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Multivalent ^{L}Kγ-PNA Oligomers Bind to a Human Telomere DNA G-Rich Sequence to Form Quadruplexes

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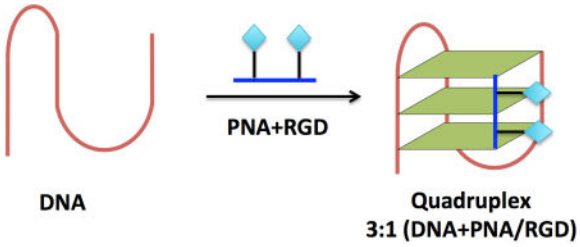
Abstract

We report G-quadruplex formation between peptide nucleic acids (PNAs) composed of ^{L}Kγ-PNA-G monomers and a known portion of human telomeric DNA that adopts three G3 tracts via intramolecular hydrogen bonding. The resulting complex is a bimolecular PNA–DNA heteroquadruplex. In this report, we show that introduction of a γ-modification and addition of a peptide ligand does not disrupt the heteroquadruplex. Although the unmodified PNA1 forms a quadruplex with itself, the γ-substituted PNAs (PNA2 – PNA6) do not form G-quadruplexes on their own, at even high concentrations. The selectivity of these PNAs could influence the design of new quadruplex-targeting molecules or allow the quadruplex structure to be used as a scaffold for multivalent display of protein binding ligands.

Graphical abstract



Keywords

Quadruplex; DNA; PNA

Guanine-rich oligonucleotides have shown the ability to form alternative secondary structures by utilizing Hoogesten base pairs to form guanine tetrads that are stabilized in the presence of potassium or sodium cations (Figure 1a).[1, 2] These guanine tetrads are able to πstack, thereby forming stable secondary structures referred to as guanine quadruplexes (G-Quadruplexes).[3, 4] These G-Quadruplexes have been proposed to assist in biological functions such as gene regulation[5–8] and chromosomal stability.[9, 10] Several studies have

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explored the biophysical and structural properties of quadruplexes both *in vivo*[9, 11] as well as *in vitro*.[10, 12, 13] There are many strategies for targeting quadruplexes, including small molecules[14], engineered DNA-binding proteins[15, 16], and complementary oligonucleotides such as locked nucleic acids (LNAs)[17, 18] and peptide nucleic acids (PNAs).[19–21]

In addition to complimentary binding, a quadruplex can be targeted by exploiting homologous recognition. By replacing one or more G_3 tracts with a short, guanine-rich PNA, a stable heteroquadruplex can be formed. An assortment of heteroquadruplexes have been reported with various structures and stoichiometries (i.e. DNA_2:PNA_2).[19, 22–28] Previous studies have investigated an alternative stoichiometry where a short G_3 PNA can form a heteroquadruplex with a DNA containing three G_3 tracts.[29] This asymmetric design suggests the possibility of building scaffolds with ligands appended to the PNA.

Much research in recent years has focused on improving the original design of aegPNA.[30–32] Current research has focused on improving the binding properties while eliminating some of the inherent weaknesses (i.e. solubility, bioavailability, and functionality). Some common strategies include conjugating moieties to PNA termini[21] and/or modifying the PNA backbone.[33–37] Modifications at the -position of the PNA backbone have been well-documented to increase binding affinity and solubility, as well as to provide a convenient handle to attach additional functionality. Previously, we have shown that ^{L}Kγ-PNA (Figure 1b) is a versatile scaffold to attach a range of functional groups and small molecules without compromising the ability of PNAs to bind to a complementary nucleic acid sequence.[38–41] Previous work has demonstrated the advantageous properties of using a PNA to target proteins via multvalent display.[38, 39, 42–47]

A family of short G-rich ^{L}Kγ-PNAs with a varying number of -modifications was designed to explore the effect on a 3+1 bimolecular quadruplex binding (PNA 4–6). Furthermore, to explore the possibility of PNA-DNA heteroquadruplexes as scaffolds for ligand display, an integrin-targeting ligand, cRGDfK, was attached to the free amine at the position (PNA 2–3) and the effects on the bimolecualar G-quadruplex with a truncated telomeric DNA (DNA1) were studied. A ^{L}Kγ-PNA-G monomer (Figure 2) was synthesized and conjugated to a small peptide (cRGDfK) via the free amine of the side chain. Then, the potential for several different PNAs (both with and without a ligand on the ^{L}Kγ-PNA sidechains) to bind to the telomeric DNA was investigated.

