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Review

Application of peptide nucleic acid towards development of nanobiosensor arrays

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ABSTRACT

Peptide nucleic acid (PNA) is the modified DNA or DNA analogue with a neutral peptide backbone instead of a negatively charged sugar phosphate. PNA exhibits chemical stability, resistant to enzymatic degradation inside living cell, recognizing specific sequences of nucleic acid, formation of stable hybrid complexes like PNA/DNA/PNA triplex, strand invasion, extraordinary thermal stability and ionic strength, and unique hybridization relative to nucleic acids. These unique physicobiochemical properties of PNA enable a new mode of detection, which is a faster and more reliable analytical process and finds applications in the molecular diagnostics and pharmaceutical fields. Besides, a variety of unique characteristic features, PNAs replace DNA as a probe for biomolecular tool in the molecular genetic diagnostics, cytogenetics, and various pharmaceutical potentials as well as for the development of sensors/arrays/chips and many more investigation purposes. This review paper discusses the various current aspects related with PNAs, making a new hot device in the commercial applications like nanobiosensor arrays.

1. Introduction

After the landmark discovery of DNA by Watson and Crick (1953), DNA-based diagnostics have evolved, which involves the isolation of DNA polymerase, RAPD (random amplified polymorphic DNA), RFLP (restriction fragment length polymorphism), and PCR (polymerase chain reaction) techniques. In the modern era of molecular diagnostics; DNA microarrays, fluorescence *in situ* hybridization (FISH), single nucleotide polymorphism (SNP) or short tandem repeat (STR) detection, and micro-electro-mechanical system (MEMS) based microfluidics (lab-on-a-chip) technology are becoming very popular worldwide [1]. DNA is a carrier of genetic information in almost all living cells from single cells to a majestic elephant. The identification of DNA sequences is laborious and time-consuming with a low hybridization efficiency and sequence discrimination. To overcome these problems, DNA sensors/arrays/chips came into existence for high throughput analysis, which in turn represents a drastic reduction in efforts, time, and cost [2].

PNA was discovered for the first time by Drs. Nielsen, Egholm, Berg, and Buchardt in 1991. PNA is a polyamide backbone i.e., modified nucleobases with exceptional chemical, physical, and biological properties such as an excellent biological stability, higher binding affinity, better specificity, cellular uptake, a probe for hybridization, blocking enzyme function, labeling of plasmids with different types of fluorescent molecules used in therapeutics, molecular diagnostics, and many more applications in biomedical sciences [3]. PNA has potential applications like antisense technology, supramolecular nanostructure, macromolecular assembly, nanoelectrochemical system, DNA computing, nanomedical applications including drug delivery and diagnostics to treat microbial infections and diseases [4]. Besides these, the knowledge and understanding of biological processes, such as gene expression and protein synthesis, help not only in the development of medical diagnostic procedures, but are also applied in medical treatment by introducing gene and antisense therapy [5]. Due to the structure similarity of PNA with DNA, PNA is able to bind to its complementary nucleic acid sequence obeying the Watson–Crick base-pairing rules. PNA has an achiral backbone containing, repeating units of N-(2-aminoethyl)-glycine (Aeg) linked by peptide bonds. Each of the four bases i.e., adenine, cytosine, guanine, thymine (A, C, G, T) is attached to the charge-neutral backbone by methylenecarbonyl linkages. PNA has an amine-terminus (or N-terminus) and a carboxy terminus (or C-terminus), corresponding with the 5'- and 3'-ends of the DNA oligonucleotide, respectively [6], which was compared structurally with DNA and protein as shown in Fig. 1.

