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DNA-based nano-sized systems for pharmaceutical and biomedical applications ☆

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ABSTRACT

DNA is one of the most important components for all living organisms and many species, including humans, use DNA to store and transmit genetic information to new generations. Recent advances in the handling of DNA have made it possible to use DNA as a building block of nano-sized materials with precisely designed architectures. Although various approaches have been proposed to obtain DNA assemblies with designed architecture in the nano- to micrometer range, there is little information about their interaction with biological components, including target molecules. Understanding the interaction between DNA assemblies and the body is highly important for successful pharmaceutical and biomedical applications. Here, we first review the basic aspects of externally administered DNA molecules, including the stability, permeability and delivery issues. Then, we discuss the unique responses observed in the interaction of structured DNA assemblies and cells expressing Toll-like receptor-9, the receptor responsible for the recognition of unmethylated CpG dinucleotides that are abundant in the DNA of invading pathogens, such as bacteria and viruses.

Contents

1. Introduction

DNA is one of the most important components of all living organisms. Genomic DNA is the archive of genetic information, which is the most important information for many species including humans, even though the sequence has always been continuously changing. This instability in terms of over millions of years has been changing the organisms, and some of those changes have allowed them to adapt to new environments and some others have resulted in their disappearance. For example, humans, other primates and guinea pigs have lost the ability to produce ascorbic acid, or vitamin C [1], even though they require the vitamin for healthy living. Genetic mutations sometimes cause diseases, even when only one base is

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deleted, or replaced by a different base if the change is critical to the expression of a functional protein at a normal level [2–5]. Most of mutations in genomic DNA are corrected by enzymatic systems to keep the information unchanged [6,7]. The double stranded structure of DNA makes this correction process precise, because the strand without mutations is used as a template for the correction.

From the structural point of view, there are many advantages in using DNA as a building block of nano-sized materials with precisely designed architecture [8–13]. In particular, the specificity of the hydrogen interactions between adenine-thymine and guanine-cytosine allows us to design the whole structure of DNA-based materials. In addition, two strands of double-stranded DNA can be covalently bound to each other using DNA ligase when the 5'-end of one strand is complimentary to the 3'-end of another strand. Oligomers or polymers of DNA can be chemically synthesized by automated machines in a large quantities, so that DNA with a proper length and sequence can be obtained with high purity in amounts required for clinical application. Under conditions where there are no enzymes degrading DNA, DNA is very stable compared with proteins, another component candidate for nano-sized materials with designed architecture. Restriction enzymes, ligases and other enzymes are available to design and modify the properties of DNA-based materials.

Recent advances in the development of DNA assemblies have greatly increased the possibility of using DNA as a building block of nano-sized systems for pharmaceutical and biomedical applications. Various methods have been proposed and unique structures, most of which could not be imagined a few years ago, have been successfully developed [14–22]. In general, such systems use the property of DNA to hybridize its complimentary sequence. Any structure can be prepared if one can imagine it, in a nano- to micro-scale. Fig. 1 shows some examples of DNA assemblies, the generation of which has been confirmed in these previous studies. These uniquely structured DNA assemblies rarely exist in nature, so that it is not clear how they are recognized by living organisms. On the other hand, they are composed of natural DNA, so that they could be handled in the same fashion as those of natural origin. Thus, it is very important to understand how DNA assemblies are recognized by the body in order to develop DNA-based nano-sized systems for pharmaceutical and biomedical use. In this review, we summarize the basic characteristics of DNA molecules, such as their stability, membrane permeability and tissue distribution properties, and discuss the use of DNA-based nano-sized systems for pharmaceutical and biomedical applications.

2. General requirements of DNA molecules for their pharmaceutical and biomedical application

To exhibit biological functions, the externally administered DNA should reach the site of action in a biologically active form, as required for other pharmaceutical compounds. Generally speaking, there are two major hurdles in such applications of DNA molecules: one is biological instability and the other is poor ability to pass through biological membranes. Even though DNA is chemically stable compared with proteins, it is easily degraded within the body. Biological fluids, such as serum and extracellular fluid, and the cytosolic compartment contain DNases, DNA degrading enzymes [23]. Therefore, once recognized by these enzymes, DNA administered as a pharmaceutical agent will be degraded and no therapeutic benefits will be obtained.

