finding the frequency of interaction of each group with the different positions of purinic and pyrimidinic bases in different databases. In particular, Allers et al. found that specific RNA recognition seems to be mediated mostly by interactions of amide and carbonyl groups in the protein backbone. In the study of Cheng et al., the possible arrangements between amino acids bonding by HB to unpaired bases have been calculated. They found 32 possible interactions that involve two or more HBs to the 4 unpaired bases and the protonated species of adenine and cytosine, 17 of which have been observed.

The inherent donor−acceptor arrangements of the nucleic bases support many possible interactions. If one looks at the ability to form a HB of the small molecule simulating the amino acid (side chain or peptide bond), three types of small ligands can be identified: those with donor/donor HB groups such as guanidinium (as in Arg), those with acceptor/acceptor HB groups such as carboxylate (as in Glu and Asp), and those with donor/acceptor HB groups such as formamide (as in Gln and Asn). In the present study, we have chosen the last case and included formic acid because, even though it is not present in biological environment, it also presents a donor/acceptor profile. The complexes with the first two types of small ligands (donor/donor and acceptor/acceptor) are the objective of ongoing work.

As starting points, we chose all those possible donor−acceptor interactions between both the donor/acceptor small ligands (formamide and formic acid) and the four RNA bases (adenine, cytosine, guanidine, and uracil). Significant contact sites for HBs are provided by the Watson−Crick boundaries of RNA bases. For the present study, we have explored all the possible positions for the formation of a HB (as donor or as acceptor) in the four bases.

Methods

The geometries of all the compounds have been fully optimized with the program Gaussian 98,[9] using the hybrid method Becke3LYP[10] with the 6-31+G**[11] basis set. In all the cases, the nature of the compounds as a potential-energy

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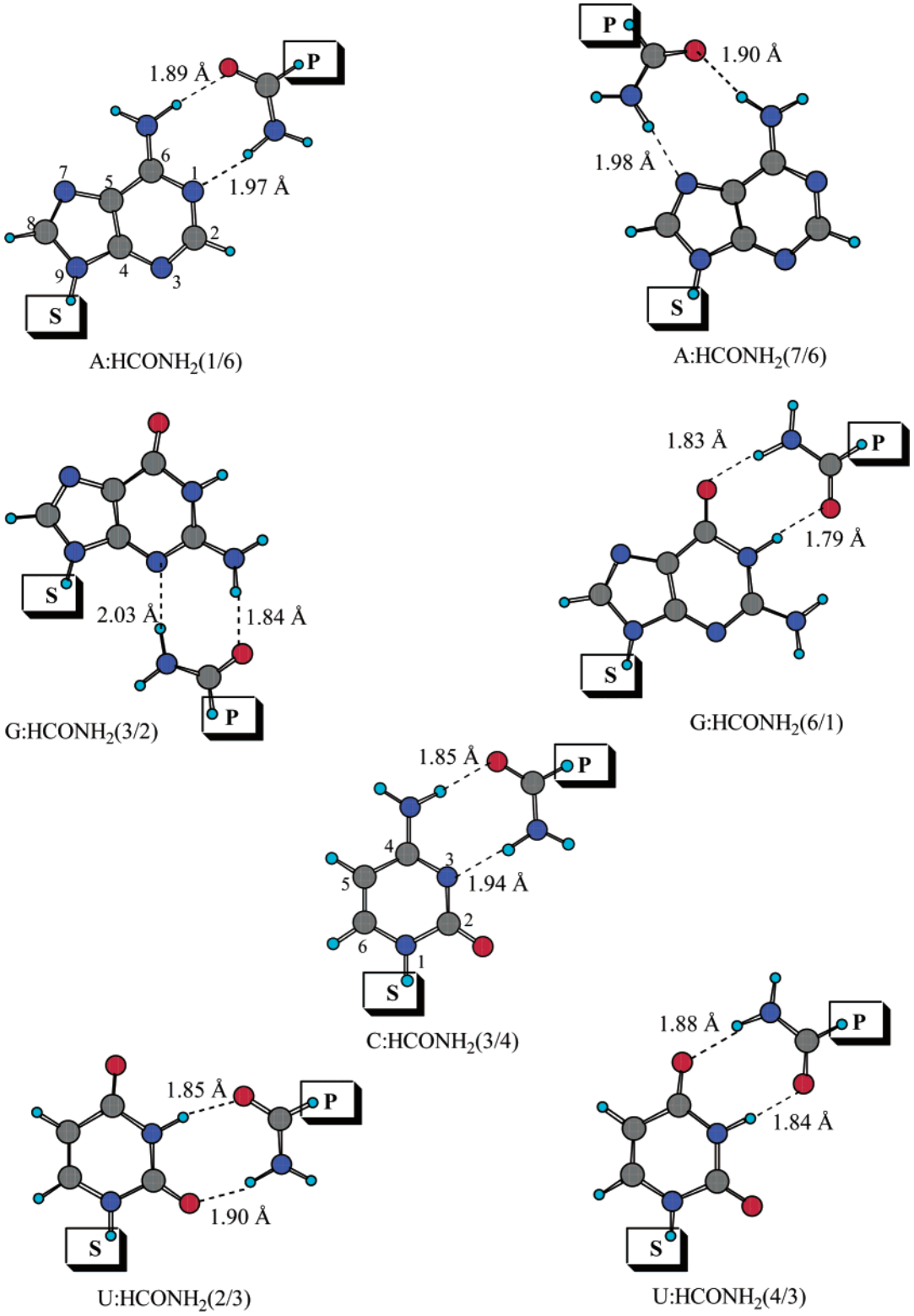

