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Structural and Engergetic Properties of Hydrogen Bonds in PNA=DNA and PNA-PNA Base Pairs: a Theoretical Study

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Abstract

The hydrogen (H)-bond properties in single base pairs of peptide nucleic acid (PNA) binding to its complementary DNA and PNA have been theoretically evaluated using B3LYP/6-31G(d,p) calculation. The *N*-(2-aminoethyl)glycine (aeg) and pyrrolidinyl 2S-aminocyclopentane-1S-carboxylic acid (acpc) groups were employed for PNA backbones. The H-bond calculations of isolated Watson–Crick base pairs and DNA-DNA base pairs were also performed for comparison. The results showed that the type of backbones does not significantly affect the H-bond geometry. The H-bond lengths and angles remain roughly constant in most AT and GC base pairs attached through various backbones, compared to the corresponding isolated base pairs. However, the backbones strongly affect the energetic property of base pairs. The H-bond strengths are significantly increased by the PNA backbones. Moreover, for the aegPNA systems the base pairs comprised of homogeneous backbones provide stronger H-bond interaction than those comprised of heterogeneous aegPNA-DNA backbones, and the calculated H-bond energies of p(purine)-d(pyrimidine) base pairs were larger negative than those of d(purine)-p(pyrimidine) base pairs. Nevertheless, the acpcPNA backbones provide the contrasty effects compared to the aegPNA backbones. This indicates that the H-bond interactions are dependent of the types of backbones and base pairs.

Keywords: H-bond energy, Peptide nucleic acid, DFT calculation

1. Introduction

Deoxyribonucleic acid (DNA) is well known to play a pivotal role in biology as the carrier of genetic information. The chemical structure of this "molecule of life" was originally discovered and reported by Watson and Crick in 1953 (1). The backbone of the DNA strand is made from alternating a negatively charged phosphate and a sugar (2-deoxyribose) groups. The sugars are joined together by phosphate groups that form phosphodiester bonds (Figure 1A). The

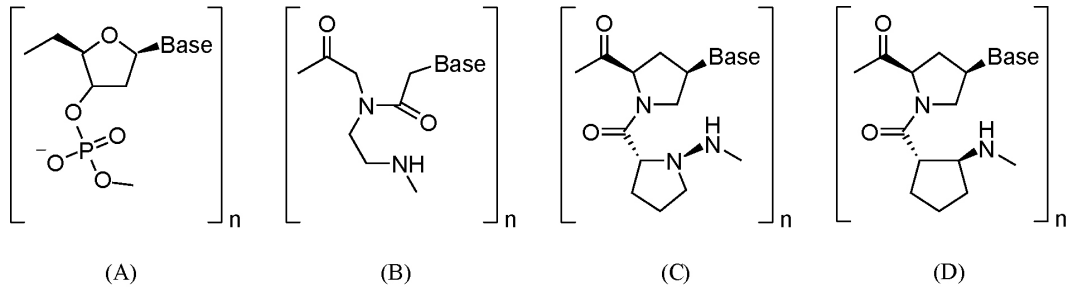

Figure 1. Monomeric units of (A) DNA, and PNA with (B) N-(2-aminoethyl)glycine (*aeg*PNA), (C) pyrrolidinyl D-aminopyrrolidine-2-carboxylic acid (*apc*PNA) and (D) pyrrolidinyl 2S-aminocyclo pentane-1S-carboxylic acid (*acpc*PNA) backbones.

anionic nature of the DNA backbone may be neutralized by associating countercations, such as Na^+ or Mg^{2+}. Four nucleobases found in DNA are adenine (A), cytosine(C), guanine (G) and thymine (T). Adenine and guanine are classified as purine bases, whereas cytosine and thymine are pyrimidine bases. Two double helices of nucleotides are stabilized by hydrogen (H) bonds between the bases attached to the two strands. The two types of purine–pyrimidine pairs form different number of hydrogen bonds that are two bonds for adenine–thymine (AT) and three bonds for guanine–cytosine (GC) pairs (2), see Figure 2. Since the H-bond interactions in DNA base pairs play a major rule in the DNA properties, it has been widely studied from both experimental and theoretical chemists; see, for example, Asensio et al. (3) and references therein. In recent years experimental techniques have been increasingly supplemented by computational and theoretical studies. This concerns application of quantum chemical as well as statistical methods. The main goal of the quantum chemical calculations is to complement experiments and provide information and predictions which are not easily accessible by experimental techniques, in order to elucidate the nature of the processes studied.

