Interaction of Protein Backbone with Nucleic Acid Bases†

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A theoretical study of the hydrogen-bonded (HB) complexes between a protein model and nucleic acid bases (NAB) has been carried out. As protein models, *N*-formylglycinamide (For-Gly-NH_2, 2-formylaminoacetamide), **1**, in β- and γ-conformations and as NABs, the isolated ones, and the AU, GC dimers in the Watson−Crick (WC) disposition have been considered. Only those dispositions with a double HB between the protein model and the nucleic acid bases have been studied. The aromatic CH groups of the nucleic acids have been included as HB donor. The results indicate that the strongest HBs between the individual NAB and the protein models involve the atoms that participate in the formation of the WC dimers. In the trimeric complexes, no significant preference is obtained for the **1**-AU trimers studied while in the **1**-GC ones the complex where formylglycinamide interacts simultaneously with the carbonyl group of guanine and the amino of cytosine is favored. The electron density of the complexes has been analyzed using the atoms in molecules methodology, finding exponential relationships between the electron density and its Laplacian vs the bond distance. Finally, the effect in the nuclear chemical shielding due to the complexation has been explored. Exponential relationships have been found for the variation of the chemical shift of the ^{1}H signal for the NH···O and NH···N interactions with the HB distance.

Introduction

Protein and nucleic acids (RNA and DNA) constitute two of the most important molecular systems in the living organism. Proteins are responsible for the catalysis of biological processes while nucleic acids store and transmit the genetic information. In several cases, both systems are simultaneously involved as, for example, in the biosynthesis of proteins or in the repairment of damaged DNA by topoisomerases.

The recognition of nucleic acids by proteins is due to the charged phosphate groups, specially in the RNA−protein complexes.[1] However, these strong interactions are not discriminative, and weaker interactions are responsible for selective recognition.[2] The interactions present in the protein−NAB complex have been analyzed on the basis of X-ray and NMR data.[3,4] A classification of the hydrogen bonds in protein−DNA complexes show that the side chains and backbone of both molecules are involved in these interactions.[5] A database of thermodynamic experimental data for protein−nucleic acids interactions (PRONIT) has been recently published.[6] In a previous article, we have studied the recognition of the WC dimers by an additional nucleic acid base in the formation of triplexes.[7]

In the present article, all the potential interaction complexes between a model of the backbone of a protein and the nucleic acid bases (NAB) monomers and the corresponding Watson−Crick (WC) dimers have been studied. Further work will treat other potential interaction in those systems such as the ones between nucleic acid bases and amino acid side chains. The complexes have been optimized with a hybrid HF-DFT method, B3LYP, and its electronic properties have been characterized using the atoms in molecules (AIM) methodology. In addition, the effect on the nuclear shieldings due to the HB complexation has been calculated with the GIAO method.

Methods

To simplify the study, two configurations of formylglycinamide (For-Gly-NH_2) **1** in β- and γ-conformations (Figure 1) have been used as peptide models. All the complexes showing two potential hydrogen bonds (HB) between the atoms of **1** (indicated with a star in Figure 1) and the nucleic acid bases have been considered. The CH groups of the NAB have been included as HB donors since previous publications indicated that this moiety can act as HB donor both in model systems[8] and in calculation of NAB complexes.[9] Other possibilities such as single HB complexes and those where the CH groups of the glycine moiety act as HB donor[10] have not been considered in order to reduce the study to the most significant aspects.

Since the β-configuration of **1** shows C_s symmetry, the same symmetry has been imposed in all its complexes. For the complexes with **1** in γ-configuration, no symmetry restriction has been adopted.

The calculation has been carried out with the Gaussian 98[11] program using the hybrid HF-DFT B3LYP method[12] and the 6-31+G** basis set.[13] This level of calculation has been shown to provide similar results to MP2 computations and to be adequate to study HB of medium strength.[14−16] The interaction energies have been corrected with the inherent basis set superposition error (BSSE) using the Boys−Bernardi counterpoise technique.[17] The interaction energies obtained are not free energies since the entropic contribution has not been included.

