HIGH-MOLECULAR WEIGHT POLYETHYLENE GLYCOLS CONJUGATED TO ANTISENSE OLIGONUCLEOTIDES

Synthesis and Applications

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1. INTRODUCTION

The oligonucleotides are short- to medium-size sequences of nucleic acids. As such they have a polianionic nature. Repeating monomer units, nucleosides, in which a ribose, or a deoxyribose unit, is joined via an N-glycosidic bond with an heterobase, purine- or pyrimidine-like, makes them. These units are connected each other by a phosphodiester bridge through the 3’- and 5’-OH functionalities of the sugars.

Usually, due to the size required for the expression of the biological activity, the average dimension of the synthetic oligonucleotides ranges between 10/12 and 18/20 nucleosides.

Taking advantage from the specificity of Watson-Crick base pairing, as originated by the formation of a regular net of hydrogen bonds between opposite heterocyclic bases of complementary nucleic acid sequences, these molecules have been tested as biological tools for elucidating gene function and as human therapeutics. It is easy to conceive that a properly designed oligonucleotide could be able to inhibit a gene by interaction with a specific part of its sequence. This inhibition of the gene expression was proposed more than 20 years ago by Zamecnik and Stephenson¹, with the purpose of blocking the effect of an exogenous viral genome inside hosting cells. This approach will be equally effective in suppressing unwanted pathological
Polyethylene Glycols Conjugated to Antisense Oligonucleotides

effects due to nucleic acid sequences, as those, for example, of some oncogene, or of any other gene-related pathology.

Since then, this so-called antisense strategy has been widely investigated, and two main mechanism of action can be described. The first one, the true antisense effect, is due to the arrest of the translation process, i.e. the production of proteins as expressed by the genetic message encoded in a RNA chain, the messenger or mRNA. (Fig.1A). In this case, the complex between the antisense oligonucleotide and the target RNA yields a stable duplex; as a consequence, the protein production is avoided, mainly through the activation of an ubiquitary enzyme, the Rnase H, that destroys the RNA part of that duplex.

In the other process, the antigene mechanism is generated by the direct complexion of the original code inside the double helix of DNA. Some special oligonucleotide sequences are able to make a triple complex, a triplex, with the double helix of the DNA target: in such a way the transcription of the message in a new mRNA chain is escaped, and the following translation in the pathological proteins is fully prevented (Fig. 1B).

![Figure 1. Antisense oligonucleotide: A. scheme of the true antisense action; B. scheme of the antigene action](image-url)