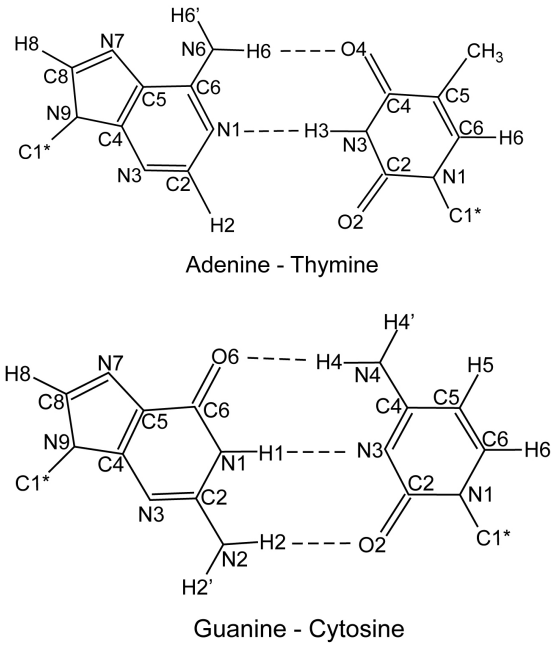

Figure 2. Watson–Crick base pairs found in double stranded DNA. C1* is a linking atom of backbone.

Peptide nucleic acid (PNA), first studied by Nielsen's group in 1991 (4), is a DNA analogue in which the negatively charged sugar-phosphate backbone of DNA is replaced by a neutral backbone of achiral poly(*N*-aminoethylglycine) with the nucleobases attached through a methylenecarbonyl linkage at the glycine nitrogen, namely *aeg*PNA (Figure 1B). The electrostatically neutral PNA molecule is capable of recognizing its complementary sequence in both DNA and RNA strands, as well as another complementary PNA strand with high sequence specificity for forming the Watson–Crick base pairs and leading to PNA-DNA, PNA-RNA and PNA-PNA hybrids, respectively. These hybrids are considerably more selectivity and stable than the corresponding DNA-DNA duplexes (5-6). Due to the unique properties of PNA, it has attracted a broad attention for applications in the fields of biotechnology and therapeutics (7-9). Great efforts have been made to design and synthesize a PNA based on the original aeg backbone to form chimeric PNA (10). Recently, Vilaivan and co-workers (11-13) have synthesized a series of conformationally constrained chiral analogs of PNA with the pyrrolidinyl D-aminopyrrolidine-2-carboxylic acid backbone, namely *apc*PNA, and pyrrolidinyl 2S-aminocyclopentane-1S-carboxylic acid backbone, namely *acpc*PNA, see Figures 1C and 1D. Their interactions with nucleic acids were studied by UV and CD spectroscopy. The homopolymeric PNAs form very stable 1:1 hybrids with their complementary DNAs as indicated by a very high melting temperature (T_m) of over 80°C for both apcPNA and acpcPNA systems. The binding of decamer TA pairs of apcPNA-DNA duplex has been theoretically studied using a molecular dynamics simulation. The apcPNA-DNA hybrid shows a higher thermodynamic stability in term of the binding free energy compared with that of the corresponding DNA-DNA complex (14). This is due to the large gas-phase energy repulsion of the two negatively charged sugar-phosphate backbones of the DNA strands. According to our knowledge, the theoretical studies of structural property and H-bond energy have so far been limited to PNA (15), especially, no such study has been performed for acpcPNA system.

In this study, we present a comparative evaluation of H-bond energy and H-bond geometry in a single base pair of aegPNA-DNA, acpcPNA-DNA, aegPNA-aegPNA and acpcPNA-acpcPNA systems using the density functional theory (DFT) calculation. The interactions in isolated Watson–Crick base pairs and DNA-DNA base pairs have also been investigated for comparison.