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† The abbreviations used are as follows: HB, hydrogen bond; NAB, nucleic acid bases; A, adenine; U, uracil; G, guanine; C, cytosine; WC, Watson−Crick; RNA, ribonucleic acid; DNA, deoxyribose nucleic acid; PRONIT, protein−nucleic acids interactions database; HF, Hartree−Fock; DFT, density functional theory; AIM, atoms in molecules; GIAO, gauge including atomic orbital; MP2, Moller-Plesset perturbation method to second order; BSSE, basis set superposition error; B3LYP, Becke 3-parameter Lee−Yang−Parr; NA, nucleic acid.

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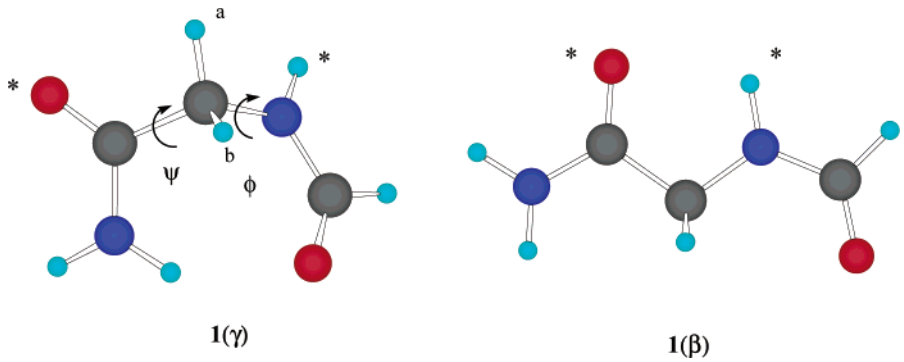

Figure 1. β- and γ-conformations of **1**. The atoms with the asterisks are those considered to form HBs with NAB.

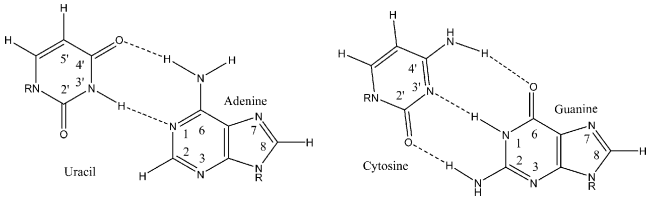

Figure 2. Schematic representation of the nucleic acid bases (NAB) and the numbering used throughout the text.

The properties of the electron density of the complexes have been analyzed using the atoms in molecules (AIM) methodology[18] and the AIMPAC programs.[19] The HB interactions have been characterized by the formation of a critical point between a hydrogen atom and an electron donor atom that are connected by the corresponding bond path.

An analysis of the shielding due to the complexation has been carried out using the GIAO method[20] at the same computational level used for the geometry optimization (B3LYP/6-31+G**). A recent report shows a good agreement between the experimental and calculated data using the B3LYP functional and the 6-31G** basis set.[21]

Results and Discussion

The two torsion angles used to describe the backbone conformation of the peptides [ϕ,ψ] (see Figure 1) are expected to have a maximum of three minima each. When dealing with simple structures, the number of minima can be reduced as in the case of the formylglycinamide **1** where only five structures are stable. In addition, two of them are mirror images of another two, leaving only three unique structures (β, γ, and δ).[22] At the B3LYP/6-31+G** computational level the most stable conformation is the γ, followed by the β (E_{rel} = 0.10 kcal/mol), the less stable one being the δ (E_{rel} = 1.97 kcal/mol). Similar results have been described for B3LYP/6-311++G** calculations.[22a] In the present article, the two configurations with the N−H and CO groups in a disposition to form double HB complexes, γ and β, have been considered as models for the interaction of the peptide backbone with nucleic acids. The calculated values, at the B3LYP/6-31+G** level, of the ψ and ϕ angles for For-Gly-NH_2 are 180 and 180° for the β-configuration and 65 and 81° for the γ one.