Figure 1. B3LYP/6-31+G**-optimized structures of the RNA bases−formamide complexes. The position of the sugar (S) in the RNA nucleoside and the rest of the amino acid (P) in the formamide moiety are indicated.

minimum has been established at the B3LYP/6-31+G** level, by verifying that all the corresponding frequencies were real.

Interaction energies, E_I, have been calculated as the difference of the total energy of the complex and the sum of the isolated monomers. They have been corrected by the inherent basis-set superposition error (BSSE) using the Boys−Bernardi counterpoise technique.[12]

The topological properties of the electron charge density (electron density at the bond critical point, ρ(BCP), and its Laplacian, $\nabla^2\rho$(BCP)) have been studied using the Atoms in Molecules methodology (AIM) with the AIMPAC program package.[13] The natural bond orbital (NBO) analysis was used to determine the nature of the interactions in the formation of the complexes. These calculations were performed with the NBO code implemented in Gaussian 98.[14]

The nomenclature of the complexes is expressed by the letter corresponding to the RNA base, then formic acid (HCO_2H) or formamide ($HCONH_2$) groups, and finally the number of the

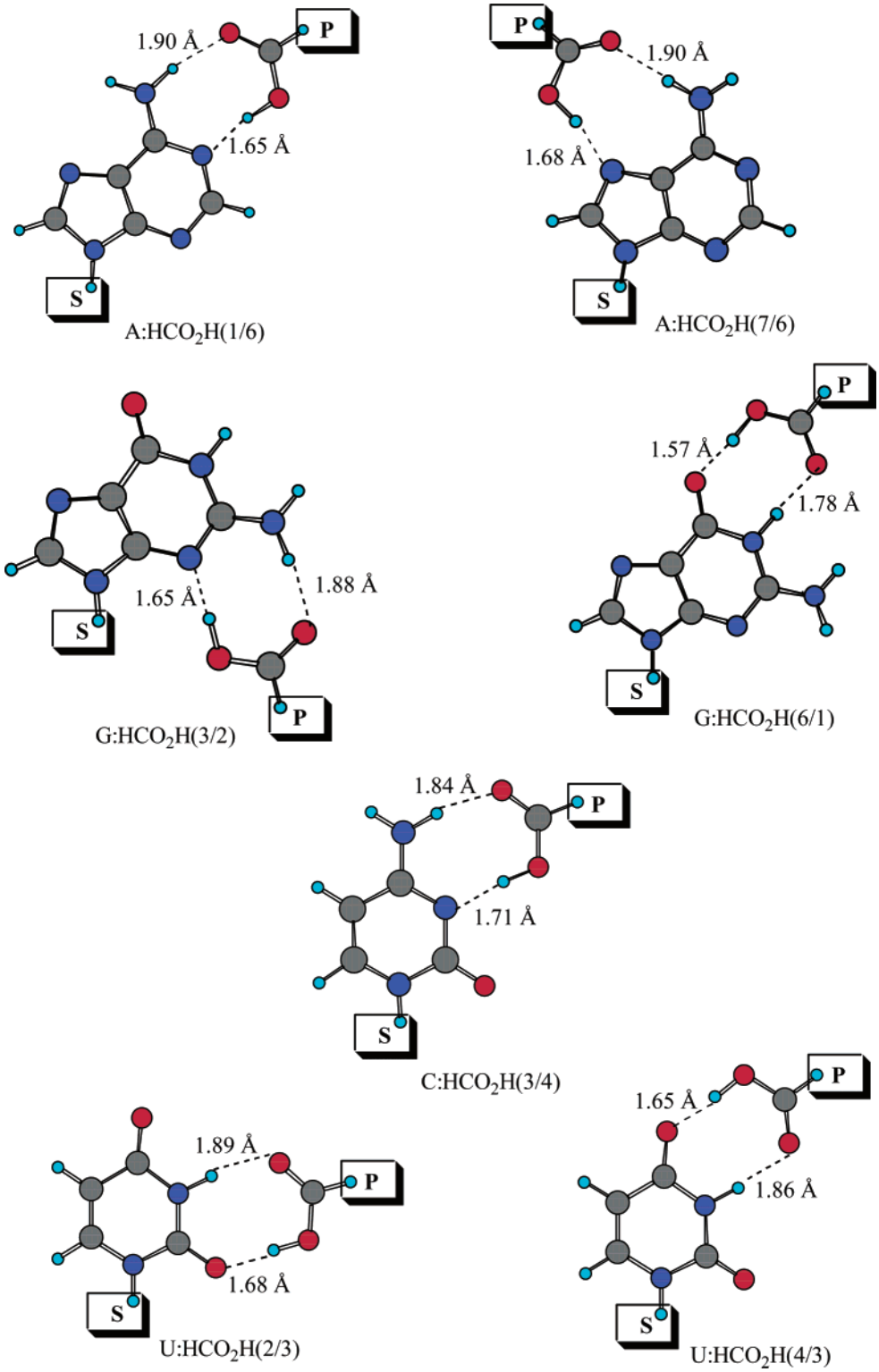

Figure 2. B3LYP/6-31+G**-optimized structures of the RNA bases−formic acid complexes. The position of the sugar (S) in the RNA nucleoside and the rest of the amino acid (P) in the formic acid moiety are indicated.

RNA bases atoms that interact with the formamide or formic acid monomers. Thus, for example the purine base complex A/$HCONH_2$ (1/6) will be formed between the N1 of adenine and the NH_2 of formamide and between the NH_2 in position 6 of adenine and the C═O group of formamide (for numeration of purine and pyrimidine rings, see Figure 1).

Results and Discussion

All the possible HB complexes between the purine and pyrimidine RNA bases and formamide and formic acid were optimized, and their structures of minimum energy are gathered in Figures 1 and 2, respectively. These complexes are formed by a double interaction between both monomers since formamide and formic acid exhibit a HB acceptor (C═O in both molecules) and a HB donor group (NH_2 and OH respectively) simultaneously, and RNA bases can also act as HB donors or acceptors depending on the orientation of the molecule.

