การศึกษาสมบัติของคู่เบสวัตสัน-คริกในไพโรลิดินิลพีเอ็นเอที่จับกับดีเอ็นเอและอาร์เอ็นเอด้วยทฤษฎีฟังก์ชันนอลความหนาแน่น

Property of Watson-Crick Base Pairs in Pyrrolidinyl PNA Binding to DNA and RNA: a Density Functional Theory Study

Nattawee Poomsuk,[1] Amphawan Maitarad,[2] Chittima Laohpongspaisan[1] and Khatcharin Siriwong[1,*]

[1] Materials Chemistry Research Center, Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

[2] Chemistry Program, Faculty of Science and Technology, Uttaradit Rajabhat University, Uttaradit 53000, Thailand.

*Corresponding Author, E-mail: skhatcha@kku.ac.th

บทคัดย่อ

ได้ศึกษาสมบัติของคู่เบสวัตสัน-คริกในกรดเปปไทด์นิวคลีอิก (PNA) ชนิดไพโรลิดินิลที่จับกับดีเอ็นเอ (DNA) และอาร์เอ็นเอ (RNA) โดยใช้การคำนวณด้วยทฤษฎีฟังก์ชันนอลความหนาแน่น แกนหลักของ PNA ที่ศึกษาได้แก่ (2′*R*,4′*R*)-โพรลิล-(1*S*,2*S*)-2-อะมิโนไซโคลบิวเทนคาร์บอกซิลิกแอซิด (acbcPNA), (2′*R*,4′*R*)-โพรลิล-(1*S*,2*S*)-2-อะมิโนไซโคลเพนเทนคาร์บอกซิลิกแอซิด (acpcPNA) และ (2′*R*,4′*S*)-โพรลิล-(1*S*,2*S*)-2-อะมิโนไซโคลเพนเทนคาร์บอกซิลิกแอซิด (*epi*-acpcPNA) ผลการคำนวณพบว่าโครงสร้างเสถียรของคู่เบสของแต่ละระบบไม่แตกต่างกันอย่างมีนัยสำคัญ แสดงให้เห็นว่าแกนหลักของกรดนิวคลีอิกต่างๆ ไม่มีผลต่อลักษณะพันธะไฮโดรเจนของคู่เบส อย่างไรก็ตามแกนหลักเหล่านี้มีผลต่อความแรงของพันธะไฮโดรเจนซึ่งพบว่าอันตรกิริยาระหว่าง acbcPNA กับ DNA หรือ RNA มีค่ามากกว่า PNA ชนิดอื่นๆ นอกจากนี้ยังได้ศึกษาผลของตัวทำละลายต่อความสามารถในการยึดจับกันของคู่เบส พบว่าอันตรกิริยาของคู่เบสลดลงประมาณ 2 เท่า เมื่อเทียบกับคู่เบสที่อยู่ในสถานะแก๊ส ข้อมูลนี้อาจนำไปสู่ความเข้าใจถึงความเสถียรของโมเลกุลสายคู่ PNA-DNA หรือ PNA-RNA ต่อไป

ABSTRACT

The property of Watson-Crick base pairs in pyrrolidinyl peptide nucleic acids (PNAs) binding to DNA and RNA were studied using density functional theory calculation. The studied PNA backbones were (2′*R*,4′*R*)-prolyl-(1*S*,2*S*)-2-aminocyclobutane carboxylic acid (acbcPNA), (2′*R*,4′*R*)-prolyl-(1*S*,2*S*)-2-aminocyclopentane carboxylic acid (acpcPNA) and (2′*R*,4′*S*)-prolyl-(1*S*,2*S*)-2-aminocyclopentane carboxylic acid (*epi*-acpcPNA). The results revealed that the optimized base pair geometries of all system were insignificantly different, implying that the nucleic acid backbones did not affect the hydrogen bond geometry of base pairs. However, the backbones affected the hydrogen bond strength. The interaction between acbcPNA and DNA/RNA was higher than those of other PNAs. The effect of solvent on binding ability of base pairs was also studied and the result revealed that the interaction of base pairs was reduced by about 2 times compared to the gas phase interaction. This information may lead to the understanding of PNA-DNA or PNA-RNA duplex stability.

