Biochemical and Biophysical Research Communications 372 (2008) 765–771

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Insight into why pyrrolidinyl peptide nucleic acid binding to DNA is more stable than the DNA·DNA duplex

Khatcharin Siriwong [a], Parawan Chuichay [b], Suwipa Saen-oon [c,d], Chaturong Suparpprom [d], Tirayut Vilaivan [d], Supot Hannongbua [d,*]

[a] Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Physics, Faculty of Science, Kasetsart University, 50 Phahon Yothin Road, Cha-tuchak, Bangkok 10900, Thailand
[c] Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[d] Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 19 May 2008
Available online 2 June 2008

Keywords:
Molecular dynamics simulation
Peptide nucleic acids
Pyrrolidinyl PNA
PNA/DNA
Binding free energy

ABSTRACT

Molecular dynamics (MD) simulations and experimental measurements of the stability of a novel pyrrolidinyl PNA binding to DNA (PNA·DNA) in both parallel and antiparallel configurations were carried out. For comparison, simulations were also performed for the DNA·DNA duplex. The conformations of the three simulated systems were found to retain well-defined base pairing and base stacking as their starting B-like structure. A large gas-phase energy repulsion of the two negatively charged sugar-phosphate backbones of the DNA strands was found to reduce the stability of the DNA·DNA duplex significantly compared with that of the PNA·DNA complexes, especially in the antiparallel binding configuration. In addition, the antiparallel PNA·DNA was observed to be less solvated than that of the other two systems. The simulated binding free energies and the experimental melting temperatures for the three investigated systems are in good agreement, indicating that the antiparallel PNA·DNA is the most stable duplex.

Peptide nucleic acid (PNA) has attracted wide attention due to its unique properties and applications in the fields of biotechnology and therapeutics (see, for example, references [1–6] for reviews). Not surprisingly, much attention has been paid to developing novel PNA analogs with the aim of achieving even better binding properties which would broaden their applications. Therefore, understanding of fundamental properties such as molecular interactions, structural data as well as ligand solvation, is recognized to be the key to success in designing and discovering a new class of PNA analogs that serves the above purpose.

PNA is a DNA analog in which the sugar-phosphate backbone of DNA is replaced by poly(*N*-aminoethylglycine) with the nucleobases attached through a methylenecarbonyl linkage at the glycine nitrogen (see Fig. 1A and B). The electrostatically neutral PNA molecule is capable of recognizing its complementary sequence in DNA with high sequence specificity, forming Watson–Crick basepairs and leading to PNA·DNA duplexes that are considerably more stable than the corresponding DNA complexes [7–9]. This is believed mainly to be due to the lack of electrostatic repulsion between the uncharged PNA and negatively charged DNA strands [7]. Moreover, a PNA strand can bind to its complementary DNA strand both in a parallel fashion where the C-terminus of the PNA strand is oriented toward the 3′end of the complementary DNA strand, and in an antiparallel fashion where the orientation is opposite, i.e., the C-terminus is oriented in the direction of the 5′ end [7,10]. The antiparallel PNA·DNA complex with achiral *N*-(2-aminoethyl)glycine (*aeg*) backbone has been found to be marginally more stable than the corresponding parallel PNA·DNA but both are much more stable than DNA·DNA duplexes [7]. PNA can also bind to its complementary strand to form PNA·PNA [11,12], as well as to PNA·DNA duplexes to form PNA·DNA·PNA triplexes [10,13,14].

Most of the newly designed PNA analogs are based on partial modification of the original *aeg* backbone to form chimeric PNA [15–17]. Recently, Vilaivan and co-workers [18–20] have synthesized a series of conformationally constrained chiral analogs of PNA based on the pyrrolidine and cyclic beta-amino acid core structures (see Fig. 1C and D), and studied their interactions with nucleic acids by UV and CD spectroscopy. 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. Theoretical studies of structural and dynamical properties have so far been limited to classical *aeg*PNA [21–25]. On the other hand, the three-dimensional structure of the pyrrolidinyl PNA·DNA double helix, both in solution and in crystal structure, is presently unknown.