2. Materials and Methods

Computational details

Theoretical studies of H-bond interactions in DNA have widely been carried out using B3LYP density functional method (16). The studies suggested that the B3LYP method yields accurate H-bond energies when used with large basis sets. Herbert and co-workers have calculated the H-bond energies of isolated AT and GC base

pairs and illustrated that 6-31+G(d,p) is near the basis set limit for the systems of nucleic acids whereas 6-31G(d,p) basis set provided an overestimated energy compared with the experimental data (15). However, using B3LYP with 6-31G(d,p) and 6-31+G(d,p) levels did not give notably different in base pair geometries. Since the goal of this study is to comparatively evaluate the H-bond interactions in single base pairs of DNA-DNA, PNA-DNA and PNA-PNA systems (where the backbones of PNA are aeg and acpc groups), the B3LYP/6-31G(d,p) level was employed for all calculations.

Starting structures of DNA-DNA base pairs were generated using the Hyper Chem 8 program (17). Two negative charges of these pairs were neutralized by adding Na^+ counterions. Each counterion was placed in bridging position between two phosphate oxygens. Starting geometries of both aegPNA and acpcPNA were built up based on the DNA-DNA pair by one-to-one mapping of the PNA backbone atoms onto DNA backbone atoms. The coordinates of the missing atoms were added using geometric calculation. Complete geometry optimizations were then performed for each of the structures at the B3LYP/6-31G(d,p) method using Gaussian 09 program (18) to reach minimum energy configurations. Since the H-bond energy, $E_{\text{H-bond}}$, in base pair is considered as the binding interaction between two bases, therefore it is evaluated in the same manner of the energy difference of dimmer (base pair) and two monomers (bases) which can be expressed as

$$E_{\text{H-bond}} = E_{\text{base pair}} - E_{\text{base 1}} - E_{\text{base 2}} \quad [1]$$

where $E_{\text{base pair}}$, $E_{\text{base 1}}$ and $E_{\text{base 2}}$ are the energies of base pair, single base 1 and base 2, respectively. The individual optimized geometries of both single bases were used for their energy calculations. This allows the relaxation of single base conformation when its complementary base is absence.

3. Results and Discussion

3.1 H-bond distances and angles

The structural property of interest in base pair binding is the H-bond geometry between Watson–Crick bases. The H-bonding region can be described by bond lengths and angles between the H-bond donors and the acceptors. The H-bond lengths and angles of AT base pair are presented in Table 1. The values for the GC base pair are presented in Table 2. As the results, our computed geometrical parameters for the isolated Watson–Crick base pairs (in which each C1* atom was replaced with hydrogen atom, see Figure 2) of both AT and GC pairs are in agreement with previously published results (19). Two H-bond lengths in AT base pair were 2.93 and 2.85 Å for N6–O4 and N1–N3 bonds, respectively, and the bond angles were 174.3° and 179.5° for N6-H6-O4 and N1-H3-N3, respectively. Taking into account the effect of sugar-phosphate backbone, i.e. dA-dT system, one can see that two bond lengths were 2.94 and 2.83 Å for N6–O4 and N1–N3 bonds, respectively, and the bond angles were 173.8° for N6-H6-O4 and 179.0° for N1-H3-N3. These values are consistent with the X-ray crystallographic measure-

Table 1. Optimized H-bond lengths and angles for AT base pairs.[a]

System		bond length (Å)		bond angle (degree)	
		N6–O4	N1–N3	N6-H6-O4	N1-H3-N3
isolated base pair[b]		2.93 (2.94)	2.85 (2.84)	174.3 (174.5)	179.5 (180.0)
DNA:[c]	dA-dT	2.94 (2.95)	2.83 (2.82)	173.8	179.0
aegPNA:	pA-pT	2.93	2.85	174.8	179.4
	pA-dT	2.93	2.85	175.0	179.6
	pT-dA	2.93	2.85	174.5	179.4
acpcPNA:	pA-pT	2.93	2.85	174.3	179.5
	pA-dT	2.91	2.87	175.8	179.2
	pT-dA	2.94	2.83	173.8	179.0

[a] Bond lengths and bond angles are read from adenine to thymine.
[b] The values in the parentheses are from the previous calculations (Bertran et al., 1998).
[c] The values in the parentheses are from the experimental data as the X-ray crystallographic measurements (2)

Table 2. Optimized H-bond lengths and angles for GC base pairs.[a]