The interaction distances obtained for the RNA base−formamide complexes are between 2.0 and 1.8 Å, in agreement with HB interactions. As expected, the shortest distances (1.79

TABLE 1: Total Energies (E_T, au) and Interaction Energies Including BSSE Corrections (BSSE, E_{I+BSSE}, kcal mol^{-1}) for the RNA Bases, Formamide, Formic Acid, and Their Corresponding Complexes Calculated at the B3LYP/6-31+G Computational Level**

	E_T	BSSE	E_{I+BSSE}
A/$HCONH_2$ (1/6)	−637.2833357	0.56	11.57
A/$HCONH_2$ (7/6)	−637.2822781	0.59	10.88
G/$HCONH_2$ (3/2)	−712.5197225	0.59	10.79
G/$HCONH_2$ (6/1)	−712.5314796	0.68	18.08
C/$HCONH_2$ (3/4)	−564.8992601	0.61	15.24
U/$HCONH_2$ (2/3)	−584.7759875	0.59	10.58
U/$HCONH_2$ (4/3)	−584.7774409	0.61	11.48
A/HCO_2H (1/6)	−657.1539446	0.79	15.07
A/HCO_2H (7/6)	−657.1530371	0.84	14.45
G/HCO_2H (3/2)	−732.3907715	0.83	14.55
G/HCO_2H (6/1)	−732.3980441	0.86	19.08
C/HCO_2H (3/4)	−584.7662428	0.84	16.46
U/HCO_2H (2/3)	−604.6437325	0.78	12.32
U/HCO_2H (4/3)	−604.6454149	0.78	13.37

to 1.90 Å, see Figure 1) correspond to N−H···O interactions, whereas the N−H···N distances are longer (1.94 to 2.03 Å, see Figure 1). In the case of the RNA base−formic acid complexes, the average interaction distances are shorter than the Formamide complexes (between 1.9 and 1.6 Å). The N−H···O distances appear in a similar range to the previous complexes (between 1.78 and 1.90 Å, see Figure 2). However, the O−H···N interactions are shorter (1.65−1.71 Å), and the O−H···O distances are even shorter (1.57−1.68 Å, see Figure 2). This is in agreement with previous results showing that O−H···O distances are the shortest of HB interactions.[15]

Regarding the interaction energies, the complexes with formic acid are between 1 and 4 kcal mol^{-1} more stable than those with formamide as can be observed in Table 1. This again confirms that formic acid is able to form stronger interactions with the bases because the OH group and the C═O group are a strong HB donor and acceptor, respectively, whereas the NH_2 group from Formamide is a slightly weaker HB donor.

From the formamide complexes, G/$HCONH_2$ (6/1) and C/$HCONH_2$ (3/4) show the largest interaction energies. The high stability of the guanine complex might be explained by a secondary interaction between the NH_2 in position 2 of the purine ring and the C═O of formamide, since that N−H···O distance is 2.68 Å (see Figure 1). This kind of complex with a bifurcated HB has been also proposed by Cheng et al.[5] The same is observed for the formic acid complexes, G/HCO2H (6/1) and C/HCO_2H (3/4) being the most stable of them. In this case, the secondary stabilizing interaction could also be established since the corresponding N−H···O distance is 2.98 Å (see Figure 2). Both guanine and adenine−$HCONH_2$ complexes correspond with two structures proposed by Cheng et al.[5] that were not found in their database. However, in the article of Allers et al.[4] is found that guanine shows HB interactions 16 times between the position 6 and the amide group (NH) of the polypeptide backbone and 9 times between the position 1 and the polypeptide backbone carbonyl group (CO). Regarding cytosine, position 4 establishes 14 HB interactions with the CO of the polypeptide backbone, 4 with Gln, and 3 with Asn, while position 3 interacts 8 times with NH groups of the backbone. Therefore, even though these complexes have not been found in experimental databases those positions are highly susceptible to form HB.

Two complexes can be formed between guanine and formamide or formic acid, those interacting in the 2 and 3 positions of the purine system and those interacting with the 1 and 6 positions of that system. The difference in E_{I+BSSE} between these two possible complexes is of 7.29 kcal mol^{-1} for the formamide complexes and 4.53 kcal mol^{-1} for the formic acid ones. It is not by chance that in both cases (formamide and formic acid) the most stable complex is the one interacting in the 1 and 6 positions of the guanine ring, which are the positions used for base pairing in RNA (as well as in DNA) cases in canonical forms.