A schematic representation of the nucleic acid bases showing the potential HB donor and acceptor sites with the numbering used is represented in Figure 2. In the present article, the interactions between **1** and the NAB in the HB complexes have been indicated by the numbers of the atoms of the NAB which interact with the CO and NH moieties of **1** respectively.

Five possible complexes have been considered for adenine (Figure 3), three for guanine and uracil and only one for cytosine, excluding in all cases the amino group used as linkage with the sugar in the nucleic acid (NA) chain. For the Watson−Crick dimers, six complexes with the AU dimer and four with the GC one have been considered.

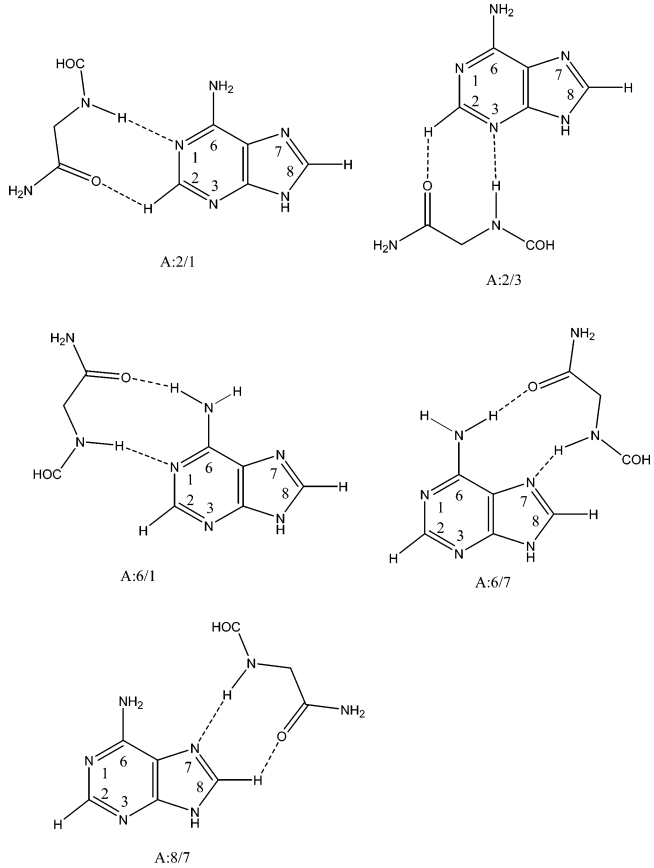

Figure 3. Schematic representation of the studied adenine complexes.

The interaction energy of the complexes is shown in Table 1. The corresponding values for the complexes between isolated NAB and **1** range between −3 kcal/mol [**1**(β)-A:2/1 complex] and −19 kcal/mol [**1**(γ)-G:1/6 complex]. Considering the strongest complex for each base as representative of this base, the interaction strength is A < U < C < G, which corresponds to the same order of the dipole moment of the isolated base (2.45, 4.65, 6.89 and 7.07 D, respectively). These results indicate the importance of the electrostatic interaction in the HB complexes due to the simultaneous polarization of the interacting systems.

An analysis of the crystallographic protein−RNA interactions shows that hydrogen bonds with guanine are preferred.[23] The weakest complexes correspond to those cases where the HB donor of the NAB is a CH group, while in the strongest

TABLE 1: Corrected Interaction Energies, E_{I+BSSE}, of the Complexes Studied (kcal/mol)[a]