คำสำคัญ: กรดเปปไทด์นิวคลีอิก คู่เบสวัตสัน-คริก การคำนวณเชิงควอนตัม พันธะไฮโดรเจน

Keywords: Peptide nucleic acid, Watson-Crick base pair, Quantum calculation, Hydrogen bond

INTRODUCTION

Watson-Crick base pairs (WC bps) are the specific hydrogen bond patterns that allow two nucleic acid strands to retain a regular helical structure. In deoxyribonucleic acid (DNA), adenine (A) binds to thymine (T) with two hydrogen bonds while guanine (G) binds to cytosine (C) with three hydrogen bonds. In ribonucleic acid (RNA), thymine is substituted by uracil (U) as shown in Fig.1 (Watson and Crick, 1953; Dahm, 2005).

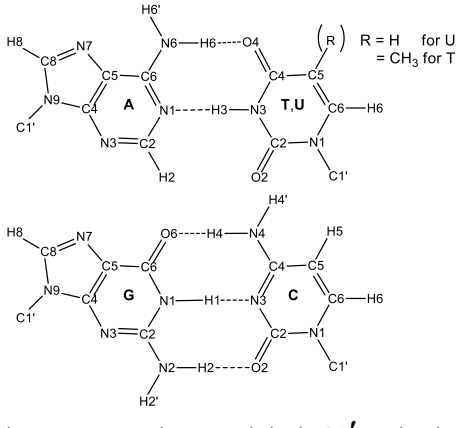

Fig. 1 Watson-Crick base pairs with atom label, C1′ is the linking atom to backbone.

Peptide nucleic acid (PNA) is an unusual DNA-like structure that has been developed for a wide range of applications. It can be used as an antisense/antigene drug for sequence specification of gene expression and also as molecular tools for probing and manipulating DNA/RNA structures (Germini et al., 2005; Ivanova et al., 2008). PNA structure is made by replacing a non-charge polyamide residue on sugar-phosphate group to create polypeptide backbone. Over 20 years ago, the nucleic acid with *N*-2 -aminoethylglycine backbone, namely aegPNA (Fig. 2), was first discovered by Nielsen's group (Nielsen et al., 1991; Egholm et al., 1992). The aegPNA was able to bind to DNA, RNA and also itself, resulting PNA-DNA, PNA-RNA and PNA-PNA duplexes, respectively, with highly binding affinity. Furthermore, PNA structure also expresses the distinct properties, for example, the highly thermal and chemical stability, the fairly stability in high ionic strength, and resistance to nucleases and proteases. However, due to the lack of electrostatic charge in PNA backbone, it has poor water solubility compared to DNA. During the past decade, a new series of pyrrolidinyl PNA consisting of pyrrolidine ring and cyclic β-amino acid spacer was developed by Vilaivan and co-workers (Vilaivan et al., 2001; Suparpprom et al., 2005; Vilaivan et al., 2011). The most powerful pyrrolidinyl PNA backbones, which is stable to salt concentration and temperature and also exhibits sequence specificity when binding to DNA or RNA, are (2′*R*,4′*R*)-prolyl-(1 *S*,2 *S*)-2 -aminocyclobutane carboxylic acid (acbcPNA), (2′*R*,4′*R*)-prolyl-(1 *S*,2 *S*)-2 -aminocyclopentane carboxylic acid (acpcPNA) and (2′*R*,4′*S*)-prolyl-(1 *S*,2 *S*)-2 -aminocyclopentane carboxylic acid (*epi*-acpcPNA) (Fig. 2) . However, the understanding insight into the structural property and molecular behavior of the pyrrolidinyl PNA is still unclear due to the unavailability of its X-ray structure.

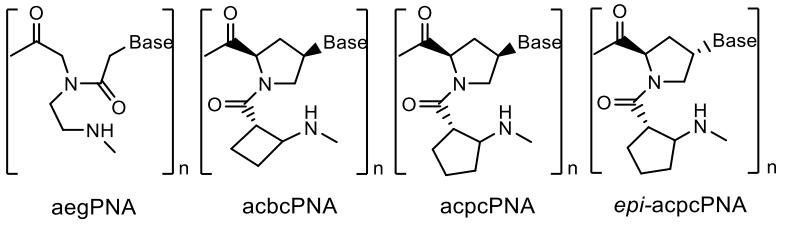

Fig. 2 The chemical structures of PNA with *N*-2-aminoethylglycine (aegPNA), (2′*R*,4′*R*)-prolyl-(1*S*,2*S*)-2-aminocyclobutane carboxylic acid (acbcPNA), (2′*R*,4′*R*)-prolyl-(1*S*,2*S*)-2-aminocyclopentane carboxylic acid (acpcPNA) and (2′*R*,4′*S*)-prolyl-(1*S*,2*S*)-2-aminocyclopentane carboxylic acid (*epi*-acpcPNA) backbones.

