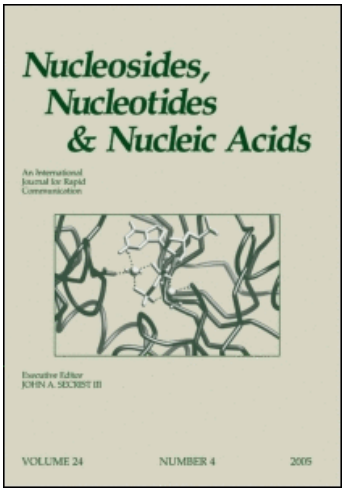

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STUDIES IN OLIGONUCLEOTIDE-BASED ARTIFICIAL NUCLEASE SYSTEMS. INTRAMOLECULAR COPPER (II) COMPLEX FORMATION IN AN OLIGONUCLEOTIDE *BIS*-PHENANTHROLINE CONJUGATE

Dmitri A. Ossipov and Roger Strömberg ▫ *Division of Organic and Bioorganic Chemistry, MBB, Scheele Laboratory, Karolinska Intitutet, Sweden*

▫ *We have recently developed oligonucleotide based artificial nuclease (OBAN) systems based on 2′-O-methyloligoribonucleotides carrying a 2,9-dimethylphenanthroline · Zn(II) complex. These hybridize to an RNA molecule with bulge formation in the central region of the target and cleave the RNA target in a catalytic manner. When studying an 11-mer 2′-O-methyloligoribonucleotide carrying two 2,9-dimethylphenanthroline moieties, located 5 base pairs apart from each other, we found that this forms a cyclic structure in the presence of Cu^{2+} ions. This is due to intramolecular Cu(2,9-dimethylphenanthroline)$_2$ complex formation, i.e., with the two ligands conjugated to the same oligonucleotide.*

INTRODUCTION

Recently we have reported on the development of OBANs that are 2′-*O*-Me-oligoribonucleotides linked to a Zn^{2+} complex of 2,9-dimethylphenanthroline (dmp).[1,2] Our OBANs hybridize giving bulge formation in the central region of the RNA target.[3] In search of optimal combination of linker and linker attachment position we have prepared novel 2′-*O*-Me-oligoribonucleotide conjugates (**IV–VI**) carrying dmp moieties. In order to further increase the OBAN efficiency we also designed an OBAN carrying two catalytic groups, which could cooperatively act on the same scissile phosphate. There are examples of oligonucleotide *bis*-chelate conjugates for metal-catalyzed phosphate transfer[4,5] (Figure 1).

Two chelating moieties within the same oligonucleotide strand could however impart specific properties to the conjugates when chelating ligands interact with each other through complexation to the same metal ion. In this study we present the observation that intramolecular Cu^{2+} complex formation occur in an 11-mer 2′-*O*-methyloligoribonucleotide carrying two 2,9-dimethylphenanthrolines located 5 base pairs apart. The formation of a Cu(2,9-dimethylphenanthroline)$_2$ complex

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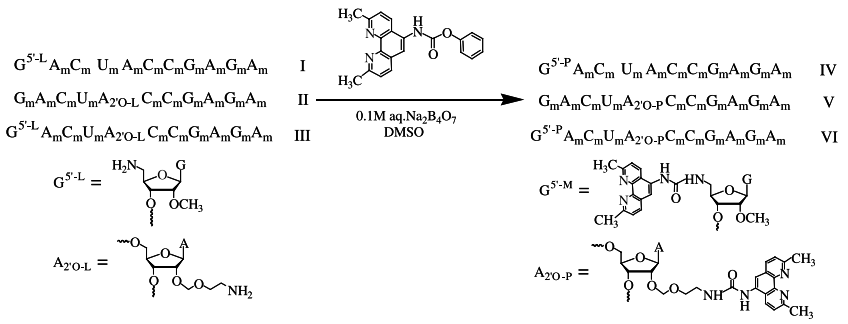

FIGURE 1

leads to cyclization within the oligonucleotide conjugate. It is a novel observation that chelating parts separated in space in an oligonucleotide can form an intramolecular bischelate in the presence of a metal ion.