System		bond length (Å)			bond angle (degree)		
		O6–N4	N1–N3	N2–O2	O6-H4-N4	N1-H1-N3	N2-H2-O2
isolated base pair[b]		2.79 (2.79)	2.95 (2.93)	2.92 (2.92)	179.6 (179.5)	177.4 (177.8)	178.8 (179.4)
DNA: [c]	dG-dC	2.81 (2.91)	2.93 (2.95)	2.90 (2.86)	179.0	177.9	179.0
aegPNA:	pG-pC	2.79	2.94	2.92	179.7	178.3	180.0
	pG-dC	2.80	2.94	2.90	179.5	176.9	177.6
	pC-dG	2.79	2.94	2.91	178.2	177.6	179.2
acpcPNA:	pG-pC	2.79	2.95	2.92	179.6	177.4	178.8
	pG-dC	2.81	2.93	2.87	179.8	177.5	180.0
	pC-dG	2.76	2.96	2.95	178.7	176.0	178.1

[a] Bond lengths and bond angles are read from guanine to cytosine.
[b] The values in the parentheses are from the previous calculations (Bertran et al., 1998).
[c] The values in the parentheses are from the experimental data as the X-ray crystallographic measurements (2)

ments (2) and are similar to those found in the isolated AT base pair. Since in the calculated model the DNA backbones connected to the nucleobases were neutralized by Na^+ ions, thus the repulsion between two negative charges of the phosphate backbones could be very small. It is important to note that the positions of Na^+ ions in the optimized structure remain in the bridging position between two phosphate oxygens. Thus, the anionic DNA backbones should be stabilized.

Similar results of the H-bond geometry affected by the backbone were obtained for

the heterogeneous acpcPNA-DNA backbones, i.e. pA-dT and pT-dA systems. The H-bond lengths and angles were deviated from those of isolated AT pair, especially in the case of pA-dT pair (Table 1), whereas the homogeneous acpcPNA backbones (pA-pT) and all systems of aegPNA backbones provided the identical bond lengths and very less deviation of bond angles compared to the values of isolated base pair. This shows that the aegPNA and homogeneous acpcPNA systems yield a less effect on the H-bond geometry.

In the case of GC base pair, for isolated Watson–Crick base pair the H-bond lengths were 2.79, 2.95 and 2.92 Å for O6–N4, N1–N3 and N2–O2, respectively, and the bond angles were 179.6°, 177.4° and 178.8° for O6-H4-N4, N1-H1-N3 and N2-H2-O2, respectively. When nucleobase attached through the backbone, a small change of the geometrical parameters compared to the isolated system was obtained from the aegPNA and homogeneous acpcPNA systems, i.e. the change of ~0.01 Å for bond length and ~1° for bond angle, see Table 2. The larger different values were found in dG-dC and heterogeneous acpcPNA-DNA systems. The change was found up to 0.05 Å for the bond lengths. The largest deviation was observed for N2–O2 bond in the pG-dC system. The effect of backbones on the GC structure is similar to that found in AT base pair. All aegPNA systems and homogeneous acpcPNA backbones do not provide a significant change in H-bond lengths and angles, whereas the DNA-DNA and heterogeneous acpcPNA-DNA backbones yield a larger effect on the geometrical parameters. Particularly, p(purine)-d(pyrimidine) systems of acpcPNA backbone result the largest effect on the geometry. Generally, however, in all systems the H-bond angles are likely linear.

Apart from the characterization of H-bond geometry, an important task is the evaluation of interaction energies of base pairs. Clearly, there is a correlation between the H-bond characterization and its interaction energy.

3.2 H-bond strengths

H-bond strengths are evaluated in term of H-bond energies according to Eq.[1] and the values were listed in Table 3. In the case of isolated Watson–Crick base pairs, the H-bond energies were –16.4 and –30.4 kcal/mol for AT and GC base pairs, respectively, indicating the larger interaction of GC over AT base pair. Clearly, the higher H-bond strength found in the GC base pair is caused by the larger number of hydrogen bonds (three bonds) and more linearity of H-bond angles compared to those of two bonds in the AT base pair (see Tables 1 and 2).

The addition of the DNA backbones to the isolated base pairs does not result a significant change in the H-bond strengths. The H-bond energies are similar to those found in isolated Watson–Crick base pairs (–16.0 and –30.3 kcal/mol for dA-dT and dG-dC pairs, respectively). As described above, the repulsion of two negative charges in phosphate backbones could be neglected because of the association of counterions.

However, a slight decrease of H-bond strength observed in DNA-DNA systems demonstrates an existence of partial repulsion.