According to Cheng et al.,[5] there are eight types of interactions with RNA bases in which amino acids form two HBs to a Watson−Crick face, of these, U/$HCONH_2$ (2/3) has been observed only in RNA. Considering that uracil is one of the RNA bases that shows larger frequency HB contacts with Asn and that some of the interaction established with formic acid are some of the shortest (O−H···O interactions of 1.65 or 1.68 Å), it is unexpected that uracil provides some of the less stable complexes with both formamide and formic acid. On the contrary, the strong HB interactions with positions 1, 2, and 6 of guanine and the 1 and 6 sites of adenine is no surprise. It is a generally known fact that has been also demonstrated in some hydration studies of DNA bases.[16]

By comparison of these density functional theory results with recent and detailed work of Cheng et al.,[5] who have listed 32 possible HB interactions between nucleic acid bases and amino acid side chains, in which 17 have been observed in highly resolved complex structures, we found that some of the complexes obtained by us have been proposed and found in their database analysis. Thus, for example, the complex A/$HCONH_2$ (1/6) was found three times in protein−DNA complexes and twice in protein−RNA complexes. These Asn (or Gln)−adenine interactions were observed in RNaseB−DNA and methyltransferase−DNA complexes. Also, complex A/$HCONH_2$ (7/6) was found 83 times in protein−DNA complexes and once in protein−RNA complexes, these Asn (Gln)−adenine interactions were also predicted by Seaman at al.[17] Finally, complex G/$HCONH_2$ (3/2) was found 7 times in protein−DNA complexes and 6 times in protein−RNA complexes. These three complexes are the ones with the less strong interactions showing E_{I+BSSE} around 10 kcal mol^{-1}.

The results obtained from the electron density analysis (see Table 2), show that a BCP is found for each of the RNA bases−formamide/formic acid interactions. The electron density values (around 10^{-2} au) as well as the sign of the Laplacians of this electron density (always positive) found in the BCPs correspond to medium to strong HB interactions.[18] This is in agreement with the observation that HB contacts are the most common interaction between RNA and proteins.[2]

In general, and as expected, the O−H···O interactions are short (average interaction distance = 1.63 Å) and show a high concentration of electron density in the BCP (average ρ(BCP) = 0.0517 au) indicating strong HBs. On the contrary, N−H···N interactions are longer and the electron density at the BCP is smaller (average d(N−H···H) = 1.98 Å, ρ(BCP) = 0.0282 au). The average distance and ρ(BCP) values found for the O−H···N interactions is 1.67 Å and 0.0562 au, respectively, whereas for the N−H···O interactions are 1.86 Å and 0.0309 au respectively. If the O−H···N values are compared with those of the O−H···O interaction, it seems that what is more relevant to the strength of a HB is the nature of the HB donor more that that of the HB acceptor. Thus, it looks like if O−H is the HB donor the HB formed will be strong independently of the HB acceptor.

In several previous articles,[19] we have found a correlation between the electron density in the BCP between two atoms and the distance between these two atoms. Again, this correlation is found in the present work. If we considered all the double

TABLE 2: Electron Density (au) and Laplacian at the BCP of the HB Found in the Complexes Optimized at B3LYP/6-31+G Level (HB Distances (Å) Are Also Shown)**