The properties of PNAs, like high binding affinity, high specificity and high sensitivity, are explored in the PNA array, which forms a strong PNA/DNA duplex due to the electrically neutral property of PNA oligomers. These strong PNA/DNA duplexes result in higher melting temperature (Tm) value i.e., approximately 1 °C higher per base pair when compared to that of the corresponding DNA/DNA duplex. Single-base mismatches have a considerably more destabilizing effect that enables to detect SNP that is not difficult for DNA-based microarrays [7–9]. High biological stability of PNA probes are resistant to enzymatic degradation because of the backbone of N-(2-

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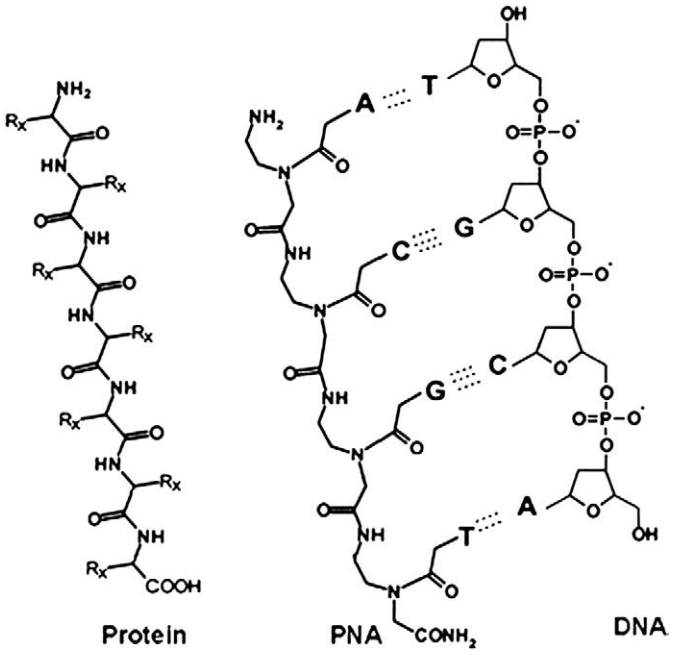

Fig. 1. The structural comparison of PNA with DNA and protein.

aminoethyl)-glycine, which is not recognized by nucleases and proteases. Since PNA probes are not degraded by the enzymes, their shelf life is considerably long over years even at room temperature [10–14]. Due to their chemical structure PNA probes are highly stable against various chemicals. PNA probes are fairly acid stable, whereas DNA probes are susceptible to depurination at acidic pH (pH 4.5–6.5). PNA probes are also stable in a wide range of temperature [15,16]. The duplexes between PNA and DNA or RNA are thermally more stable than the corresponding DNA–DNA or DNA–RNA duplexes, but the thermal stability of PNA–PNA duplexes exceeds that of PNA–RNA duplexes, which again are more stable than PNA–DNA duplexes (PNA–PNA duplexes>PNA–RNA duplexes>PNA–DNA duplexes) [17]. Thus, the advancement in nucleic acid chemistry and in technologies such as PCR and antisense studies, has opened a new advance interdisciplinary dimension in biotechnological research. These properties make PNA a powerful tool for molecular biology, genomics, therapeutics and diagnostics based on the development of DNA/PNA biosensors/biochips/microarrays [18,19].

2. PNA bioelectrochemistry

Recently, several methods for enhancing the sensitivity of electrochemical DNA biosensor have been reported. Specifically, the integration of nanotechnology, microfabrication techniques, and miniaturized devices with novel biochemical detection methodologies, leads to very sensitive and fast assays for the detection of desired analytes related with various commercial sectors. Wang et al. reported for the first time that PNA probes (10–15-mer) offer an efficient surface hybridization in combination with the high specificity of DNA binding in the solution phase. They detected for the first time single-base mismatches (point mutation) by an electrochemical technique using the Co $(phen)_3^{3+}$ as an electroactive redox indicator (for high hybridization signal events) and a carbon paste electrode as a transducer [20]. Conducting polymer based nucleic acid biosensor could be suitable for environmental toxicant detection. Arora et al. immobilized ds-CT-DNA onto electrochemically prepared polypyrrole–polyvinylsulphonate (PPy–PVS) films. They investigated the amperometric response studies of the DNA/PPY–PVS electrodes at 25 °C as a function of 2-aminoanthracene (2-AA) concentration (0.01–20 ppm) and ortho-chlorophenol (OCP, 0.1–30 ppm) respectively [21]. Furthermore, Prabhakar et al. reported twenty-bases long NH_2-modified DNA and PNA probes specific to a pathogen (*Mycobacterium tuberculosis*), covalently immobilized onto a polyaniline (PANI)/Au electrode can detect nucleic acid with complementary, one-base mismatch and noncomplementary targets within 30 s using methylene blue as an electroactive redox indicator [22]. Fig. 2 (A) and (B) revealed, the general modified schematic representation of polyaniline and self-assembled monolayer based biosensor for the detection of target nucleic acid base sequences respectively. The principle of detection was based on the electrochemical measurement of hybridization events in both schemes. The gold modified PANi film was subjected to glutaraldehyde (as cross-linker) with either DNA or PNA (-NH_2 group of guanine) to form stable probe (either DNA/PANI/Au or PNA/PANI/Au) for the target probe to generate enhancing hybridization signal, which was electrochemically measured in supporting electrolytes.