complex	**1**(β)	**1**(γ)
A:2/1	−2.92	−6.43
A:2/3	−3.55	−6.46
A:6/1	−7.34	−11.49
A:6/7	−6.79	−10.45
A:8/7	−4.48	−8.51
C:4/3	−10.44	−17.30
G:1/6	−12.34	−19.08
G:2/3	−6.41	−10.69
G:7/8	−7.09	−11.99
U:3/2	−8.15	−10.65
U:3/4	−8.66	−11.55
U:5/4	−5.83	−9.88
AU:2/3	−14.64 (−2.42)	
AU:8/7	−16.58 (−4.36)	−20.88 (−8.65)
AU:6/7	−18.32 (−6.09)	−21.03 (−8.80)
AU:2/2′	−14.47 (−2.24)	
AU:6/4′	−16.90 (−4.67)	−21.07 (−8.84)
AU:5′/4′	−16.83 (−4.61)	−21.17 (−8.94)
GC:2/3	−30.64 (−4.98)	−34.82 (−9.16)
GC:2/2′	−30.13 (−4.47)	−34.51 (−8.85)
GC:6/4′	−34.82 (−9.16)	−42.75 (−17.09)
GC:7/8	−33.53 (−7.87)	−37.54 (−11.88)

[a] The values of the E_{I+BSSE} of the AU and GC WC dimers are −12.23 and −25.66 kcal/mol, respectively. In parentheses is given the interaction energy obtained considering two subsystems, the WC dimer and **1**.

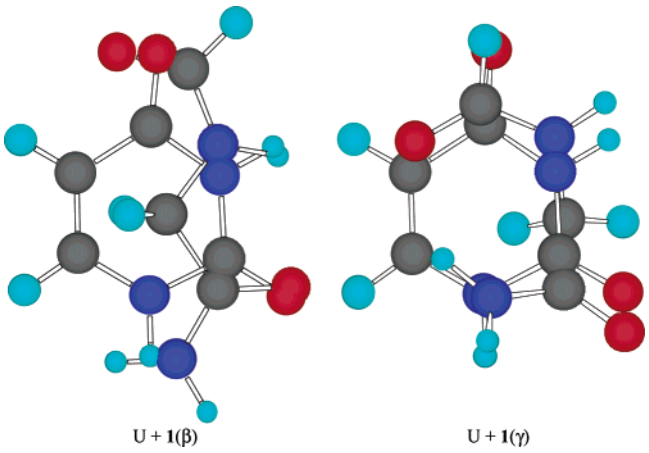

Figure 4. Superposition of uracil with **1**(γ) and **1**(β).

complexes the atoms involved are those responsible of the formation of the WC dimers.

In all cases, the complexes with **1** in γ-configuration are more stable than the ones with those in β-configuration. To test the importance of mimicking the geometry of the complementarily NAB by the peptides, both configurations of **1** have been superimposed with the structure of uracil using the four atoms of the carbonyl and amino groups (Figure 4). The results indicate that while in the β-configuration the terminal atoms of these groups (O and H respectively) in the two molecules are closer, in the γ-configuration the corresponding CO and NH groups are located almost parallel to those in the uracil molecule, favoring a better interaction.

From the analysis of the interacting groups from the NAB and the corresponding interaction energy, the following average trend is followed: CH/N < NH_2/N ≤ NH/O where the first groups act as HB donor and the second as HB acceptor. However, in some particular cases complexes of the first group can show stronger interaction energies than the ones in the second or even the third group (see for instance **1**-G:2/3 vs **1**-G:7/8 and **1**−U:3/2).

TABLE 2: Geometrical Parameters (Å and deg) of the HB Formed in the Complexes[a]