Recently, hydrogen bond (H-bond) interaction in aegPNA-DNA single base pair was comparatively studied using quantum calculation via density functional theory (DFT) method (Herbert, 2006). The polarizable continuum model (PCM) was also employed to evaluate solvation effect on the interaction energy. For pyrrolidinyl PNA, the structural and energetic properties of single WC bps of acpcPNA binding to DNA and itself were reported previously. (Chaiyatoom and Siriwong, 2011). The calculated results were consistent with the experimental observation, where the H-bond strength of acpcPNA-DNA was stronger than that of aegPNA-DNA, while self-paring of acpcPNA was weaker than aegPNA. Nevertheless, insight into the geometry of PNA backbone is still undescribed. In addition, acbcPNA and *epi*-acpcPNA backbones as well as PNA-RNA hybrid have not been studied.

In this work, the WC bps in PNA-DNA and PNA-RNA systems, using acbcPNA, acpcPNA and *epi*-acpcPNA as PNA backbones, were studied with DFT calculation. The geometry of Watson-Crick H-bond and PNA backbone and also binding affinity between nucleobases were also considered. Since the solvent plays an important role in nucleic acid stability, the optimization and energy calculation were also performed in solvation state using PCM approach to investigate the solvation effect on base pair stability.

DETAILS OF CALCULATION

Various single WC bps of PNA-DNA and PNA-RNA systems were constructed using HyperChem 8.0 program (Hypercube, 2006). To neutralize a negative charge of phosphate group containing in DNA/RNA backbone, Na^+ was added by placing as a bridging position between two ionized oxygens (O^-) of phosphate group (Herbert, 2006). The isolated base pairs, i.e. without backbone, were also studied for comparison. All prepared structures were fully optimized using DFT method at B3LYP method (Becke, 1988; Lee et al., 1988) with the 6-31G(d) basis set (Rassolov et al., 2001). The optimized structures were then employed to calculate single point energy (SPE) using B3LYP/6-31+G(d,p) method (Clark et al., 1983). The SPE was corrected with zero point energy (ZPE) calculated at B3LYP/6-31G(d) level and basis set superposition error (BSSE) obtained from B3LYP/6-31+G(d,p) calculation. All calculations were carried out using Gaussian 09 program (Frisch et al., 2009). The interaction energy (ΔE) which is mainly contributed from hydrogen bonding of base pair was obtained according to equation

$$\Delta E = E_{\text{base pair}} - (E_{\text{base1}} + E_{\text{base2}})$$

where $E_{\text{base pair}}$, E_{base1} and E_{base2} are the corrected SPE of base pair, single base 1 and

single base 2, respectively, based on the same optimized structure.

Because the applications of PNAs as molecular tools or as therapeutic agents do not generally take place in the gas phase, the solvation effect is therefore clearly important as mentioned above (Tomasi et al., 2005). Here, the conductor-like PCM was employed to investigate the stability of base pair in aqueous environment.