The ^{L}Kγ-PNA backbone (**1**) was synthesized as reported previously.[25] The G(Cbz)-acetic acid was then coupled to ^{L}Kγ-PNA backbone (**1**) producing the ^{L}Kγ-PNA-G monomer ester (**2**) in high yield (70%). Removal of the allyl group using catalytic palladium provided the ^{L}Kγ-PNA-G monomer (**3**) (Scheme 1). The family of PNAs (Table 1) was synthesized using a standard PNA procedure on Applied BioSystems 433A automated peptide synthesizer, purified by HPLC, and characterized by mass spectrometry as previously reported.[26] To attach the ligand, the purified PNA was reacted with diethyl squarate to form an amide with the amines on the -sidechains. Then, the purified intermediate was reacted with an amine-modified cRGDfK ligand to yield the RGD-PNA conjugate.

The thermodynamic properties were assessed using UV melting curve analysis monitoring the hypochromic shift at 295 nm and 305 nm. The T_m was defined as the temperature at which half of the oligonucleotides were folded and was determined by the minimum of the 1st derivative. First, the thermal stability of the truncated telomeric DNA (DNA1) alone was determined to be 58.9 °C. Then, the truncated DNA1 was hybridized to the unmodified PNA1 (1:1 / PNA1 : DNA1), and the T_m was 57.3 °C. The T_m values are consistent with results obtained by Paul. Et al.[29] Next, the effect of increasing - modifications with PNA 4–6 was analyzed. When annealed with DNA1, PNAs with increasing ^{L}Kγ-PNA-G monomers bearing a free amine at the - position (PNA4, PNA5, PNA6) showed a melting transition (T_m = 60.2, 58.4, and 59.1 °C, respectively). The recorded T_m's were similar, suggesting that a - modification has minimal effect on the quadruplex stability. Introducing one or two ^{L}Kγ-PNA-G-RGD monomers (**G^{R}**) in PNA (PNA2 and PNA3) has very minor effects on the melting transition when annealed with DNA1 (T_m = 58.7 °C and T_m = 57.3 °C, respectively). Overall, the UV melting curve analysis indicates that the bimolecular quadruplex was formed and addition of a ^{L}Kγ-PNA-G monomer and/or a ligand does not perturb the stability.

The secondary structures of the oligonucleotides were analyzed using CD spectropolarimetry. DNA1 exhibited a CD signature with two maxima at λ260 and λ295 nm, which are characteristic of a mixture of parallel and antiparallel G-quadruplex conformations, respectively (Figure 5). The CD profile of the unmodified PNA1-DNA1 quadruplex showed a slightly larger maxima at λ 260 nm and λ 295 nm compared to DNA1 alone (Figure 3). Likewise, the CD profile of PNA2-DNA1 and PNA3-DNA1 showed strong maxima at λ260 nm and λ295 nm, although PNA2 and PNA3 also exhibited strong CD signals alone (Figure S1). The CD profile of PNA4, PNA5, and PNA6, in complex with DNA1 showed strong maxima at λ 260 nm and λ295 nm and weaker signals in the absence of DNA, thus indicating formation of a hybrid PNA-DNA quadruplex structure.

