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Review

DNA nanotechnology-based development of delivery systems for bioactive compounds

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ABSTRACT

Nucleic acids, DNA and RNA, not only allow transfer and replication of densely coded genetic information, but also act as danger signals triggering innate immune response. Recent progress in the design and construction of nano-sized structures using DNA has opened a new field of nanotechnology. The unique properties of nano-sized DNA constructs can be exploited to develop programmable materials for efficient delivery of bioactive compounds. In this review, recent advances in DNA nanotechnology and its applications as delivery systems are summarized. In particular, we focus on the delivery of DNA containing unmethylated cytosine-phosphate-guanine (CpG) dinucleotide, or CpG motif, to immune cells expressing Toll-like receptor 9. Recent studies have shown that precisely designed DNA constructs, such as multi-branched DNA, polyhedral DNA, and DNA origami, can be used to enhance the biological activity of CpG DNA.

Contents

1. Introduction

For millions of years, living organisms have used nucleic acids, DNA and RNA, to encode, transfer, decode, and transcribe genetic information. This is largely attributed to the nature of the nucleic acids to form a double-stranded structures with a complementary strand (Watson and Crick, 1974), and the double-strand formation keeps the genetic information unchanged (Crick, 1970). Recently, this ability to form double-stranded structures has been exploited to construct a variety of unnatural tertiary DNA structures—DNA-based nano-scale materials. To date, DNA nanotechnology has been used in optical detection, diagnostics, and drug delivery (Seeman, 2010; Roh et al., 2011b; Hartman et al., 2013).

From a structural perspective, there are many advantages of using DNA as a building block for developing diagnostic and therapeutic materials. The classical right-handed double helical DNA, B-DNA, is a nano-scale species; the diameter and helical rise

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of B-DNA is about 2 nm and 0.34 nm/base, respectively (Leslie et al., 1980). Two DNA molecules can be ligated to form one species via DNA ligase-catalyzed reactions (Fig. 1A). Single-stranded overhangs, called sticky ends, are used for the ligation of DNA molecules (Wu and Wallace, 1989). This permits the custom-fabrication of readily programmable intermolecular connections and allows us to synthesize desired DNA structures with varying functions (Lee et al., 2009). Branched DNA with junction points has also been used as building blocks. A typical branched DNA forms a four-armed Holliday junction, an intermediate structure formed during the process of genetic recombination (Holliday, 1964; Shinagawa and Iwasaki, 1995). In addition, Y-shaped (Y-DNA) and X-shaped (X-DNA) DNAs have been designed and synthesized using three or four oligodeoxynucleotides (ODNs), respectively, with the halves of each ODN being partially complementary to a half of each of the other two ODNs (Kallenbach et al., 1983; Ma et al., 1986) (Fig. 1B). The branched DNA can be used to create two- or three-dimensional networked structures (Seeman, 1982).

These approaches enable us to develop several types of self-assembled and bottom-up tertiary DNA structures. DNA is a highly homogeneous and inexpensive material which can be obtained by total synthesis. In addition, DNA is a chemically stable molecule. Chemical modification of the functional groups of DNA can also be possible. These properties of DNA are the advantages of DNA-based delivery systems.

Recent studies have found that DNA nanostructures are useful for pharmaceutical and biomedical applications (Nishikawa et al., 2010). DNA or RNA containing specific sequences, such as antisense ODN, decoy ODN, small interfering RNA (siRNA), and unmethylated cytosine-phosphate-guanine (CpG) ODN, has several biological functions, including the inhibition of expression of target molecules and the activation of innate immunity (Dove, 2002; Fattal and Bochot, 2006; Krishnamachari and Salem, 2009; Tomita et al., 2003). DNA nanostructures constructed using functional nucleic acids could improve or strengthen their biological activity. Nucleic acids are degraded by nucleases in vivo (Takakura et al., 2001). This biodegradability assures that DNA and RNA are biocompatible. Therefore, oligomers and polymers consisting of DNA can be used for the development of biodegradable, biocompatible, and safe nano-scale materials. In this review, approaches to use nano-scale DNA structures exhibiting unique structural properties as drug delivery systems (DDS) are summarized.