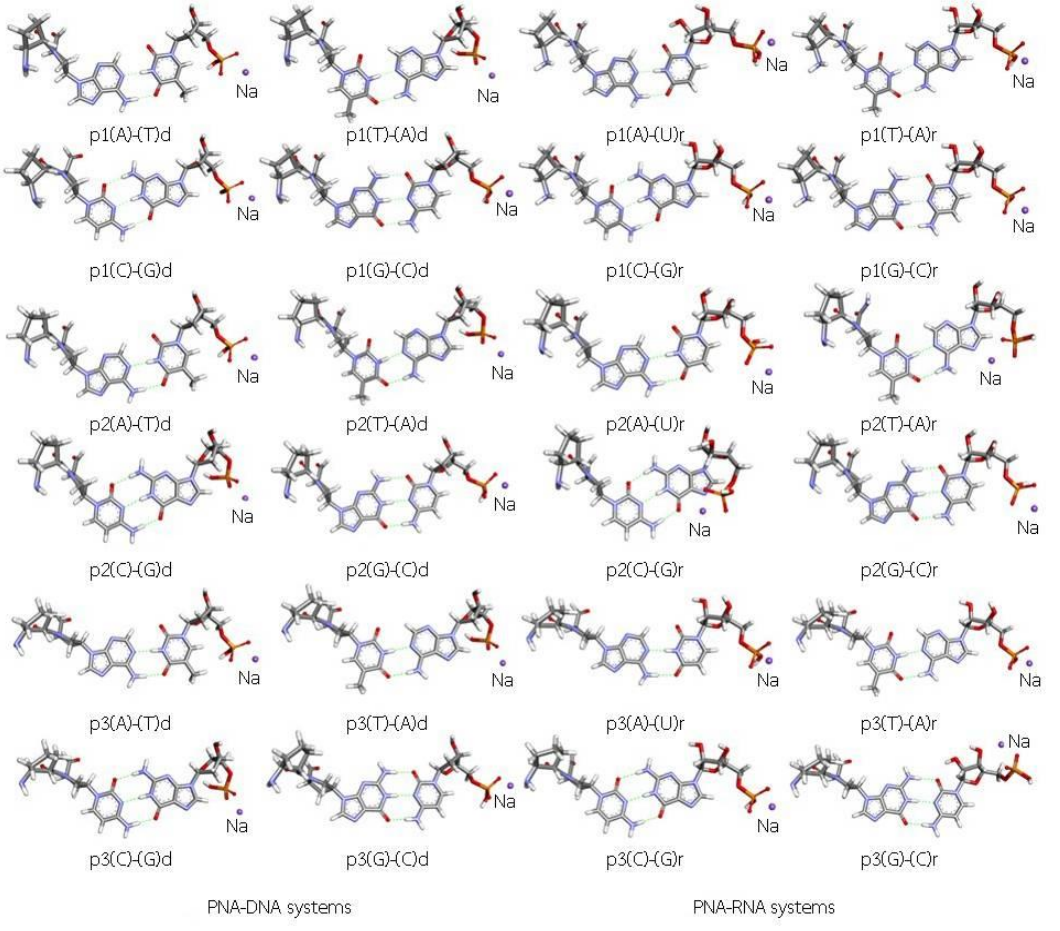

Fig. 3 Optimized structures of various PNA-DNA and PNA-RNA single base pairs (p1, p2, p3, d and r stand for acbcPNA, acpcPNA, *epi*-acpcPNA, DNA and RNA backbones, respectively).

RESULTS AND DISCUSSION

1. H-bond geometry

The optimized structures of single base pairs with various backbones obtained from the DFT calculation were presented in Fig. 3. The H-bond geometry was considered in terms of bond length and bond angle between H-bond donor and acceptor (see Fig. 1 for atom

label). As the results, the optimized structures of isolated base pairs agreed very well with the experiment. The bond length and bond angle measurements observed from the optimized structures were very close to the X-ray crystallographic structure (Yanson et al., 1979). For both isolated A-T and G-C base pairs, bond length was in the range of 2.8–3.0 Å and bond angle was almost linear (174–180°). When the backbones were added, despite of the type of backbone, the H-bond geometry was still roughly constant as found in isolated A-T and G-C base pairs. This indicated that the type of backbone did not affect the hydrogen bond geometry of Watson-Crick base pair.

For A-T system, the hydrogen bond lengths of N6···O4 varied from 2.91 to 2.96 Å whereas those of N1···N3 were slightly shorter (varying from 2.87 to 2.91 Å). The bond angles of N6–H6···O4 exhibited slightly larger deviation from linearity with the values of about 174° to 177°, compared with those of N1···H3–N3 where the angles were very close to the linearity with the values larger than 178°. For G-C system, the bond distance of O6···N4 seemed to be slightly shorter (~2.8 Å) than those of N1···N3 and N2···O2 (~3.0 Å) whereas all bond angles were similar.