To understand the origin of the higher stability of pyrrolidinyl PNA·DNA in comparison with the DNA·DNA duplex, molecular dynamics (MD) simulations were carried out for the DNA·DNA, PNA·DNA parallel, and PNA·DNA antiparallel systems (for the sche-

* Corresponding author. Fax: + 66 2 2187603.
E-mail address: supot.h@chula.ac.th (S. Hannongbua).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.102

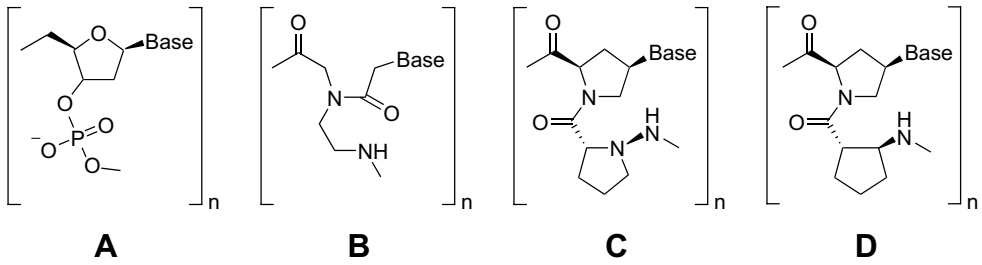

Fig. 1. Monomeric units of (A) DNA, and PNA with (B) *N*-(2-aminoethyl)glycine, (C) pyrrolidinyl D-aminopyrrolidine-2-carboxylic acid and (D) pyrrolidinyl 2*S*-amino-cyclopentane-1*S*-carboxylic acid backbones.

matic representations of the PNA·DNA and DNA·DNA duplexes studied, see Fig. SM1 in Supporting information). The results were investigated at the molecular level in terms of structural properties, solvation and binding free energies of the duplexes. In addition, the preferred direction of binding between PNA and DNA (parallel vs. antiparallel) was predicted and experimentally verified.

Materials and methods

Molecular dynamics simulations. Three MD simulations have been carried out using the AMBER 8 program [26]. The first two systems were the parallel and antiparallel PNA·DNA duplexes, where the PNA strand consists of the chiral pyrrolidinyl PNA backbone with decamer thymine bases (Fig. 1C), and its complementary DNA strand contains decamer adenine bases. The last system was an antiparallel DNA·DNA duplex with the same sequence for comparison. All starting coordinates were based on the canonical B-DNA prepared as follows. The B-form of a DNA·DNA duplex with 10 A·T pairs was generated using the NUGEN module of AMBER 8. Since the three-dimensional structure of pyrrolidinyl PNA is not available, its starting structure was built up based on that of the DNA·DNA duplex by one-to-one mapping of the PNA backbone atoms onto DNA backbone atoms [21]. The coordinates of the missing atoms were added using geometric calculation. To improve the structure of this modified backbone, partial optimization was then performed using molecular mechanics calculations. The force field parameters of PNA are not available in the AMBER package. They were prepared within the standard procedure (for details see Supporting information).

The MD simulations were performed in aqueous solution using the standard condition (truncated octahedral box containing TIP3P water molecules [27], neutralized the system with sodium ions, AMBER-parm99 force field [28] complemented with the prepared parameters, SHAKE algorithm [29] employed for all hydrogen atoms, time step of 2 fs, temperature of 300 K, pressure of 1 atm, periodic boundary conditions, cutoff of 9 Å for nonbonded interactions, and particle mesh Ewald method to account for long-range interactions [30]). The simulation protocol has followed the standard procedures described elsewhere [24]. After achieving equilibration, unrestrained MD simulations were initiated and after the first nanosecond the coordinates were stored every 1 ps for 2 ns. The trajectories over these last 2 ns were then analyzed.