Further, Wang et al. described that the replacement of the DNA recognition layer with a PNA, greatly improved the distinction between closely related sequences, as well as several other attractive advantages like the use of PNA recognition layers in DNA diagnostics [23]. Fojta et al. reported the adsorption behavior of PNA and DNA decamer sequences on a mercury surface using AC impedance measurements at a hanging mercury drop electrode. They found that the adsorption behavior of the PNA with an electrically neutral backbone differs greatly from that of the DNA whereas the DNA–PNA hybrid shows intermediate behavior [24]. Furthermore, Fojta et al. also reported cathodic or anodic stripping voltammetry for a highly sensitive determination of nucleobases, nucleosides, nucleotides or acid-hydrolyzed NAs, based on the formation of sparingly soluble complexes of the nucleic acid constituents with electrochemically generated mercury or copper (I) ions. DNAs, RNAs and their synthetic analogues, either unmodified or labeled with electroactive markers, have been analyzed by adsorptive stripping (AdS) techniques with mercury, mercury film, amalgam, and carbon-based electrodes [25]. Wang et al. reported the adsorption behavior of PNA oligomers onto carbon paste electrodes and displayed a strong adsorption in comparison to DNA. The strong adsorption of PNA is exploited for an effective preconcentration step prior to the chronopotentiometric measurement as desired for future diagnostic, pharmaceutical, and biological applications [26]. Wang et al. also reported a 17-mer PNA as the recognition layer of an electrochemical biosensor for detecting a specific mutation in the p53 gene. The sequence-specific analysis of the p53 gene can thus become extremely useful to help the monitoring of cancer progress and patient therapy [27]. Tomschik et al. reported the behavior of oligomers of ssDNA and ssPNA with homopurine and mixed sequences. They showed that ssPNA produced qualitatively the same CV responses at the mercury electrode, i.e., the cathodic peak of adenine and cytosine and the anodic peak of guanine. The heights and potentials of these peaks of ssPNA differ from those of ssDNA and some relations between the oligonucleotide base content and electrochemical responses are evident. At carbon electrodes oxidation peaks of guanine and adenine are observed at low concentrations of DNA or PNA with both chronopotentiometry and voltammetry if a proper baseline correction technique is applied [28]. Gasser et al. reported for the first time, the facile synthesis of a new alkyne-substituted PNA monomer building block that can be inserted at any given place within any desired PNA sequence on the automated DNA/PNA synthesizer by using the click chemistry methodology. They speculated that the new PNA bio-organometallic conjugates act as an electrochemical probe [29]. Li et al. reported ferrocenyl–azobenzene labeled PNA monomer of thymine (Fc–Azo–T) as an effective electrochemical and photochemical probe respectively and shows a highly electrochemical and photochemical activity using differential pulsed voltammogram (DPV) and UV–VIS spectroscopy [30]. Palecek et al. recently reported that a single-base mismatch detection in DNA hybridization sensors based on a simple method of covalent end labeling of PNA with osmium tetroxide, 2, 2′-bipyridine (Os, bipy). Os,