2. Torsion angle of PNA

As the experimental 3D structures of pyrrolidinyl PNAs are unavailable, the torsion angles ϕ and θ (see Fig.4) of optimized PNA structure were considered by comparing with the X-ray structures of *trans*-acbc (Fernandes et al., 2010) and *trans*-acpc (Appella et al., 1999) oligomers. As listed in Table 1, it was found that ϕ angle in both oligomers was insignificantly different whereas θ angle of *trans*-acpc was smaller. The modeled PNAs yielded the deviation of both ϕ and θ angles compared with the experimental data. This should be due to unconstrained terminals of PNA backbone in single base pair allowing a free movement of the backbone, while the movement of backbone in oligomer was restrained by neighboring units. Therefore the large deviation of the torsion angle was observed in the optimized PNAs. Furthermore, both ϕ and θ angles of *epi*-acpcPNA backbone was rather different from those of acpcPNA, indicating the effect of stereochemistry on PNA structure.

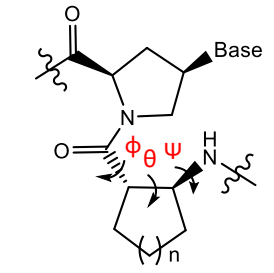

Fig. 4 Definition of torsion angles for PNA backbone.

Table 1 Torsion angles[a] (in degree) of PNA obtained from the optimized PNA-DNA and PNA-RNA single base pairs.

System		ϕ	θ
Oligomer:	*trans*-acbc	–99	102
	trans-acpc	–100	94
PNA-DNA:	acbcPNA	–106(7)	100(0)
	acpcPNA	–107(1)	74(0)
	epi-acpcPNA	–161(0)	69(0)
PNA-RNA:	acbcPNA	–92(1)	95(0)
	acpcPNA	–99(3)	101(10)
	epi-acpcPNA	–159(5)	72(8)

[a] The values were averaged over all A-T and G-C pairs including PNA-DNA and PNA-RNA backbones (standard deviations are in the parentheses).

3. Interaction Energy

The interaction energy (ΔE) refers to the interaction between two molecules. For nucleic acid, the higher stability of nucleic acid double helix is owing to the stronger interaction of WC bps which is mainly contributed by hydrogen bonding between base pairs. The calculated ΔE obtained from optimized structures were listed in Table 2.

Note that the reported experimental data of ΔE in gas phase for isolated A-T and G-C base pairs were –13 and –21 kcal/mol, respectively, (Yanson et al., 1979) implying that the calculated energy was under estimation for A-T and over estimation for G-C base pair (see Table 2). Such deviation caused by the calculation method was also reported by Herbert and co-workers (Herbert et al., 2006). However, for a comparative study, the B3LYP method with 6-31G(d) or 6-31+G(d,p) basis set can provide a reasonable result.

By considering the calculated gas phase energy of A-T(U) system, it was found that DNA and RNA backbones slightly

destabilized the interaction of isolated base pair, i.e. decreases in interaction energies of about 0.6 and 0.4 kcal/mol were observed for d(A)-(T)d and r(A)-(U)r, respectively. This is not surprising because both sides of base pair are negatively charged backbones which are repulsive. Although the backbones are neutralized with Na^+, the repulsion can take place. When replacing one sugar-phosphate backbone in DNA-DNA or RNA-RNA pair with PNA backbone, the base pair interactions were increased slightly, especially for all PNA-RNA systems where the calculated energies were found to be larger than –12 kcal/mol. Moreover, acbcPNA seemed to yield slightly larger interaction compared to five-membered ring PNAs. This was in agreement with the experimental melting temperature (T_m) where the T_m value of acbcPNA-DNA was higher than those of acpcPNA-DNA and *epi*-acpcPNA-DNA duplexes containing the same base sequence (Vilaivan et al., 2011).

Table 2 The interaction energy (Δ*E*, in kcal/mol) corrected with ZPE and BSSE calculations in gas phase and aqueous phase.

