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Artificial Ribonucleases

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Abstract: Chemically modified oligonucleotides serve as tools in fundamental research as diagnostic reagents and pharmaceutical compounds. Various chemical groups can nowadays be linked to oligonucleotides. In particular, synthetic constructs of oligonucleotides and lanthanide complexes can serve as artificial ribonucleases. In this paper, some applications of modified oligonucleotides originating from our research efforts directed at the development of artificial ribonucleases are summarized.

Keywords: Artificial ribonucleases · Lanthanide complexes · Oligonucleotide · RNA · RNA cleavage

Due to its significant role in many biological processes, RNA is a subject of ongoing intensive investigation. New insights into the structural and functional properties of various RNAs may lead to the development of new drugs targeting viral [1] and microbial [2] RNAs. In addition, understanding the function of expressed mRNAs is of relevance for the field of functional genomics [3]. Antisense oligonucleotides [4–6] offer a highly specific way of inhibiting gene expression by binding to the mRNA coding for a given protein and are, thus, useful tools for validation of new therapeutic targets [7]. Several research groups have pursued the goal of cleaving RNA in a sequence-specific way [8][9]. A method which allows the cleavage of an RNA molecule of interest at a single site offers potential benefits both for therapeutic and diagnostic purposes. Furthermore, the development of artificial ribonucleases also leads to new tools for the study of the structure and function of RNA.

For the successful construction of an artificial ribonuclease, two minimal requirements have to be fulfilled: i) the specific recognition and binding of the nuclease to the RNA target site and ii) the efficient chemical cleavage of the target's phosphodiester backbone. The ability of metals and their complexes to catalyze the transesterification of phosphodiesters is well known. The mechanism by which metals catalyze the transesterification of RNA has been investigated in detail and can be illustrated as shown in Scheme 1A [10][11]. The principal driving force that allows RNA to readily undergo strand cleavage upon coordination of the phosphate group by a Lewis acid is the presence of the 2'-hydroxyl group. The latter acts as the nucleophile in the subsequent displacement reaction. This transesterification reaction gives rise to two cleavage products, one fragment bearing a 2',3'-cyclophosphate, the other a free 5'-OH group. In particular, lanthanide complexes have been shown to induce efficient backbone cleavage of ribonucleic acid [12][13]. Therefore, our own research efforts have concentrated on using conjugates of macrocyclic lanthanide complexes of type 1 linked to oligonucleotides. Scheme 1B illustrates the process of the sequence specific cleavage of a target RNA by such a oligonucleotide–metal conjugate. Synthesis of the terpyridine moiety and complex formation follows the procedure described by Constable and Holmes [14]. Due to the remarkable stability of the metal complex, oligonucleotide conjugates can be cleaved from the solid support, deprotected and purified by standard chromatographic or electrophoretic methods without concomitant loss of the metal.

The conjugates thus obtained efficiently induce RNA cleavage [15]. Typically, the artificial ribonucleases cleave the target with a half-life of 6–10 h at 37 °C (pH 7.4). Cleavage occurs in the single stranded region of the RNA target, as illustrated in Scheme 2. 2'-O-(2-methoxyethyl)-modified cleaver conjugates (see Scheme 2B) cleave RNA with equal efficiency and at essentially the same site as unmodified (deoxyoligonucleotide) conjugates. The efficiency of the cleavage reaction is dependent on several factors [16] such as the type of metal, the substitution of the ligand, and the sequence of the RNA target.

As mentioned above, cleavage takes place in the single stranded region of the RNA. It is a well known fact that double stranded RNA is considerably more resistant to metal ion promoted transesterification than its single stranded counterpart [17] rendering cleavage of RNA within a duplex difficult or impossible. The potency of such artificial ribonucleases might be raised if the cleavage process could be extended into the duplex region. This would increase the number of possible cleavage sites and, perhaps more importantly, offer the possibility of catalytic turnover.

We found that cleavage can be directed into a double stranded region of RNA by introduction of bulges and internal loops in the RNA target strand [18]. Thus, lanthanide complexes were linked to the oligonucleotides at sites in the major or the minor groove which position the metal complex near the bulge [19].

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Scheme 1. A) Metal complex induced phosphodiester cleavage in RNA. B) Schematic representation of the sequence specific cleavage of an RNA target by an oligonucleotide lanthanide conjugate.

Scheme 2. Lanthanide induced backbone cleavage of an oligoribonucleotide by complementary oligodeoxynucleotide (A) and 2'-O-(2-methoxyethyl)-modified oligonucleo-tide (B) conjugates. Arrows indicate the site of cleavage; the size of the arrow is drawn proportional to the cleavage intensity.

Scheme 3. A) RNA cleavage (arrows) at bulged nucleotides. B) Artificial chemical ribonuclease acting with multiple turnover.

Treatment of the target RNA takes place at or near the bulge (Scheme 3A). Using such a system, it was possible to find 2'-O-(2-methoxyethyl)-modified conjugates acting with multiple turnover (Scheme 3B) [20].

Applications of artificial ribonucleases of the type described above may find application in the cleavage of large, biologically relevant RNA, such as messenger RNA. Experiments evaluating the potential of oligonucleotide–metal conjugates for such applications are currently underway.

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