		ρ(BCP)	$\nabla^2\rho$(BCP)	d(X···H)
A/$HCONH_2$ (1/6)	NH···O	0.0293	0.0819	1.89
	N···HN	0.0292	0.0688	1.97
A/$HCONH_2$ (7/6)	NH···O	0.0278	0.0813	1.90
	N···HN	0.0277	0.0686	1.98
G/$HCONH_2$ (3/2)	NH···O	0.0322	0.0917	1.84
	N···HN	0.0256	0.0617	2.03
G/$HCONH_2$ (6/1)	NH···O	0.0374	0.1044	1.79
	O···HN	0.0336	0.0950	1.83
C/$HCONH_2$ (3/4)	NH···O	0.0320	0.0900	1.85
	N···HN	0.0303	0.0743	1.94
U/$HCONH_2$ (2/3)	NH···O	0.0309	0.0876	1.85
	O···HN	0.0274	0.0813	1.90
U/$HCONH_2$ (4/3)	NH···O	0.0321	0.0905	1.84
	O···HN	0.0291	0.0855	1.88
A/HCO_2H (1/6)	NH···O	0.0285	0.0815	1.90
	N···HO	0.0599	0.1040	1.65
A/HCO_2H (7/6)	NH···O	0.0270	0.0806	1.90
	N···HO	0.0543	0.1085	1.68
G/HCO_2H (3/2)	NH···O	0.0292	0.0837	1.88
	N···HO	0.0590	0.1074	1.65
G/HCO_2H (6/1)	NH···O	0.0375	0.1076	1.78
	O···HO	0.0614	0.1524	1.57
C/HCO_2H (3/4)	NH···O	0.0326	0.0943	1.84
	N···HO	0.0518	0.1011	1.71
U/HCO_2H (2/3)	NH···O	0.0282	0.0811	1.89
	O···HO	0.0449	0.1337	1.68
U/HCO_2H (4/3)	NH···O	0.0302	0.0867	1.86
	O···HO	0.0489	0.1408	1.65

TABLE 3: Charge Transfer (e) and Orbital Interaction Energy (kcal mol^{-1}) Calculated at the B3LYP/6-31+G Level with the NBO Method**

complex	charge transfer	E(2)
A/$HCONH_2$ (1/6)	−0.005	NH···O 11.84
		N···HN 17.38
A/$HCONH_2$ (7/6)	−0.004	NH···O 9.97
		N···HN 16.67
G/$HCONH_2$ (3/2)	0.006	NH···O 13.73
		N···HN 14.71
G/$HCONH_2$ (6/1)	0.011	NH···O 18.83
		O···HN 14.15
C/$HCONH_2$ (3/4)	−0.005	NH···O 13.90
		N···HN 17.88
U/$HCONH_2$ (2/3)	0.015	NH···O 12.99
		O···HN 8.46
U/$HCONH_2$ (4/3)	0.013	NH···O 14.17
		O···HN 9.60
A/HCO_2H (1/6)	−0.065	NH···O 10.41
		N···HO 44.94
A/HCO_2H (7/6)	−0.055	NH···O 8.71
		N···H(···O) 57.14
		(N···)**H···O** 384.12
G/HCO_2H (3/2)	−0.062	N−H···O 10.91
		N···H−O 44.78
G/HCO_2H (6/1)	−0.037	N−H···O 18.17
		O···H−O 37.96
C/HCO_2H (3/4)	−0.046	N−H···O 13.35
		N···H−O 37.76
U/HCO_2H (2/3)	−0.021	N−H···O 10.05
		O···H−O 20.60
U/HCO_2H (4/3)	−0.026	N−H···O 11.77
		O···H−O 25.34

interactions established between RNA bases and formamide or formic acid, we obtained an acceptable logarithmic correlation: $d(X-H\cdots Y) = -0.40 \ln[\rho(BCP)] + 0.50$, $R^2 = 0.90$, $n = 28$. Furthermore, considering the most typical interactions between these two monomers, the HBs between a N and an O atom, the correlation is much improved as can be seen in the following equation: $d(NH\cdots O, OH\cdots N) = -0.32 \ln[\rho(BCP)] + 0.75$, $R^2 = 0.99$, $n = 21$.

Also, as previously, we found a good exponential correlation between the Laplacian of the electron density at the BCP and the distance, both for the O···H and N···H HB interactions. Thus, for the O···H we obtained the following equation: $\nabla^2\rho(BCP) = 4.35\, e^{-2.10d(O\cdots H)}$, $R^2 = 0.99$, whereas for the N···H interaction, the following correlation was obtained: $\nabla^2\rho(BCP) = 1.12\, e^{-1.41d(N\cdots H)}$, $R^2 = 0.99$.

Finally, the nature of the HBs formed was analyzed by using the NBO approach (see Table 3). First, the charge transfer that occurs with the formation of both HBs in each complex was evaluated. In general, the charge is transferred from the RNA base to the formamide or formic acid molecules with the exception of guanine and uracil complexes with formamide. The charge transfer calculated for the RNA bases−formamide complexes is rather small, especially in the case of the adenine and cytosine complexes. The formation of the RNA−formic acid complexes involves a larger amount of charge transferred in agreement with the larger stabilization of these complexes compared with the RNA−formamide ones.