Systems	Base pairs	Δ*E*		Base pairs	Δ*E*	
		Gas	Aqueous		Gas	Aqueous
Isolated base pair:	(A)-(T)	–11.81	–6.24	(G)-(C)	–25.94	–10.70
	(A)-(U)	–11.99	–6.29			
DNA-DNA:	d(A)-(T)d	–11.24	–6.30	d(G)-(C)d	–26.08	–11.01
RNA-RNA:	r(A)-(U)r	–11.55	–6.37	r(G)-(C)r	–26.06	–10.87
acbcPNA-DNA:	p1(A)-(T)d	–12.09	–6.51	p1(C)-(G)d	–24.95	–11.44
	p1(T)-(A)d	–12.63	–6.60	p1(G)-(C)d	–27.85	–11.97
acpcPNA-DNA:	p2(A)-(T)d	–11.96	–6.57	p2(C)-(G)d	–24.37	–11.72
	p2(T)-(A)d	–11.62	–6.49	p2(G)-(C)d	–27.76	–12.33
epi-acpcPNA-DNA:	p3(A)-(T)d	–11.82	–6.36	p3(C)-(G)d	–24.93	–11.71
	p3(T)-(A)d	–12.21	–6.44	p3(G)-(C)d	–27.29	–11.97
acbcPNA-RNA:	p1(A)-(U)r	–12.66	–6.85	p1(C)-(G)r	–25.51	–11.64
	p1(T)-(A)r	–12.27	–6.50	p1(G)-(C)r	–26.10	–11.87
acpcPNA-RNA:	p2(A)-(U)r	–12.11	–6.64	p2(C)-(G)r	–23.87	–11.63
	p2(T)-(A)r	–12.15	–6.13	p2(G)-(C)r	–28.10	–11.78
epi-acpcPNA-RNA:	p3(A)-(U)r	–12.35	–6.57	p3(C)-(G)r	–25.65	–11.93
	p3(T)-(A)r	–12.22	–6.51	p3(G)-(C)r	–23.63	–11.86

For G-C base pair, the calculated energies were about two times larger than those of A-T(U) system due to more hydrogen bonds in G-C base pair. For both DNA-DNA and RNA- RNA backbones, the energy was about –26 kcal/mol, which was very close to isolated

G-C base pair. This implied that the DNA and RNA backbones neutralized with the Na^+ did not affect to the binding ability of base pairs, unlike A-T(U) system. Interestingly, when one side of DNA-DNA or RNA-RNA hybrid was replaced with PNA backbone, the energy changed remarkably, especially when guanine was located in PNA backbone (except for p3(G)-(C)r base pair). This evident was not found in A-T(U) system. Such finding indicates that the pyrrolidinyl PNA backbone plays an important role on electron density of G and C nucleobases, and thus directly affects the binding ability of G-C base pair.

It is worth noting that the values of BSSE used to correct the interaction energy of all systems were about 0.7 to 0.8 kcal/mol, indicating that the type of backbone does not significantly affect the BSSE calculation. This implies that BSSE correction can be neglected for a comparative study on the interaction energy because this calculation is computational time consuming.

In aqueous phase, the ΔE values were significantly reduced from the gas phase energy, about 1.8–2.0 times for A-T(U) and 2.0–2.3 times for G-C base pairs. This is consistent with solvent interactions with the solute dipoles that decrease the strength of hydrogen bonding (Herbert et al., 2006). A larger number of hydrogen bonds of G-C base pair compared to A-T(U) base pair (three *vs*. two) was accounted for the larger decrease in its stability. As clearly seen in Table 2, the decrease of base pair stability of PNA-DNA and PNA-RNA hybrids due to solvent effect was not significantly different, confirming that charge screening of backbones is less than that of nucleobases.

CONCLUSION

In this work, the single base pairs of PNA-DNA and PNA-RNA hybrids were studied using quantum calculation via DFT method. The pyrrolidinyl PNA backbones used in this studied were acbcPNA, acpcPNA and *epi*-acpcPNA. The calculated results demonstrated that the backbones did not affect the hydrogen bond geometry of Watson-Crick base pairs. However, the calculated interaction energy revealed that all PNA backbones directly affected the binding ability of base pair. It was found that acbcPNA provided larger interaction compare to other PNAs. In addition, solvation effect could greatly destabilize base pair interaction. It significantly screened the hydrogen bond region compared to backbone region by reducing the effective dipole of each hydrogen bond. This yielded the decrease of interaction energy of about 2 times compared to the base pair in gas phase. This information may lead to the understanding of PNA-DNA or PNA-RNA duplex stability.

ACKNOWLEDGMENTS

This work has been financially supported by Science Achievement Scholarship of Thailand, Materials Chemistry Research Center, Khon Kaen University, and Uttaradit Rajabhat University. National e-Science Infrastructure Consortium (URL: http://www.e-science.in.th) is acknowledged for computing resources.

REFERENCES

Appella, D.H., Christianson, L.A., Karle, I.L., Powell, D.R. and Gellman, S.H. (1999). Synthesis and characterization of trans-2-aminocyclo hexanecarboxylic acid oligomers: an unnatural helical secondary structure and implications for β-peptide tertiary structure. J Am Chem Soc. 121: 6206–6212.

