CHAPTER 11

PNAs as Novel Cancer Therapeutics

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Abstract

Peptide nucleic acid (PNA) is a hybrid compound with nucleoside bases linked to a peptide-like amide backbone. PNA is capable of sequence-specific base pairing and forms highly stable double and triple helices with natural nucleic acids (DNA, RNA). PNA forms stable hydrogen bonds and is resistant to degradation by nucleases and proteases. Because of these physicochemical properties, PNA has attracted great attention, since its first description in 1991, as a potential gene-specific drug and a versatile molecular biology tool. More and more laboratories are working with PNA and the number of applications in which PNA proves useful continues to increase.

In this chapter, we describe aspects of the biochemistry of peptide nucleic acids and their use as a molecular biology reagent, and then focus on the antisense and anti-gene activity of PNA, with special reference to studies of medical interest, in particular in the PML/RARA and the bcl-2 systems.

Introduction

It has been known for a long time that naturally occurring "antisense" RNA can anneal to complementary "sense" transcripts, thus preventing them from being translated into the corresponding polypeptide. Decades ago, the first work appeared that exploited nucleic acid hybridization to inhibit gene expression by means of an "antisense" DNA oligo-deoxynucleotide (ODN). Since that time, a perennial hope for gene-specific inhibitors of expression has arisen, which could represent "magic bullets" for diseases in which gene expression is specifically upregulated. A huge scientific effort has led to the development of antisense ODNs as experimental anti-cancer drugs, some of which are currently undergoing clinical evaluation.

Despite the elegant mechanism underlying specific gene expression inhibition by antisense ODNs, many problems are associated with in vivo applications of this strategy. One of these difficulties is related to the instability of ODNs in biological fluids; in particular, the phosphodiester bond linking deoxyribose units is susceptible to cleavage by nucleases, which rapidly degrade conventional ODNs. This can be overcome by modifications of the backbone to obtain a noncleavable ODN. One such modification is the phosphorothioate bond, where a sulphur atom is substituted for an oxygen in the phosphate group. Phosphorothioate (PS-) ODNs are much more stable and they still activate RNase H upon binding to complementary RNA sequences.

Although PS-ODNs are the most widely used ODNs in basic and clinical research, unfortunately PS-ODNs have some unexpected properties. First, they can have pro-inflammatory activity, especially when they contain the CpG motif, which is a CG dinucleotide preceded by two purines (A or G) and followed by two pyrimidines (T or C). This pro-inflammatory effect appears to be mainly due to stimulation of B cell proliferation. Second, PS-ODNs can bind nonspecifically to several proteins, including the complement negative regulator, Factor H.
This leads to abnormal activation of the complement cascade, with the potential for acute inflammatory responses and ultimately cardiovascular toxicity. Another antisense-independent activity of this type of ODNs is observed with oligomers carrying four consecutive guanines, the G-quartet motif, that mediates ODN binding to a specific set of proteins and RNAs, producing particular pharmacological effects. More adverse effects include inhibition of the clotting cascade and end-organ toxicity, particularly to the liver and bone marrow. Other modified backbones have been devised in order to improve antisense technology; for example, the phosphoramic acid, the α-phosphodiester, the 2′-O-methyl and the methylphosphonate linkages confer increased stability and cellular uptake. Despite growing optimism, whether antisense compounds will become commonly used drugs in the future remains an open question.

In 1991 a group of Danish chemists led by Dr. Ole Buchardt and Dr. Peter Nielsen described a new example of a synthetic molecule, which they called peptide nucleic acid (PNA), that behaves like a nucleic acid (Fig. 1). This innovative report followed results reported several years earlier by Dr. James Summerton in the USA on another DNA mimic with a nonphosphodiester, nonribose backbone (morpholino). Due to its high affinity and selectivity for complementary nucleic acid sequences and its resistance to enzymatic degradation, PNA is being studied both as a molecular probe and a possible antisense oligomer for eventual clinical use.

**Biochemistry of Peptide Nucleic Acids**

PNA has a modified peptide (poly-2-aminoethyl-glycine) backbone, where nucleoside bases are linked to the amino nitrogens via a methyl carbonyl group. This artificial molecule is able to bind DNA and RNA in a sequence-specific manner. In contrast to natural nucleic acids, PNA has no phosphate diester bonds and therefore is uncharged (nonpolar). This feature makes PNA/DNA and PNA/RNA hybrids more thermostable than double-stranded (ds) DNA, due to lack of electrostatic repulsion between the two strands. For the same reason, PNA complexes are not affected to a large extent by conditions of low ionic strength. Despite their strong

![Figure 1. Chemical structure of PNA compared to protein and DNA. The amide bond, present in proteins and PNAs, is boxed.](image)