Table 3. H-bond energies (in kcal/mol) of the AT and GC base pairs.[a]

Systems		A(X)-T(Y)	G(X)-C(Y)
isolated base pair		–16.4	–30.4
DNA:	dX-dY	–16.0	–30.3
aegPNA:	pX-pY	–26.5	–33.5
	pX-dY	–22.5	–32.6
	dX-pY	–19.9	–31.5
acpcPNA:	pX-pY	–16.8	–29.7
	pX-dY	–17.0	–32.1
	dX-pY	–20.9	–32.5

[a] Purines are represented by X and pyrimidines are represented by Y. Negative numbers indicate stable conformations.

In all systems comprised of PNA backbones, the interaction energies are significantly larger negative values than those observed for the corresponding base pairs in DNA-DNA systems. For AT base pairs, the H-bond energies were –19.9 to –26.5 kcal/mol and were –16.8 to –20.9 kcal/mol for aegPNA and acpcPNA backbones, respectively. As expected, the stronger H-bond strength is found in GC base pairs (–31.5 to –33.5 kcal/mol for aegPNA and –29.7 to –32.5 kcal/mol for acpcPNA). Interestingly, for the aegPNA systems the heterogeneous backbones, i.e. p(purine)-d(pyrimidine) and d(purine)-p(pyrimidine) systems, yield a weaker H-bond interaction compared to the homogeneous backbones, p(purine)-p(pyrimidine) base pairs. For instance, the computed H-bond energies (–22.5 and –19.9 kcal/mol for pA-dT and dA-pT base pairs, respectively) are less negative values than the energy of pA-pT base pair (–26.5 kcal/mol). This suggests that the H-bond strength in homogeneous base pair is higher than that in heterogeneous base pair. Moreover, the heterogeneous p(purine)-d(pyrimidine) base pairs provide stronger interaction than d(purine)-p(pyrimidine) base pairs; –22.5 kcal/mol for pA-dT *vs*. –19.9 kcal/mol for dA-pT, and –32.6 kcal/mol for pG-dC *vs*. –31.5 kcal/mol for dG-pC. These results are consistent with the experimental data reported previously that the order of thermal stabilities was PNA-PNA > PNA-DNA > DNA-DNA duplexes. In addition, for PNA-DNA duplexes, the higher melting temperatures were obtained when the PNA strands consisted of rich purine bases (20).

In contrast, for acpcPNA backbone, the heterogeneous base pairs result larger H-bond strength than the homogeneous base pairs. The H-bond energies were –17.0 and –20.9 kcal/mol for heterogeneous pA-dT and dA-pT base pairs, respectively, *vs*. –16.8 kcal/mol for homogeneous pA-pT base pair. Also, the computed energies of heterogeneous pG-dC and dG-pC base pairs were about –32 kcal/mol *vs*. –29.7 kcal/mol for homogeneous pG-pC base pair. Furthermore, d(purine)-p(pyrimidine) base pairs trend to provide lager H-bond interaction than p(purine)-d(pyrimidine) base pairs, unlike the aegPNA backbone. Consequently,

one can see that the types of backbones and base pairs significantly affect the H-bond strength. Therefore, one can predict that the stability of PNA-DNA and PNA-PNA double strands should be dependent of the backbones and base pair sequences.

4. Conclusions

We performed a computational work to study the structural and energetic properties of H-bonds in isolated Watson–Crick base pairs, DNA-DNA, PNA-DNA and PNA-PNA base pairs. The aeg and acpc groups were employed for PNA backbones. The H-bond lengths and angles remain roughly constant in most AT and GC base pairs comprised of various backbones, compared to the corresponding isolated base pairs. This indicates that the type of backbones does not provide a significant change in the H-bond geometry. On the other hand, the H-bond strengths are significantly increased when the sugar-phosphate backbone was replaced with the PNA backbone. In addition, the homogeneous aegPNA systems yield stronger H-bond interaction than the heterogeneous aegPNA-DNA hybrids, and the computed energies of p(purine)-d(pyrimidine) base pairs were larger negative than those of d(purine)-p(pyrimidine) base pairs. However, the contrasty results were observed for acpcPNA backbones. This indicates the effect of the types of backbones and base pairs on the H-bond strength, and thus on the stability of PNA-DNA and PNA-PNA double strands.

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