It was observed that PNA1 forms quadruplex with itself at high concentrations, (Figure S3) and does not have a significant CD signal (Figure S4). These properties were consistent with the literature[48]. Interestingly, the UV-temperature profile of PNA2 and PNA3 at 295 nm indicates that they do not form quadruplexes in the same buffer at concentrations up to 200 μM. Likewise, the UV-temperature profiles of PNA4, PNA5, and PNA6 at 295 nm indicate they do not form quadruplexes in the absence of DNA1, even at high concentrations.

We have reported the synthesis of ^{L}Kγ-PNA-G monomers to target a human telomeric DNA sequence, and we have shown that γ-substituted PNA can form a hybrid quadruplex with the DNA. Interestingly, ^{L}Kγ-PNAs do not form G-quadruplexes alone, even at high concentrations, while unmodified PNA1 forms a quadruplex at 200 μM. The selectivity of these PNAs could open up new possibilities to design quadruplex-targeting ligands. This work broadens the types of secondary structures that can be used for ligand display and future studies should explore the efficacy of these PNA-DNA heteroquadruplexes as scaffolds for ligand display.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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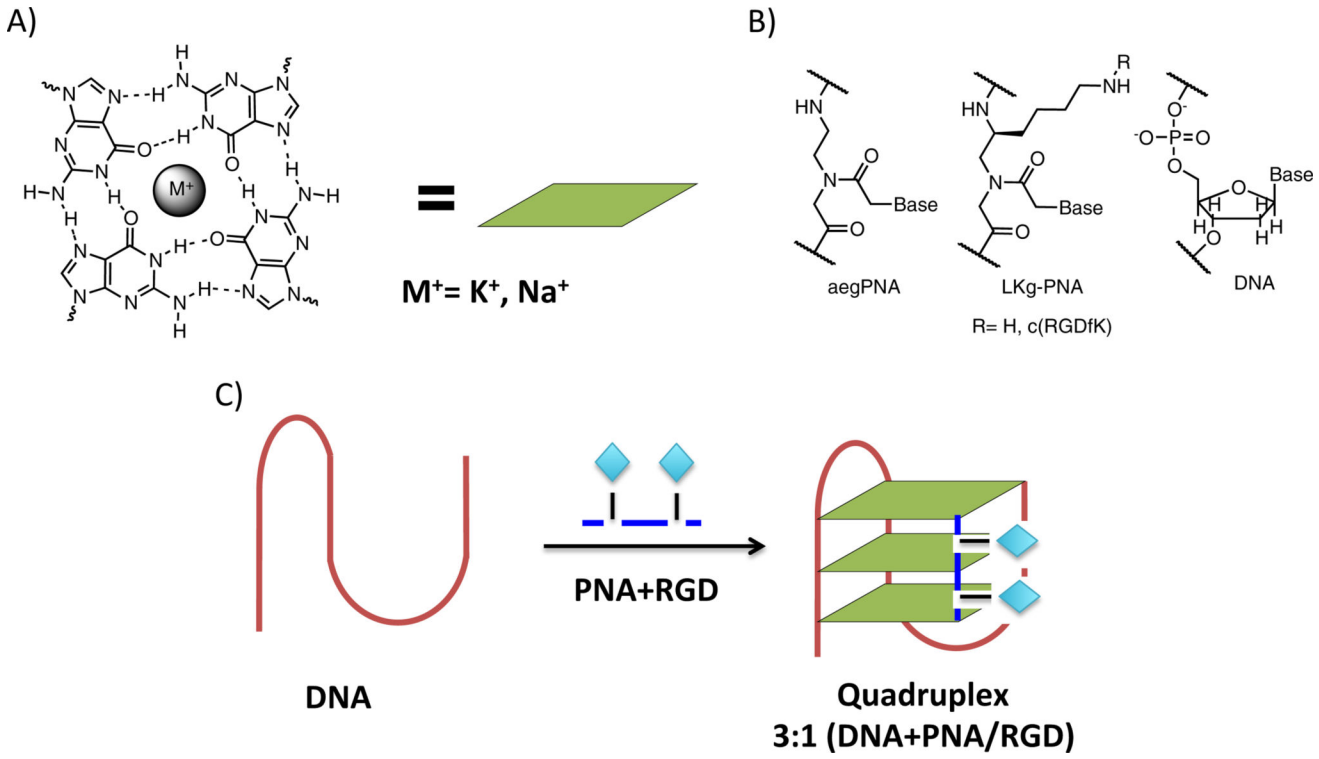
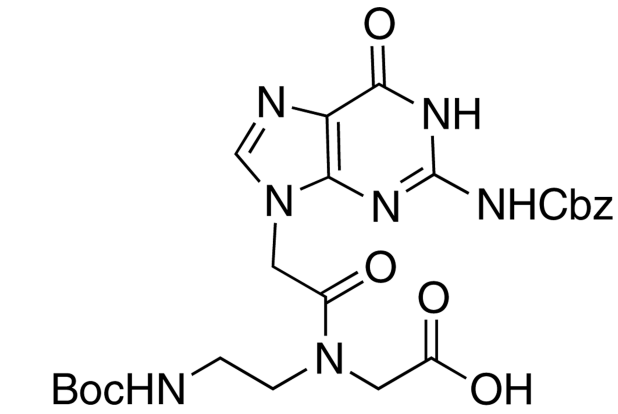
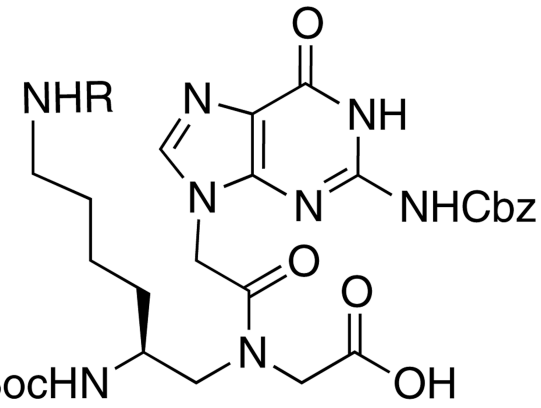


