Sensitive fluorescent sensing for DNA assay

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This review focuses on novel fluorescent sensing systems for rapid, sensitive, reliable and cost-effective DNA detection in clinical diagnostics. Several materials, including conjugated polyelectrolytes, nanomaterials and molecular beacons, which exhibit attractive properties, are involved to stabilize and to amplify the signal. We describe and evaluate these different DNA analytical methods and give some pointers as to the likely directions of future developments.

Keywords: Fluorescent; DNA; Conjugated polyelectrolyte; Nanomaterial; Molecular beacon; Review

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1. Introduction

The enormous amount of information generated in the Human Genome Project prompted the development of DNA sensors and high-density DNA arrays [1–4]. The ability to sense and to detect ultralow concentrations of specific DNA sequences is important in clinical diagnostics, gene therapy, food safety, environment, and biodefense applications [5–11], so many researchers have turned their attention to the investigation of DNA analysis and monitoring.

Methods of detection are normally based on identifying a specific sequence by hybridizing a sequence probe to the target analyte. With or without labeling, the measurement signals can be acquired {by, e.g., optical, surface-plasmon resonance (SPR), piezoelectric or electronic-transduction techniques} [12–18]. Not only are fast, precise and cost-effective methodologies required, but highly selective, sensitive techniques are also needed for detection assays.

Conventional methods that use radioactive [^{32}P]-labeled nucleic-acid (NA) probes or the polymerase-chain reaction (PCR) coupled with molecular fluorophore assays offer high sensitivity of detection, but they suffer from several drawbacks (e.g., complex handling procedures, easy contamination, high cost and lack of portability) [19–22]. Recently, great efforts have been made to develop biotechnologies to improve the sensitivity, the selectivity and the ease of operation for NA analysis [23–33]. Among them, DNA hybridization, offering excellent selectivity using the DNA base pair coupled with optical detection, is one of the most widely-used methods [34–36]. Usually, a fluorescent dye molecule is utilized to signal the hybridization.

In ultratrace gene analysis, signal amplification is the most critical issue and is typically achieved by coupling fluorophores (e.g., organic dyes) to the DNA probes. However, two major difficulties limit the sensitivity when these fluorophores are used. The first is the relatively low signal amplification. Because a DNA probe can be labeled with only one fluorophore or a few fluorophores, the fluorescence signal is too weak to be detected when the target concentration is low. The second challenge is the poor photostability of many fluorophores. Most organic dyes suffer serious photobleaching, resulting in irreproducible signals for ultratrace bioanalysis.

To achieve strong, photostable fluorescence signals, several novel materials {e.g., conjugated polyelectrolytes (CPs), nanomaterials [e.g., silica, gold nanoparticles (GNPs), quantum dots (QDs) and carbon nanotubes (CNTs)], and molecular beacons (MBs)} have been introduced for bioanalysis because of their many attractive properties, including superior optical properties, substantially greater chemical

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stability, and stability against photobleaching [37–41].

In this article, we summarize analytical applications using these novel materials in DNA detection. They cover several areas in dire need of intense investigation [e.g., classical DNA analysis, detection of single-nucleotide polymorphisms (SNPs), DNA-ligation or cleavage monitoring, and DNA delivery].

2. Conjugated polyelectrolytes

CPs are versatile new materials that combine features of conventional conjugated polymers and polyelectrolytes, resulting in unique electrical and optical properties that have made them ideally suited for applications in electrooptic devices, biosensors and drug delivery [42–45]. Modification of CPs with anionic or cationic functional groups yields materials that possess the properties of conjugated polymers but are also water soluble, which is essential for interfacing with biological substrates (e.g., proteins and DNA). These water-soluble conjugated polymers are attractive sensor materials because their electrical, optical and optoelectric properties can be greatly modified by small perturbations of the local environment [46].

