Trends in
Biochemical Sciences

CellPress

Spotlight

Pre-twisting for improved genome modification and miRNA targeting

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Two reports by Dhuri *et al*. and Oyaghire *et al*., respectively, show that, through installing chiral centers at the backbone of the artificial nucleic acid, peptide nucleic acid (PNA), enhanced miRNA targeting and genome modification can be achieved, with important implications in fighting cancers and β-thalassemia.

Regulatory tools precisely targeting DNA and RNA sequences are needed as new therapeutic modalities. Initially developed in 1991 by Nielsen and coworkers, PNA (Figure 1A,B) [1], shows great potential in targeting disease-related DNA and RNA sequences. PNAs can pair with natural DNA/RNA sequences forming PNA-DNA and PNA-RNA duplexes. Compared with the sugar ring moiety of DNA/RNA with three or four chiral centers, canonical PNA has no ring structure or chiral center. The relatively flexible and charge-neutral backbone makes PNA advantageous in the recognition of DNA/RNA sequences through Watson-Crick base pairing with both parallel and antiparallel strand orientations with enhanced binding affinity. Furthermore, PNAs recognize DNA and RNA sequences through triplex structure formation (e.g., PNA•DNA-PNA, PNA•RNA-PNA, PNA•RNA-RNA, and PNA•DNA-DNA triplexes; here - and • denote Watson-Crick and Hoogsteen pairs, respectively). Typically, PNAs show no significant binding with proteins and thus are non-immunogenic and resistant against proteases and nucleases. Delivery of PNAs has been facilitated by assembly with nanoparticles including block copolymer poly(lactic-co-glycolic acid) (PLGA) (Figure 1C) [2,3] and by conjugating with cell-penetrating molecules, including the pH-low insertion peptide (pHLIP) (Figure 1D) [4].

During the past decades, a tremendous amount of work on the molecular engineering of canonical PNA, including both the backbone and nucleobase, has resulted in new promising PNA-based molecular platforms in targeted DNA correction and RNA binding. For example, functionalizations at α, β, and γ carbon atoms, generating chiral centers, in the backbone of PNA give rise to pre-twisted right-handed structure in PNA (Figure 1A,B) and thus enhanced hybridization strength with DNA/RNA targets [5]. γPNAs with a diethylene glycol (mini poly ethylene glycol, mp) moiety (with an R-configuration of the γ carbon, designated as ^{MP}γPNA, Figure 1A) are relatively more biocompatible with improved solubility and biological activity [6]. Interestingly, a simple hydroxymethyl group attachment [serine modification γPNA (sγPNA), Figure 1A] also improves the biological activity in targeting RNA [3].

Various γPNAs have shown potential in biomedical applications such as gene editing and anti-miRNA technology (Figure 1C,D) [2–4,6,7], though it was not clear how the chemistry and bulkiness of the newly installed functional groups in the PNA backbone affect the binding and biological activities. Recently, two head-to-head comparison studies of ^{MP}γPNA and sγPNA have shown that sγPNA is superior compared with ^{MP}γPNA in DNA invasion-based genome correction of a single mutation in β-thalassemia (Figure 1C) and in targeting the seed region of an oncomir miR-155 (Figure 1D) [2,4].

Genomic DNA modification

Early work has shown that PNAs can invade DNA duplex through PNA•DNA-PNA triplex formation (Figure 1C). The invaded structure is recognized as DNA damage that allows precise DNA correction through a DNA repair and recombination process [2,7]. Accordingly, γPNAs have shown efficient gene correction and low off-target effects in hematopoietic stem cells [7]. Thus, PNAs may serve as complementary tools of CRISPR-Cas system for genomic DNA editing, with the advantages of relatively small molecular weight and low immunogenicity for PNAs. It has been reported that the DNA editing precision of CRISPR-Cas is affected by DNA topology, with supercoiled DNA causing an increased off-targeting effect [8]. DNA invasion by PNAs may result in the relaxation of DNA supercoiling and thus potentially is advantageous. Oyaghire *et al*. recently reported a head-to-head comparison of ^{MP}γPNA and sγPNA assembled with PLGA nanoparticles for DNA correction in mouse bone marrow cells [2]. Interestingly, compared with ^{MP}γPNA, sγPNA has an enhanced binding and a higher frequency (1.5-fold) in stimulating gene modification of a mutation that causes β-thalassemia.

Oncogenic miRNA targeting

miRNAs overexpressed in cancer cells may be targeted through miRNA-PNA duplex formation. Conjugating pHLIP and γPNA (pHLIP-γPNA) allows selective targeting of miRNAs in the acidic tumor microenvironment but not the normal cells (Figure 1D) [4]. Dhuri *et al*. reported that a pHLIP-sγPNA conjugate is more advantageous compared with pHLIP-^{MP}γPNA conjugate in targeting the seed region of miR-155 for restricting the U2932 lymphoma cell proliferation *in vitro* and in a mouse model [4].