2. DNA nanostructures for drug delivery systems

Pioneering work by Seeman and coworker, who constructed a DNA cube, laid the foundation for DNA nanotechnology (Chen and Seeman, 1991). Since then, a number of self-assembled polyhedral DNA nanostructures have been developed (He et al., 2008, 2010; Zhang et al., 2008, 2009, 2010; Li et al., 2012) (Fig. 1C).

A group of researchers from Cornell University developed dendrimer-like DNA (DL-DNA) through the ligation of Y-DNA building blocks (Li et al., 2004; Freedman et al., 2005). Three ODNs with the halves of each ODN being complementary to a half of the other two ODNs are used to construct Y-DNA, and which are ligated to each other through the adhesive terminal ends using T4 DNA ligase. The numerous terminals of the DL-DNA can be used to conjugate any functional moieties, including fluorescent probes. Rothemund (2006) prepared materials of arbitrary two-dimensional shapes, including a smiley face, using a 7-kilobase single-stranded scaffold and over 200 short 'staple strands' to hold the scaffold in place. The multiple staple strands were hybridized to the long scaffold strand by simple annealing. This method, known as "DNA origami", allows the arrangement of a large number of ODNs with nano- to micro-meter precision and made it possible to create more complex and larger structures. The DNA nanostructures developed thus far have shown enormous potential for applications in a number of fields.

Synthetic or semi-synthetic DNA or RNA has the potential to interact with target molecules, such as endogenous DNA, RNA, and proteins. Such nucleic acids can be used as therapeutic agents for the treatment of diseases. Because there are several barriers for the delivery of functional DNA and RNA, a variety of delivery systems, including liposomes, nanoparticles, polymers, and molecular conjugates, have been developed (Ma et al., 2005; Lee et al., 2012c; Tsai et al., 2012; Lambert et al., 2001). Although

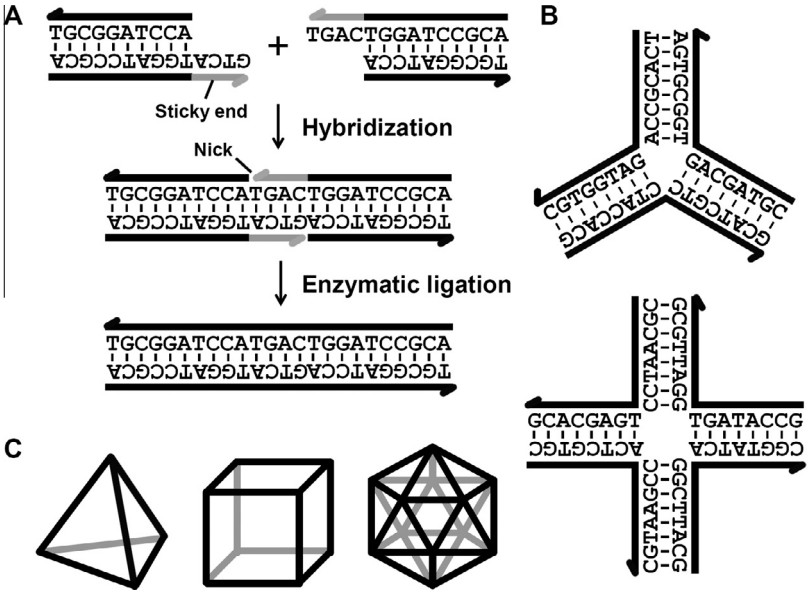

Fig. 1. DNA ligase-catalyzed reaction and schematic representations of DNA assemblies. In most cases, double-strand formation between two DNA molecules acts as the driving force for the formation of DNA assemblies. (A) Two double-stranded DNA molecules can be connected by hybridization through the sticky ends (5′-GTCA-3′ and 5′-TGAC-3′ in this case). The nick between the two double-stranded DNA molecules can be enzymatically ligated, to produce one double-stranded DNA. (B) The sequence and planar presentation of Y-DNA and X-DNA. (C) Schematic representations of a variety of DNA assemblies. Tetrahedral DNA (left) (He et al., 2008), hexahedral DNA (middle) (Chen and Seeman, 1991), and icosahedral DNA (right) (He et al., 2010).