The nature of the interactions established between RNA bases and formamide or formic acid was determined by looking at the orbital interaction energy (E(2) in NBO method). The values obtained depend greatly on the nature of the atoms involved in the HB interaction. Thus, the average E(2) value found for the NH···O interactions is of 12.4 kcal mol^{-1}, whereas for the NH···N interactions it is 16.66, for the OH···N interactions is 42.49, and for the OH···O interactions is 27.97 kcal mol^{-1}. All these interactions occur between the lone pair of the HB donor (=O or =N) and one unoccupied (N),(O)-H molecular orbital. It is particularly interesting that O−H···N interactions have substantially larger E(2) energies than O−H···O interactions. Weinhold et al.[20] have used the energy and diffuseness of the occupied orbital to explain the preference of the OC···HF complex vs the CO···HF one. In our case, the energy values of the N and O lone pair orbitals are very similar; therefore, the mentioned explanation cannot be used here.

One case should be looked at carefully. This is one of the HB formed in the A/HCO_2H (7/6) complex, in particular the interaction between the N7 of the purine ring and the OH of the formic acid. This HB is particularly short (only 1.68 Å) and when analyzing the orbital interaction energy, two different values were observed, one of 57.14 for an interaction between the lone pair of N7 and an orbital of the hydroxyl H atom and a value of 384.12 kcal mol^{-1} for an interaction between that H orbital and the lone pair of the hydroxyl O atom. This could indicate that the H atom is being transferred to the N of the purinic RNA base.

It could be interesting to compare the charges obtained by the two methods here, the AIM and NBO methods. We have chosen some examples to carry out such a comparison, the A/$HCONH_2$ (1/6), A/HCO_2H (1/6), G/$HCONH_2$ (6/1), and G/HCO_2H (6/1) complexes. The atomic charges obtained for the atoms involved in the HBs established in those complexes are shown in Table 4. In general, the atomic charges calculated by the AIM method are larger than those computed by using the NBO approach. This difference is more noticeable in the case of O and N atoms. For the H atoms, it was possible to find a good correlation between both sets of charges (Q[NBO] = 0.5221Q[AIM] + 0.1874, $R^2 = 0.979$). Wiberg et al.[21] analyzed different kinds of charges, and they observed the same tendency that we have found; the AIM charges of electronegative atoms are much larger than those calculated with the NBO approach.

TABLE 4: Atomic Charges (*e*) Calculated at the B3LYP/6-31+G Level with the AIM and NBO Methods for Some of the Complexes Studied**

	X···H	*Q*[AIM]	*Q*[NBO]
A/$HCONH_2$ (1/6)	**N**···(H)	−1.227	−0.601
	(N)···**H**	0.515	0.452
	H···(O)	0.526	0.460
	(H)···**O**	−1.218	−0.666
A/HCO_2H (1/6)	**N**···(H)	−1.241	−0.610
	(N)···**H**	0.525	0.462
	H···(O)	0.639	0.519
	(H)···**O**	−1.225	−0.661
G/$HCONH_2$ (6/1)	**O**···(H)	−1.218	−0.664
	(O)···**H**	0.519	0.464
	H···(O)	0.539	0.463
	(H)···**O**	−1.231	−0.693
G/HCO_2H (6/1)	**O**···(H)	−1.206	−0.667
	(O)···**H**	0.523	0.468
	H···(O)	0.664	0.536
	(H)···**O**	−1.236	−0.683