Becke, A.D. (1988). Density-functional exchange-energy approximation with correct asymptotic behavior. Phys Rev A 38: 3098–3100.

Chaiyatoom, W. and Siriwong, K. (2011). Structural and energetic properties of hydrogen bonds in PNA-DNA and PNA-PNA base pairs: a theoretical study. KKU Res J. 16: 587–595.

Clark, T., Chandrasekhar, J., Spitznagel, G.W. and Schleyer, P.v.R. (1983). Efficient diffuse function-augmented basis sets for anion calculations. III. The 3-21+G basis set for first-row elements, Li–F. J Comp Chem. 4: 294–301.

Dahm, R. (2005). Friedrich Miescher and the discovery of DNA. Dev Biol. 278: 274–288.

Egholm, M., Buchardt, O., Nielsen, P.E. and Berg, R.H. (1992). Peptide nucleic acids (PNA). Oligonucleotide analogs with an achiral peptide backbone. J Am Chem Soc. 114(5): 1895-1897.

Fernandes, C., Faure, S., Pereira, E., Théry, V., Declerck, V., Guillot, R. and Aitken, D.J. (2010). 12-helix folding of cyclobutane β-amino acid oligomers. Org Lett. 12: 3606–3609.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Cheeseman, J.R. and Scalmani, G., et al. (2009). Gaussian 09 Revision B. 01. Wallingford CT: Gaussian.

Germini, A., Rossi, S., Zanetti, A., Corradini, R., Fogher, C. and Marchelli, R. (2005). Development of a peptide nucleic acid array platform for the detection of genetically modified organisms in food. J Agric Food Chem. 53: 3958–3962.

Herbert, H.E., Halls, M.D., Hratchian, H.P. and Raghavachari, K. (2006). Hydrogen-bonding interactions in peptide nucleic acid and deoxyribonucleic acid: a comparative study. J Phys Chem B 110(7): 3336–3343.

HyperChem Professional 8 .0. (2006). USA, Florida: Hypercube.

Ivanova, G.D., Fabani, M.M., Arzumanov, A.A., Abes, R., Yin, H. and Lebleu, B., et.al. (2008). PNA-peptide conjugates as intracellular gene control agents. Nucleic Acids Symp Ser. 52: 31–32.

Lee, C., Yang, W. and Parr, R. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B 37: 785–789.

Nielsen, P.E., Egholm, M. and Berg, R.H., Buchardt, O. (1991). Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science, 254(5037): 1497-1500.

Rassolov, V.A., Ratner, M.A., Pople, J. A., Redfern, P.C. and Curtiss, L.A. (2001). 6-31G* Basis set for

third-row atoms. J Comp Chem. 22: 976–984.

Saenger, W. (1984). Principles of nucleic acid structure. New York: Springer Verlag.

Suparpprom, C., Srisuwannaket, C., Sangvanich, P. and Vilaivan, T. (2005). Synthesis and oligodeoxynucleotide binding properties of pyrrolidinyl peptide nucleic acids bearing prolyl-2-aminocyclopentanecarboxylic acid (ACPC) backbones. Tetrahedron Lett. 46: 2833–2837.

Tomasi, J., Mennucci, B. and Cammi, R. (2005). Quantum mechanical continuum solvation models. Chem Rev. 105: 2999–3093.

Vilaivan, T., Suparpprom, C., Harnyuttanakorn, P. and Lowe, G. (2001). Synthesis and properties of novel pyrrolidinyl PNA carrying β-amino acid spacers. Tetrahedron Lett. 42: 5533–5536.

Vilaivan, C., Srisuwannaket, C., Ananthanawat, C., Suparpprom, C., Kawakami, J. and Yamaguchi, Y., et al. (2011). Pyrrolidinyl peptide nucleic acid with α/β-peptide backbone: a conformationally constrained PNA with unusual hybridization properties. Artif *DNA PNA XNA* 2: 50–59.

Watson, J. and Crick, F. (1953). Genetical implications of the structure of deoxyribonucleic acid. Nature 171: 964–967.

Yanson, I.K., Teplitsky, A.B. and Sukhodub, L.F. (1979). Experimental studies of molecular interactions between nitrogen bases of nucleic acids. Biopolymers 18: 1149–1170.

❑❑❑❑❑