CPs are water-soluble polymers that contain a π-delocalized backbone bearing pendant ionic functionalities [47–50]. Some CPs, including polythiophene [51], poly-fluorene [52], poly (*p*-phenylenevinylene) [53], poly (*p*-phenylene ethynylene) [54], and oligonucleotide-functionalized polymer, were synthesized and their sensing properties were investigated. Compared with small-molecule dyes, their advantages stem from the light-harvesting and optical-signal-amplification properties of conjugated backbones with efficient intrachain and interchain energy-transfer mechanisms. CPs became of particular interest in recent years for reporting sequence-specific DNA or RNA recognition events via homogeneous assays based on fluorescence resonance energy transfer (FRET) [55–57]. Table 1 summarizes some FRET DNA detections using CPs.

Fig. 1 shows the principle of cationic CP (CCP) assays [58]. They usually employ a CCP and a fluorophore (C^*) labeled probe (e.g., PNA-C^*, DNA-C^* or RNA-C^*), which are chosen to favor FRET from the CCP (donor) to C^* (acceptor). FRET efficiency is proportional to $1/r^6$, where r is the donor–acceptor distance, and the integral of spectral overlap between donor and acceptor. Thus, as shown in situation A of Fig. 1, stronger electrostatic attractions between the CCP and dsDNA-C^* (dsDNA: double-stranded DNA) usually induce closer donor–acceptor distance and preferential FRET to dsDNA-C^*, as compared with ssDNA-C^* (ssDNA: single-stranded DNA) or (ssDNA-C^* + $ssDNA_{NC}$) ($ssDNA_{NC}$: non-complementary ssDNA) in situation B. Moreover, that the emission of C^* by efficient FRET from the CCP was considerably more intense than that obtained by direct excitation at the absorption maximum of C^* demonstrated the optical-signal-amplification properties of the CCP. We can thus monitor the presence of specific complementary DNA sequences by monitoring the CCP-to-C^* emission ratio in real time. Peng et al. synthesized a novel cationic, water-soluble, conjugated polymer, poly({2,5–bis[3-(N,N-diethylamino)-1-oxapropyl]-para-phenylenevinylene}-alt-para-phenylenevinylene) dibromide ($PPVNEt_2Br_2$), and used it to develop a simple label-free DNA-detection assay [59]. The results illustrated that it was possible to detect specific DNA fragments in solution by using a single Cy3-labelled oligonucleotide-probe strand. The limit of detection (LOD) for complementary target DNA was 7 nM. Further optimization of the structure and the properties of CCPs may lead to simple, practical analysis platforms for DNA hybridization.

Anionic CPs (ACPs) can also provide an important optical platform for DNA analysis. Lee et al. developed a biosynthetic anionic poly(phenyleneethynylene) (PPE)-DNA sensor for efficient self-signal-amplifying DNA detection in aqueous solution (Fig. 2) [60]. By using a simple carboiimide chemistry, PPE was successfully conjugated to DNA molecules by amide-bond formation. The resulting single-stranded DNA (ssDNA) coupled at the end of the polymer chains selectively hybridized with HEX (hexachlorofluorescein, a fluorescent dye)-labeled target complementary DNA. A large amount of fluorescence energy from the PPE was efficiently transferred to the target HEX-DNA upon DNA/DNA hybridization,

Table 1. One-step or two-step fluorescent resonance-energy transfer (FRET) process to detect DNA using conjugated polyelectrolytes (CPs)

	Donor 1	Acceptor 1 (or as Donor 2)	Acceptor 2	Ref.
CCPs	Poly(*p*-phenylenevinylene)	Cy3	—	[59]
	Poly(fluorene-co-phenylene)	Fluorescein	Ethidium bromide	[55]
		Fluorescein	—	[48,66]
		Thiazole orange	—	[64]
		Texas Red	—	[65]
		Cy5	—	[67]
	Polythiophene	Alexa Fluor 546	—	[45]
ACPs	Poly (*p*-phenylene ethynylene)	Hexachloro-fluorescein	—	[60]
Complex of CCPs and ACPs	Poly(fluorene-co-phenylene) ACP:CCP = 85:15	Benzothiadiazole	Texas Red	[56]