	1(β)				**1**(γ)			
	CO···X		NH···X		CO···X		NH···X	
	dist	angle	dist	angle	dist	angle	dist	angle
A:2/1	2.306	148.3	2.334	158.0	2.799	160.1	1.994	167.2
A:2/3	2.380	140.7	2.194	157.9	2.764	158.8	1.997	165.3
A:6/1	1.908	178.4	2.219	155.8	1.943	169.2	1.986	164.3
A:6/7	1.948	166.6	2.265	151.3	1.990	153.9	1.988	167.5
A:8/7	2.229	139.9	2.264	160.3	2.237	160.7	2.034	157.2
C:4/3	1.878	179.5	2.200	159.5	1.867	166.4	1.962	175.2
G:1/6	1.840	172.2	1.959	154.0	1.900	160.6	1.886	157.8
G:2/3	1.881	175.0	2.409	155.6	1.912	167.5	2.009	161.2
G:7/8	2.380	161.5	2.048	164.7	2.277	151.7	2.041	151.4
U:3/2	1.895	167.5	2.019	156.9	1.864	173.4	2.016	145.8
U:3/4	1.882	169.9	1.993	156.6	1.855	174.2	2.002	145.0
U:5/4	2.250	158.5	2.069	148.3	2.246	176.4	1.932	164.8
AU:2/3	2.407	135.0	2.197	159.8				
AU:8/7	2.221	140.6	2.276	160.7	2.253	159.7	2.032	157.5
AU:6/7	1.953	167.0	2.265	152.3	2.020	154.6	1.991	167.1
AU:2/2′	2.437	150.5	2.154	147.4				
AU:6/4′	2.266	119.0	2.251	149.3	2.108	143.1	1.930	162.6
AU:5′/4′	2.124	160.1	2.168	146.3	2.288	172.5	1.913	170.4
GC:2/3	1.940	176.2	2.422	155.1	2.022	168.3	1.959	165.1
GC:2/2′	2.192	129.0	2.308	144.0	2.050	152.1	1.919	166.6
GC:6/4′	2.126	128.4	2.155	154.8	1.959	152.9	1.885	155.3
GC:7/8	2.427	125.3	2.031	163.1	2.325	151.9	1.991	153.5

[a] The indicated CO and NH moieties of **1** correspond to those indicated in Figure 1.

In the case of the complexes between the WC dimers and **1**, two interaction energies have been considered: the first one was calculated considering the contribution of the three molecules and the second one considering the interaction of two subsystems, the WC dimer and **1**. For the purposes of the present article, the second interaction is more interesting since it can be compared to the interaction of proteins with DNA.

As in the case of the monomers, the strongest complexes correspond to the more polar WC dimers (their dipole moment are 1.76 and 6.07 D for the AU and GC dimers, respectively).

The comparison of the interaction energies between **1** and the isolated NAB or the WC dimers for analogous complexes show that, in almost all of the cases, their values are smaller in the latter case than in the first as an indication of no cooperativity effects due to the formation of the WC dimers. The exceptions are the **1**(γ)-AU:8/7 complex where a small energetic gain is observed (−0.14 kcal/mol) and **1**(β)-GC:7/8 where the relative stabilization is larger (−0.78 kcal/mol).

The interaction of **1** simultaneously with the NAB WC pairs provides very variable energetic results. Thus, in the case of **1**(γ)-AU:2/2′ no double HB is formed while in **1**(γ)-GC:6/4′ the interaction energy is −17 kcal/mol with respect to the WC dimer.

A comparison of the energetic values for the interaction of **1** in the different potential sites, shows that for the **1**-AU complex no clear preference is found with four complexes with similar energies while in the case of **1**-GC, the 6/4′ complex is preferred, especially for the **1**(γ) configuration. The interaction energies obtained when the CH groups act as HB donors are noteworthy.

The interaction distances (Table 2) obtained range between 1.84 and 2.80 Å being within the limits of what is considered as a HB interaction, with the exception of the longest distances that correspond to some CH···O contacts. In general, the longest distances are those corresponding to CH···O HB, followed by NH···N, and the shortest ones are the NH···O interactions. The HB angles are clearly influenced by the fact that a double HB

TABLE 3: Electron Density and Its Laplacian at the Hydrogen Bond Critical Points (au)[a]