Figure 1.

A) Arrangement of guanines around a central cation (M^+) resulting in guanine tetrad eliciting G-quadruplex formation. B) Comparison of aminoethylgycine (aeg) PNA, $^{L}K\gamma$-PNA and DNA. C) Proposed PNA-RGD conjugate binding to G-rich DNA region.



Boc aegPNA-G(Cbz) Monomer



Boc ^{L}Kγ-PNA-G(Cbz) Monomer
R= H, c(RGDfK)

Figure 2.
The Boc-aeg PNA-G(Cbz) and Boc ^{L}Kγ-PNA-G(NH_2/$(mPEG)_2$-RGD) monomers suitable for Boc mediated solid-phase peptide synthesis.

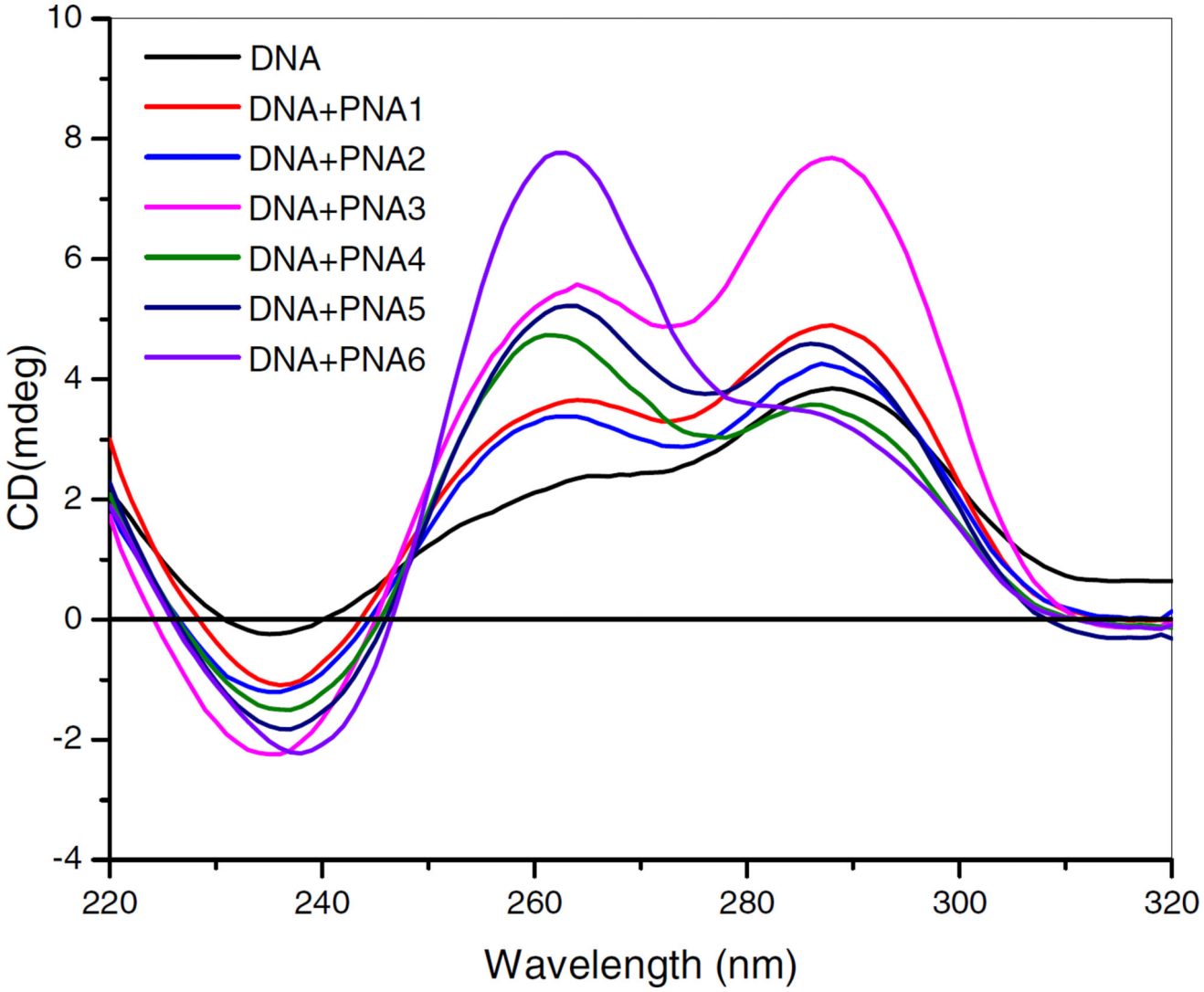
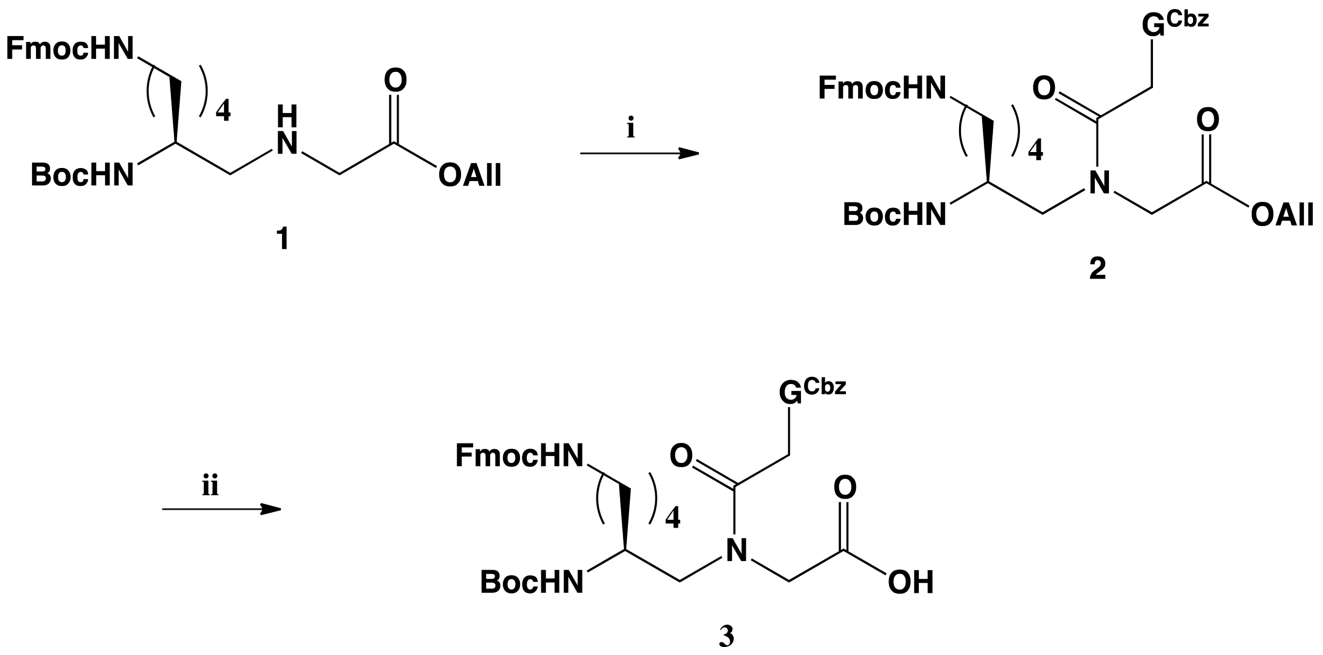