Binding free energy calculations. The binding free energy, $\Delta G_{\text{binding}}$, *for a double helix can be calculated as the difference of free energies,* G^x, where *x* stands for duplex, strand1 or strand2, expressed as [31–33]

$$\triangle G_{\text{binding}} = G^{\text{duplex}} - G^{\text{strand1}} - G^{\text{strand2}}. \quad (1)$$

Each free energy G^x was computed as an average over snapshots from the MD trajectories, according to the following equation:

$$G^x = H^x_{\text{gas}} + H^x_{\text{trans/rot}} + G^x_{\text{solvation}} - TS^x, \quad (2)$$

where H_{gas} is the gas-phase energy calculated using the molecular mechanics (MM) method, and $H_{\text{trans/rot}}$ corresponds to the energy due to six translational and rotational degrees of freedom, which is $6 \times \frac{1}{2}RT$ and equals 1.79 kcal/mol at 300 K. In addition, $G_{\text{solvation}}$ is the solvation free energy estimated using a continuum approach based on the generalized Born/surface area (GB/SA) model developed by Onufriev et al. [34]. More details of the calculation and applied parameters have been described elsewhere [33].

The last, entropic term *TS* in Eq. (2), can be estimated by normal mode (NMODE) analysis [35]. A distance-dependent dielectric constant ($\varepsilon = 4R_{ij}$, where R_{ij} is the distance between atoms *i* and *j*) was applied for minimizations and the NMODE frequencies were calculated at 300 K. In all cases, the free energies were determined for all snapshots of 2 ns MD trajectories.

Synthesis and UV melting studies of parallel and antiparallel PNA·DNA hybrids. Pyrrolidinyl PNA (Fig. 1C) was prepared with two sequences, Ac-TATATTTT-LysNH$_2$ (**c1**) and Ac-TTTTATAT-LysNH$_2$ (**c2**), by solid phase peptide synthesis from the corresponding Fmoc-protected monomers, following the published procedure [18]. In brief, the PNA syntheses were carried out on 1 μmol scales on Tentagel S RAM resin preloaded with Fmoc-Lys(Boc)-OPfp. The synthesis cycle is as follows: deprotection: 20% piperidine in DMF (1.0 mL, 15 min), wash (DMF), coupling [Pfp activated monomers/HOAt/DIEA (1:1:1) in DMF, 4 equiv, 30 min], wash (DMF), capping (4 equiv lauroyl chloride/DIEA in DMF), wash (DMF). The coupling reaction was monitored by measurement of the amounts of dibenzofulvene piperidine adduct released upon deprotection at 264 nm. After addition of the final residue was completed, the N-terminal Fmoc group was removed by 20% piperidine in DMF and acetylated. The benzoyl protecting group at adenine N^6 was removed by treatment with 1:1 concentrated aqueous ammonia:dioxane at 55 °C for 6 h. The PNA was released from the resin by treatment with 95% trifluoroacetic acid (room temperature, 1 h). The cleavage solution was evaporated to dryness, followed by precipitation of the PNA with ether. The crude PNAs were purified by reversed-phase HPLC using a gradient of water–acetonitrile containing 0.1% trifluoroacetic acid (monitoring by UV absorbance at 270 nm). The identities of the two PNAs were confirmed by MALDI-TOF mass spectrometry using a CCA matrix: **c1**: *m/z* calc. 2872.988, found 2872.222; **c2**: *m/z* calc. 2872.988, found 2874.024.

Then T_m experiments were performed on a CARY 100 UV Spectrophotometer (Varian Ltd.) equipped with a thermal melt system. The sample for T_m measurement was prepared by mixing equimolar quantities of the stock oligonucleotide and PNA solutions together to give the final concentration of PNA strands of 1 μM in sodium phosphate buffer (pH 7.0). The A_{260} was recorded in steps from 20–90 °C (block temperature) with a temperature increment of 1 °C /min. The results were normalized by dividing the absorbance at each temperature by the initial absorbance. Finally, T_m