	1(β)				**1**(γ)			
	CO···X		NH···X		CO···X		NH···X	
	ρ	$\nabla^2\rho$	ρ	$\nabla^2\rho$	ρ	$\nabla^2\rho$	ρ	$\nabla^2\rho$
A:2/1	0.012	0.041	0.013	0.036	0.005	0.017	0.028	0.067
A:2/3	0.010	0.037	0.018	0.046	0.005	0.018	0.027	0.068
A:6/1	0.024	0.083	0.018	0.044	0.025	0.072	0.029	0.068
A:6/7	0.021	0.077	0.015	0.042	0.023	0.067	0.027	0.069
A:8/7	0.018	0.053	0.010	0.034	0.015	0.043	0.025	0.064
C:4/3	0.026	0.089	0.018	0.046	0.029	0.087	0.026	0.068
G:1/6	0.030	0.099	0.026	0.071	0.029	0.082	0.028	0.087
G:2/3	0.026	0.089	0.012	0.032	0.027	0.078	0.027	0.066
G:7/8	0.010	0.039	0.023	0.062	0.014	0.041	0.025	0.065
U:3/2	0.024	0.086	0.021	0.063	0.029	0.086	0.020	0.069
U:3/4	0.025	0.088	0.023	0.066	0.030	0.088	0.021	0.071
U:5/4	0.012	0.044	0.019	0.058	0.014	0.041	0.025	0.077
AU:2/3	0.009	0.035	0.018	0.046				
AU:8/7	0.014	0.049	0.014	0.040	0.014	0.042	0.025	0.064
AU:6/7	0.021	0.076	0.015	0.042	0.021	0.062	0.027	0.069
AU:2/2′	0.008	0.031	0.014	0.051				
AU:6/4′	0.012	0.050	0.012	0.041	0.018	0.055	0.025	0.078
AU:5′/4′	0.014	0.046	0.016	0.047	0.013	0.039	0.027	0.079
GC:2/3	0.022	0.078	0.011	0.031	0.021	0.060	0.030	0.072
GC:2/2′	0.013	0.054	0.010	0.038	0.020	0.060	0.025	0.081
GC:6/4′	0.016	0.062	0.015	0.048	0.025	0.073	0.029	0.088
GC:7/8	0.009	0.036	0.024	0.064	0.012	0.037	0.028	0.071

[a] The indicated CO and NH moieties of **1** correspond to those indicated in Figure 1.

is formed, and this fact limits the possible disposition of the interacting groups, forcing in some cases angles larger than expected.

A comparison of the HB distances in the β and γ complexes shows in all cases shorter NH···X distances in the latter, up to 0.46 Å, except for the U:3/4 complexes. The differences of the CO···X distances are more variable, and they range between +0.49 and −0.17 Å. In general, the shortening of the HB distance in this complexes is associated with more linear HBs in agreement with the general correlations between HB distance and angle.

The effect of the complexation with the WC dimers does not affect significantly their HB structure. Thus, the interatomic distance of the nitrogens that defined the major and minor groove varies less than 0.1 Å in the cases studied.

The analysis of the electron density indicates the presence of bond critical points (bcp) in all the HBs considered (Table 3). The small value of the electron density and the positive value of the Laplacian correspond to a closed shell interaction as the HB. The large number of HBs studied (59 and 24 with O···H and N···H contacts, respectively) and their broad distance range (from 1.84 to 2.80 Å) have allowed to verify the correlations found previously between the electron density and its Laplacian vs the HB distance, *d*.[24] The results obtained for O···H (eqs 1 and 2) and N··H HB (eqs 3 and 4) in this case confirms the generality of the mentioned relationships. Other correlations as those between the electronic parameters and the interaction energy have not been considered since the latter is a contribution of several HB in each case explored here.