These two studies on sγPNA on genome modification and miRNA targeting [2,4] suggest that there is significant room for improving the physical and binding properties and biological activities of PNAs by carrying out interdisciplinary medicinal chemistry campaigns. A close inspection orientated of the 3D structure of PNA-

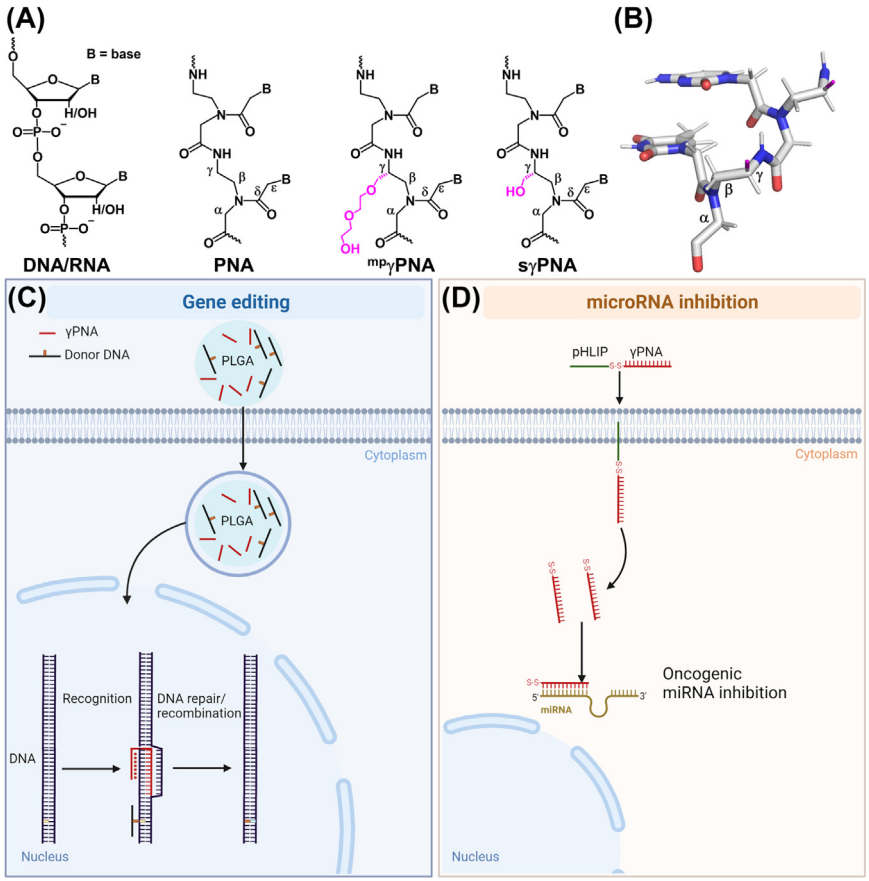

Figure 1. Peptide nucleic acid (PNA) structures and potential applications. (A) Chemical structures of DNA/RNA, canonical PNA, and γPNA. PNA has an aminoethylglycine building block and a methylene carbonyl linker replacing the standard phosphate-sugar backbone of DNA/RNA. Both the backbones of DNA/RNA and PNA contain six atoms with a two-atom linker connecting the nucleobases. A chiral center on the γ carbon is generated by attaching miniPEG (^{MP}γPNA) or hydroxymethyl group (sγPNA). (B) 3D structure of a helical PNA strand extracted from a crystal structure of PNA-DNA duplex (PDB: 1PNN). The hydrogen atoms on the γ carbons to be replaced by functional groups for pre-twisting the PNA strand into a right-handed structure are shown in magenta. The carbon, nitrogen, and oxygen atoms are shown in white, blue, and red, respectively. (C) Schematic of γPNA invasion of DNA for inducing DNA editing. Formation of a PNA•DNA-PNA structure results in correction of a disease-causing mutation through a donor DNA. The Watson-Crick and Hoogsteen pairs are indicated by - and •, respectively. (D) Schematic of γPNA targeting the seed sequence of a miRNA. Acidic microenvironment of tumors facilitates the formation of α-helical structure for the pH-low insertion peptide (pHLIP) for targeted delivery of γPNA into cancer cells. The γPNA is released by the cleavage of the disulfide bond with a reducing cancer cell environment.

DNA duplexes shows that the installed groups on α and β/γ carbons are orientated toward the major and minor grooves, respectively (Figure 1B). Compared with a relatively small hydroxymethyl group, the bulky and hydrophilic diethylene glycol moiety exposed on the minor groove side may disrupt the hydration layer of the PNA-DNA and PNA-RNA duplexes. Clearly, optimization of the chemistry and the size of the installed group on the γ position would generate further advanced PNAs.

In the two recent studies [2,4], only the PNA segment forming Watson-Crick pairs contains γ position modifications. It would be interesting to see how γ position modifications on the strand forming Hoogsteen pairs may affect the binding and activity [5,9]. It is conceivable that careful structure-based engineering of the chiralities of α and β as well as other positions (Figure 1A) may offer rich opportunities in tuning PNAs for improved targeting of DNA and RNA. Furthermore, the Hoogsteen pair-forming strand may be engineered with base modifications to enhance the binding strength and specificity [9]. In addition, efforts on engineering the delivery vectors including PLGA nanoparticles and pHLIP may also be worthwhile as vectors may have complex interactions in biological environments [10].

In summary, the two reports by Dhuri *et al*. and Oyaghire *et al*. [2,4] have shown that advanced modular synthesis techniques combined with biophysical and biological activity studies may allow precise molecular engineering of PNAs for enhanced reprogramming of the functions of DNA and RNA in diseased cells for fighting β-thalassemia, cancers, and many other diseases.

Acknowledgments

We thank the support from National Natural Science Foundation of China (NSFC) project (Grant 22177098), Shenzhen-Hong Kong Cooperation Zone for Technology and Innovation (HZQB-KCZYB-2020056), Shenzhen Third People's Hospital Research Fund, and China Postdoctoral Science Foundation funded project (2023M742416).

Declaration of interests

The authors declare no competing interests.

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https://doi.org/10.1016/j.tibs.2024.01.002

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