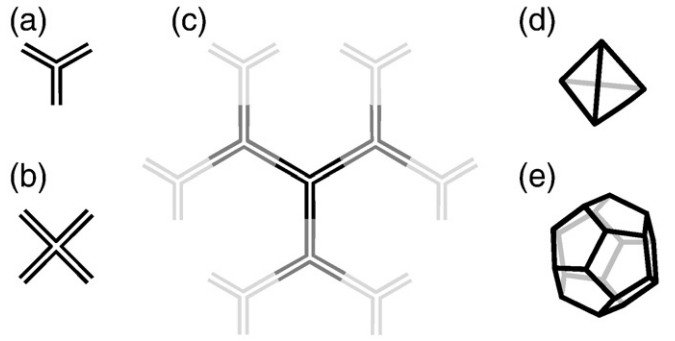

Fig. 1. Schematic presentation of a variety of DNA assemblies. In most cases, double strand formation between two DNA molecules is the driving force to form DNA assemblies. (a) Y-DNA, (b) X-DNA, (c) dendrimaer-like DNA (Y-DNA based), (d) DNA tetrahedron, and (e) DNA dodecahedron. Each line represents one (a-c) or more (d,e) DNA strands.

DNA interacts with its target molecule once it reaches its site of action. If a DNA molecule is designed to interact with complementary DNA or RNA, the length of the DNA should be 15 nucleotides or longer to guarantee the specificity of binding to the target molecule [24]. This size limitation is determined by the size of the human genome. In addition, this length is also required to give the DNA a melting temperature high enough to form a stable duplex within cells under physiological conditions. However, these requirements are dependent on the type of nucleic acids.

An exception of DNA-based drugs is CpG DNA, an immunostimulatory compound, which exhibits its biological activity through the interaction with Toll-like receptor 9 (TLR9) [25]. TLR9 is localized in the endoplasmic reticulum and the endosomal/lysosomal compartments. Therefore, endocytic uptake, a major route for cellular uptake of macromolecules including DNA molecules, can sort CpG DNA to the subcellular compartments where TLR9 is localized. In this case, the membrane permeability does not prevent DNA from exhibiting its biological activity, and DNA as long as several thousand base pairs can induce a large amount of proinflammatory cytokines when its degradation is inhibited [26].

2.1. Stability

The problem of the biological instability of short strand DNA, or oligodeoxynucleotide (ODN), has often been solved by chemical modification. Various forms of chemically modified ODN have been developed, includig phosphorothioate ODN, 2'-O-methyl ODN, morpholino ODN, methylphosphonate, phosphoramidate ODN and locked nucleic acid (Fig. 2) [27–30]. Peptide nucleic acids (PNA), which are not DNA or RNA but can hybridize with a complementary DNA/RNA, have also been developed [31]. The binding affinity of PNA to complementary nucleic acids is much greater than DNA or RNA of the same sequence because of the absence of the negative charge in the PNA strand. Covalent linkage of other molecules, such as cholesterol, has been found to be another approach to increasing the stability of ODN [32,33]. These chemically modified ODNs are more stable than the natural phosphodiester ODN, because the recognition and cleavage by nucleases is at least partly impaired by such modifications.

Attention should be paid to these modifications, because some of them, if not all, can alter the specificity of ODN to interact with target molecules. In addition, the physiochemical characteristics of ODN will be greatly changed by these modifications which, in turn, affect their pharmacokinetic profile after in vivo administration. For instance, phosphorothioate ODN is extensively bound to proteins compared with phosphodiester ODN [34]. This change in the interaction with biological components can be advantageous for an increase in the biological half-life of ODN but, at the same time, it could be a cause of tissue damage when chemically modified ODN is extensively bound to the cell surface. The stable nature of modified ODN could increase the damage compared with that induced by phosphodiester ODN.

2.2. Permeability

Another problem, that of 'poor permeability', is difficult to solve, because it is closely related to the physicochemical nature of DNA/