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Peptide nucleic acids (PNAs) and locked nucleic acids (LNAs) application in bacterial gene editing and biosensor production

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Abstract

Nucleic acid analogues are used in protein synthesis and their subsequent effects on gene expression play essential role during transcriptional and posttranscriptional events. Due to the many remaining challenges associated with genetic engineering, current research mainly focuses on new materials such as peptide nucleic acids [PNA] and locked nucleic acids [LNA]. PNAs and LNAs have been developed to mimic the chemical characteristics of DNA and RNA and to show improved properties. They represent synthetic DN/RNA analogues in which the phosphodiester backbone is replaced with unchanged aminoethylglycine units, being very resistant to enzymatic degradation by proteases and nucleases. This review summarizes the application of nucleic acid analogues in the inhibition of gene expression in bacterial genomes, as well as the usage in the development of high-performance affinity biosensors.

Keywords: Peptide Nucleic Acids; Locked Nucleic Acids; biosensor; bactericidal agents; gene editing

1. Peptide nucleic acids (PNAs)

Peptide nucleic acid (PNA) is a known artificially synthesized polymer, similar to DNA and RNA molecules [1]. With respect to the nucleic acid structure used for protein synthesis and the subsequent effects of on gene expression, they play a crucial and important role during the transcription and post-transcriptional events. Point mutations that happen in RNA and DNA sequences can produce numerous diseases, such as cancer and rare genetic disorders. Many small molecules containing drugs targeting nucleic acids are known as anticancer therapeutics [2]. Although promising results have been obtained, the off-target toxicity produced by the small molecule often limits their clinical application. Another way to achieve this is to use the specificity of the complementary binding of nucleic acids to attack the disease at the gene level and easily minimize off-target effects. Approaches to gene therapy are based on targeted reagents, those of which easily recognize DNA and RNA sequences. Most of the bioorganic reagents are designed to mimic the chemical properties of DNA and RNA, and to show enhanced properties. Some examples are locked nucleic acids (LNA), morpholinos and phosphorothioates [3]. These nucleic acid analogs provide improved properties such as resistance to enzymatic degradation, reduced off-target binding, and improved aqueous solubility.

PNAs have been studied as antigene agents, usually used for targeting genomic DNA, as well as antisense agents, used for targeting mRNA and microRNAs, to easily control regulation and gene expression. The applications are transcriptional arrest, PCR clamping direction, PNA-DNA complexes interacted by DNA binding ligands, P-loops formation as artificial transcription promoters, and many more. Numerous strategies have been used to improve PNA delivery into cells, like transfection-based methods, electroporation, and entrapment of PNA in polymer nanoparticles, inclusion of cations peptides and usage of chemically modified PNAs. PNA delivery has faced several limitations, and one of them is the usage of PNAs as antibiotic uptake

by host cells, because the components are nonionic structure and high molecular weight [4, 5]. Although PNA has been proven in *in vitro* studies, due to its biophysical properties, it is limitation to uptake into cells makes it very difficult to achieve a wider range of applications. In order to increase those uptakes, many methods have been tested to increase PNA transport across the cell membrane. Few studies have shown that the antigene and antisense properties of PNAs are simply inserted by microinjecting into the fibroblast cells [6]. Other studies have demonstrated that co-transfection of PNA/DNA complexes can easily lead to significant uptake inside the cells [7]. For example, using the streptolycin-O during in the direct permeabilization can easily increase permeability to PNAs, which will lead to PNA based mutations [8]. Another strategy involves the conjugation to cell penetrating peptides. PNAs that are delivered by this method are usually entrapped in endocytic vesicles, where they are easily degraded or recycle back to the cell surface [9]. Many studies showed that PNAs are more toxic, due to their nature of PNA peptide conjugates, the ones which results in permeabilization or disruption of cell membranes [10].

1.1 PNAs as bactericidal agents

PNA is a group of components that bind more easily and tightly to single-stranded DNA and RNA than complementary DNA [11]. These components were first identified by Nielsen et al. in 1996 [12], and after, in 2002 it was developed by Buchardt et al and Egholm et al [13]. The sugar phosphate backbone that is located in the nucleotide bases are then replaced with repeating N-(2-amino-ethyl) glycine units which are tightly connected by the help of methylene carbonyl linker [4]. PNAs are highly resistant to enzymatic degradation by proteases and nucleases. All these things make PNAs a very good candidate for many biomedical and therapeutic applications.

There are three main factors used in the design of PNAs for some activity against inhibition of bacterial growth. Those are:

1) the Length and base sequence of PNAs;
2) Membrane-penetrating peptide position in structure of peptide-PNA that studied demonstrated that 5'-end position of peptide is more active; and
3) Presence or absence of linker between peptide conjugated to PNA and sequence of PNA [14].

In recent years, PNAs applications were developed, and the components were used extensions in bacteria such as detection and identification of bacteria based on the fluorescence in situ hybridization, or gene knockdown, used as antibacterial components. Many studies showed that both gram positive and negative bacteria are susceptible to PNAs as antisense antibiotics [15, 16, 17, 18, and 19].

2. Locked nucleic acids (LNAs)

In the past two decades, Locked nucleic acids (LNAs) are noted as the most promising nucleic acid analogues. LNAs are made of many nucleotide monomers containing bicyclic furanose unit that are locked in RNA, thus mimicking sugar conformation [20]. LNA oligonucleotides exhibit unique hybridization affinity with complementary single- or double-stranded DNA and complementary single-stranded RNA [21]. Many studies have shown that LNA oligonucleotides tempt Type A (RNA-like) duplex conformations. The broad applicability of LNA oligonucleotides used for gene silencing, as well as for their research and diagnostic purposes are documented in several recent reports, some of which are described in this paper.