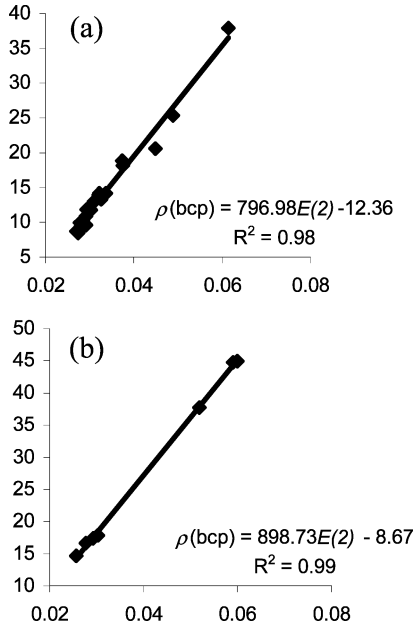

Figure 3. Plots of the correlations found between electron densities and orbital interaction energies corresponding to interactions with =O (a) and =N (b) as HB acceptors.

Considering that the electron density in the BCP indicates the strength of the HB formed, this value should correlate with the orbital interaction energy, which gives an idea of the nature of the bond studied. Thus, the values of this $E(2)$ energy were compared with those of the ρ(BCP) and the following correlation was found: $\rho(\mathrm{BCP}) = 929.90E(2) - 15.08$, $R^2 = 0.90$, $n = 27$. The plot obtained showed clearly two parallel sets of points. Taking this in consideration and if the nature of the HB acceptor is taken into account (=O or =N), two different correlations can be found (see Figure 3).

Correlation between electron densities and orbital interaction energies corresponding to interactions with =O as a HB acceptor produced the following equation: $\rho(\mathrm{BCP}) = 796.98E(2) - 12.36$, $R^2 = 0.98$, $n = 20$ (see Figure 3a). The corresponding data for interactions with =N as a HB acceptor provided the following correlation: $\rho(\mathrm{BCP}) = 898.73E(2) - 8.67$, $R^2 = 0.99$, $n = 7$ (see Figure 3b).

Conclusions

All the possible complexes formed between RNA bases and the donor/acceptor molecules formamide or formic acid by means of double interactions have been optimized at the B3LYP/6-31+G** level. The properties of the electron density at the BCP have been evaluated as well as the nature of the interactions by using the NBO approach. All those studies have confirmed that the double interactions are HBs of medium strength and are established by the interaction between a lone pair of the HB acceptor and an unoccupied bond orbital of the H−X group (HB donor).

It is interesting that the charge transfer observed in the RNA bases−formamide/formic acid complexes is relatively small compared with the large interaction energies computed for these complexes. Thus, the stabilization attained by these double interaction complexes seems to be more than that of a charge-transfer process or ionic interaction.

Geometrical, energy, electron density, and natural bond orbital results show that the G/[$HCONH_2$][HCO_2H] (6/1) complexes are the most stable, and this is in agreement with the experimental observation that shows that some of the HB contacts found with highest frequency are those between guanine and Asn and guanine and Glu. Uracil also shows a high frequency of HB contacts with Asn. This also would justify the short HB distances found for the U/$HCONH_2$ complexes studied and the medium interaction energies obtained. Cytosine shows a medium frequency distribution of HB contacts with Asn and Glu, and its complexes with formic and formamide are the second in stability. However, the only complexes found several times in protein−RNA databases are A/$HCONH_2$ (1/6), A/$HCONH_2$ (7/6), and G/$HCONH_2$ (3/2), which show the lowest interaction energies (around 10 kcal mol^{-1}). The positions of the RNA bases considered for the acceptor/donor interaction have all experimentally exhibited a high incidence of HB interactions, mainly with the NH and CO groups of the polypeptide backbone.

Good correlations have been found between the logarithm of the electron density at the BCP and the HB distance as well as between the ρ(BCP) and the orbital interaction energy $E(2)$. The logarithmic correlation has been found previously in other HB complexes. However, it is the first time that a correlation between electron density and orbital interaction energy has been reported. This is a very relevant finding since both parameters provide an idea of the nature and strength of a bond but from very different sources. The ρ(BCP) parameter is derived from the electron density of the complex and reflects its value in a particular point between two interacting atoms, whereas the $E(2)$ parameter looks at the orbital interaction between two interacting groups. Thus, for the first time both electron density and orbital interactions have shown to correlate confirming that both parameters provide information relevant for the classification and quantification of HBs.

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