Figure 3.
CD spectra DNA1 and DNA1:PNA complexes (20 μM) in 100 mM potassium phosphate buffer containing 100 mM KCl at pH 7.4 (x-axis is wavelength (nm), y-axis is millidegrees).



Scheme 1.

Reagents and conditions: (i) 2-N-(Benzhydryloxycarbonyl)guanine-9-acetic acid, DHBT, EDC, DMF, 40°C, 12h, 87%; (ii) $Pd(PPh_3)_4$, *N*-ethyl aniline, THF, RT, 3h, 70%.

Table 1

List of DNA/PNA used in this study.

DNA/PNA	Sequence[a]	# of γ units
DNA 1	d(GGGTTAGGGTTAGGGT)	N/A
PNA 1	Ac-HN -TGGGT-Lys-$CONH_2$	0
PNA 2	Ac-HN -TG**$\underline{G}^{R}$**GT-Lys-$CONH_2$	1
PNA 3	Ac-HN -T**$\underline{G}^{R}$**G**$\underline{G}^{R}$**T-Lys-$CONH_2$	2
PNA 4	Ac-HN-TG**$\underline{G}$**GT-Lys-$CONH_2$	1
PNA 5	Ac-HN-T**$\underline{G}$**G**$\underline{G}$**T-Lys-$CONH_2$	2
PNA 6	Ac-HN-T**$\underline{GGG}$**T-Lys-CONH2	3

[a]PNA oligomers are written from N terminus to C terminus. The ^{L}Kγ-PNA-G-RGD monomers (**$\underline{G}^{R}$**) are bold and underlined for clarity, ^{L}Kγ-PNA-G monomers (**$\underline{G}$**) have a free amine on the side chain.

Table 2

Melting temperatures (T_m) of DNA and DNA/PNA quadruplexes in 100 mM potassium phosphate buffer + 100 mM KCl (pH 7.4). [DNA]= 20μM; [PNA]= 20μM.

Complex	T_M [°C]
DNA1	58.9±0.3
DNA1 + PNA1	57.3±0.4
DNA1 + PNA2	58.7±2.2
DNA1 + PNA3	57.3±0.3
DNA1 + PNA4	60.2±1.2
DNA1 + PNA5	58.4±0.3
DNA1 + PNA6	59.1±0.7