$$\rho = 1.0775 \exp(-1.9574d), \quad R^2 = 0.966 \qquad (1)$$

$$\nabla^2\rho = 2.3026 \exp(-1.7571d), \quad R^2 = 0.974 \qquad (2)$$

$$\rho = 1.7523 \exp(-2.0879d), \quad R^2 = 0.996 \qquad (3)$$

$$\nabla^2\rho = 2.8455 \exp(-1.8706d), \quad R^2 = 0.997 \qquad (4)$$

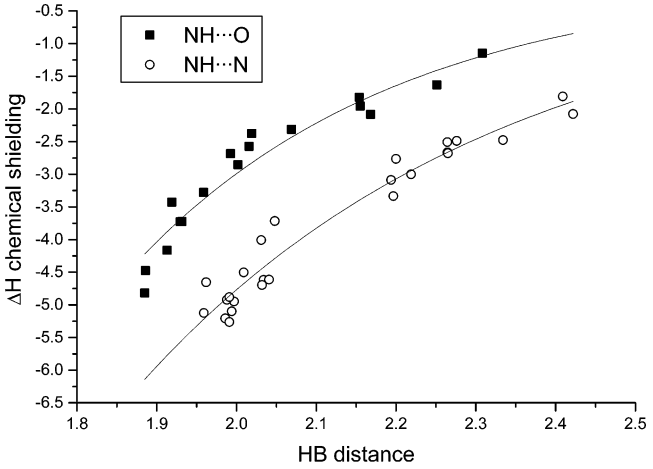

Figure 5. Δ^1H chemical shielding (ppm) vs HB distance (Å), *d*, for the NH···N and NH···O contacts. The adjusted curves corresponds to the following equations: $\Delta^1H = -1190.8 \pm 615.7 \exp(-2.9\pm0.3d)$, $R^2 = 0.93$, and $\Delta^1H = -388.4 \pm 98.3 \exp(-2.2\pm0.1d)$, $R^2 = 0.95$, for the NH···O and NH···N contacts, respectively.

The chemical shieldings calculated for the isolated structure of **1** and within the complexes have been deposited as Supporting Information. As mentioned previously, the values obtained for the different configurations of **1** allow to determine their geometrical characteristics. Thus, in **1**(γ) the chemical shielding values obtained are larger than the ones in **1**(β). In addition, the hydrogen atoms attached to the Cα are different depending if they point toward the γ-turn or not. The formation of HB complexes produce a "negative" shielding in all the cases studied, with the exception of the nitrogen shielding in the **1**(β) complexes (it can be positive or negative) and in the Cα shielding of **1**(γ) (it is always positive). Important variations are observed in the donor hydrogen atom (N**H**) reaching values up to −5.2 ppm. The variation obtained for this atom depends on the group acting as HB acceptor (N or O) and the HB distance (Figure 5). Thus, for a given interatomic distance, it is possible to differentiate the atom that act as HB donor, N or O. Exponential relationships between the bond distance and the chemical shift variation can be obtained for each HB acceptor. The fitted curves show a parallel behavior in the HB distance range studied indicating that the chemical shielding difference in this two groups of HB's are maintained along the distance. Similar relationships have been found for strong ionic HBs.[25]

The remaining chemical shieldings vary with the strength of the interaction but their dispersion is large. The only exception corresponds to the H^b (see Figure 1) that does not suffer almost any change due to the complex formation. In contrast, the H^a changes up to 0.95 ppm in the complexes **1**(γ):C4/3 due to its proximity to the N3 of the cytosine.

Conclusions

A theoretical study of the interaction of a peptide model, formylglycinamide, with isolated and WC nucleic acid bases has been carried out. The hybrid HF-DFT, B3LYP method has been used in the calculation. The geometrical, energetic, electron density, and chemical shielding of the complexes have been analyzed. The smallest interaction energies are found in those complexes which present a CH group as HB donor; however, their values indicate that in some cases it can be important for the recognition process. In the dimeric complexes, the strongest interactions correspond to those structures where **1** adopts a disposition similar to the corresponding NAB in the WC dimers. In the trimeric complexes, the **1**-AU cases studied present similar

interaction energies while in the **1**-GC ones there is one more stable than the rest.

The interaction of the protein model to the WC dimers do not show any cooperativity effect and the structure of the WC dimers is not significantly altered by the additional interaction.

The electron density characterization of the complexes shows the presence of bcp in the new HB formed. The value of the electron density and its Laplacian at the bcp shows exponential behavior vs the interatomic distance of the atoms responsible of the contact.

Finally, the effect of the complexation in the chemical shielding has been evaluated with the GIAO method. An exponential relationship has been found between the variation of the ^{1}H chemical shielding in NH···H and NH···O HBs and the interatomic distance. The rest of the nuclei notice the strength of the complexation but their dispersion is larger.

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Supporting Information Available: Table showing the variation of the chemical shielding of **1** due to the complex formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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