3. Biosensor development with nucleic acid analogues

In 1999, the IUPAC defined the biosensors as "Compact analytical device containing biological element, either integrated or associated with a physicochemical transducer" [22]. There are two main families of biosensors that are based on biocatalyst and bio-based affinity processes, thus including different types of probe molecules or bioreceptors, as well as whole cells or fragments of tissues [23]. DNA, RNA or an artificial polymer are the probe molecule used for nucleic acid-based biosensors. Nucleic acid biosensors are commonly utilized in different disciplines of genomics. The utilization of PNAs and LNAs allows high biosensor specificity and sensitivity to be easily achieved; however their unique features also introduce a few obstacles of their use. A neutral PNA backbone has promoted the usage of PNA oligomers as a probe in optoelectronic, microarray-based biosensors and lots of different types of sensors. On the other side, LNA-

based biosensors can design chimeric molecules which include both LNA nucleotides, as well as DNA/RNA nucleotides [24].

3.1. PNAs in biosensor development

Peptide nucleic acid (PNA) has great affinity for its complementary DNA/RNA molecules because of lack of the electrostatic repulsion between the natural nucleic acid and uncharged PNA backbone. In the 1991, the Nielsen has designed the PNA, and in that time has been evident that any single-stranded PNA oligomer have a greater affinity for its complementary DNA molecule than the equivalent ssDNA strain for the same target [25]. The hybridization in the antiparallel orientation is more stable, while in the parallel orientation, the sequence-specific binding is also possible [26]. The thermodynamics of hybridization of PNA/DNA heteroduplexes have been investigated in solution with the use of absorption hypochromicity melting curves and isothermal titration calorimetry. Many techniques (X-ray crystallography, NMR) have shown that the structures of PNA/DNA heteroduplexes easily extend the double helices whose features are intermediated between A and B forms of dsDNA. The interaction between PNA/DNA and PNA/RNA heteroduplexes is greater than the corresponding homoduplexes [27]. PNA have excellent thermal and chemical stability and are sensitive to enzymatic biodegradation by peptidases or nucleases. Due to its uncharged nature, PNA/DNA hybridization is highly insensitive to many changes in pH or ionic strength [28]. The SAMs of PNA on the surface tend to interact with complementary nucleic acid molecules which are very useful for biosensing applications [29]. All these properties make PNA an optimum probe molecule for the development of different types of affinity biosensors.

3.2. LNAs in biosensor development

Locked nucleic acids (LNA) were developed in 1997 by the Imanishi and Wengel groups [30]. LNAs are very helpful during improvement of sensitivity and specificity of FISH-related methods, PCR, microarrays, and others oligonucleotide-based techniques. The limited 3'-endo conformation of the ribose ring significantly reduces the flexibility of LNA conformation. According to Watson and Crick's rules, this nucleic acid may form specific base pairs with DNA/RNA [31]. It has also an excellent affinity for complementary sequences present in naturally occurring nucleic acids. LNA has very low toxicity, is resistant to nuclease digestion, can promotes triplex formation when hybridized with dsDNA, and can be synthesized by strand chemical methods [32]. In example that is reported by Fang et al., LNA microarrays detected several miRNAs [33]. On the other side, the Diercks et al. reported controversial results that LNAs do mnot improve the DNA properties of microarray-based biosensors, resulting in reduced sensitivity, stability and specificity [34]. LNA has also been introduced in DNA/RNA aptamers designed for biosensing applications. The reason for this is that fixed nucleotides not only increase the thermal stability of aptamers, but also improve their in vivo resistance to nuclease digestion [35].

4. Advantages of PNAs and LNAs

PNAs and LNAs have many advantages compared with DNA in designing probe molecules that are useful in biosensing applications. They include:

- PNA/LNA shorter than comparable DNA/RNA probes due to the extraordinary thermodynamics' stability of heteroduplexes containing PNAs and LNAs formed by native nucleic acids compare to the corresponding homoduplexes which can be used as a probe.
- The higher destabilizing effect of base mismatches on heterodimers containing PNAs/LNAs slightly improves the identification of genotyping.
- The high chemical stability and resistance to enzymatic degradation of PNA/LNA-based biosensors to be used in a wide range of biological samples [36].

5. Differences between PNAs and LNAs

There are also very important differences between PNAs and LNAs that affect the limit of detection when they are used with biosensors. The LNA molecules have a negatively charged phosphate-sugar backbone, while the PNA backbone, which is uncharged, enables hybridization with DNA/RNA molecule under low or no salt conditions. Because of different electrical nature, LNA do not have the strand-invasion properties, found in PNAs, and its hybridization to dsDNA targets is less efficient. Furthermore, the PNAs are assembled

using strand peptide synthesis procedures and is much easier to append peptide motifs on to PNA molecules than on to LNA oligomers [36, 37].

6. Conclusions

PNA technology is used in many biomedical applications. The intracellular delivery of PNA is a very important issue that needs to be solved for clinical applications. Many peptide-based methods have been used to deliver PNA to cells, but this task remains difficult due to lack of toxicity and specificity. For these reasons, recent attention has focused on nanoparticle-based delivery methods which supply the potential for targeted delivery of PNA therapeutics. The limitations in the sequence of PNA/LNA oligomers used as probes in biosensors can impair detection of some mutations in genes of interest. That's why it can be concluded that PNA and LNA share some similarities, as well as important differences between them that suggest the use of one or other depending on the desired application. In addition, both analogues have certain disadvantages compared to using DNA to develop biosensors for the construction of high-throughput microarrays. Further studies of PNA and LNA probes in biosensors are expected to provide additional information on their practical usefulness and limitations. It is expected that further studies will provide additional information on the practical usefulness and limitations of PNA.

7. References

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