RESULTS AND DISCUSSION

The oligonucleotide conjugates **IV–VI** were synthesized by reacting 5-*N*-phenylcarbamoyl-5-amino-2,9-dimethylphenanthroline with oligonucleotides **I–III** according to the previously published procedure.[2] Oligonucleotides **I–III** where synthesized using standard phosphoramidite methodology (on an Applied Biosystems 392 DNA/RNA Synthesizer) and preparation of the 3′-modified building block is described elsewhere.[3] Synthesis of the modified guanosine building block was carried from the isobutyryl protected 2′-*O*-methylguanosine by iodination[6] followed by substitution of the 5′-iodo with azide,[7] which was then converted to the 5′-amino derivative by hydrogenation. Reaction of the resulting amine with 4-methoxytrityl chloride and subsequent phosphitylation afforded the desired amidite building block.[8]

We observed that the *bis*-2,9-dimethylphenanthroline conjugate **VI** behaves differently in the presence of Cu^{2+} ion compared to the mono-2,9-dimethylphenanthroline-oligonucleotide analogs **IV** and **V**. In the HPLC analysis of OBANs **IV–VI** we observed that for OBAN **VI** a couple of peaks of almost equal intensity with a difference in retention times of ~2.5 min appeared in the chromatogram. The LC-MS analysis of those peaks revealed that only the less retarded peak belongs to OBAN **VI** whereas the other peak arises from a species with the mass exceeding that of OBAN **VI** by 63 Dalton, the atomic weight of Cu. The same mass was obtained when 0.5 nmol of OBAN **VI** was mixed with 0.5 nmol of $CuCl_2$ and analyzed by MS, while oligonucleotides **IV** or **V** bearing only one dmp (2,9-dimethylphenanthroline) residue do not give an increased mass when mixed with $CuCl_2$. A possible explanation for these results is that oligonucleotide **VI** binds Cu^{2+} ions more strongly and that the **VI** · Cu^{2+} species is stable enough to be detected by ES-TOF MS.

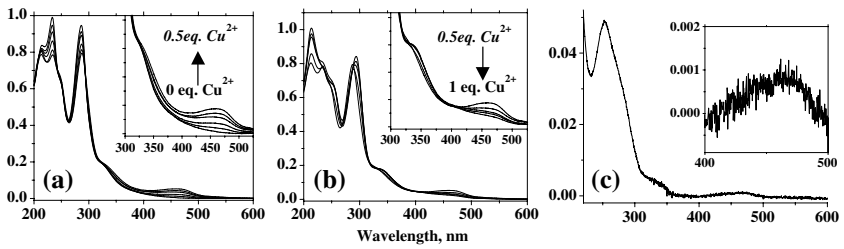

FIGURE 2 Electronic absorption spectra of (a) 25 μM of dmp in the presence of 0–0.5 equivalents of $CuCl_2$, (b) dmp in the presence of 0.5–1 equivalents of $CuCl_2$, (c) **VI** · Cu^{2+} isolated by IE-HPLC followed by RP-HPLC.

The stepwise stability constants of $Cu(dmp)^{2+}$(aq) and $Cu(dmp)_2{}^{2+}$(aq) are 1.6×10^6 and 6.3×10^6 M^{-1}, respectively, indicating that the first ligand facilitates uptake of the second dmp molecule.[9] This supports the interpretation that the two dmp moieties of the bis-conjugate **VI** may coordinate the same Cu^{2+} ion. To obtain further evidence we investigated the coordination of 5-amino-2,9-dimethylphenanthroline (NH_2dmp) to Cu^{2+} by UV/Vis and MS spectroscopy. Upon the titration of NH_2dmp with Cu^{2+} a prominent increase of the absorption band intensity at 460.7 nm was observed until the [NH_2dmp]/[Cu^{2+}] ratio became 2:1 (Figure 2a), while further increase of [Cu^{2+}] was characterized by decrease of the absorption at 460.7 nm (Figure 2b). A change in band wavelengths and intensities was also detected in the UV region of < 350 nm. This observation points at the formation of two kinds of species, most likely $Cu(NH_2dmp)_2{}^{2+}$ and $Cu(NH_2dmp)^{2+}$. However, for both extreme cases, i.e., for 2(NH_2dmp) + Cu^{2+} and NH_2dmp + Cu^{2+} mixtures, MS analysis gave a *m/z* of 509.6 which corresponds to the $Cu(NH_2dmp)_2{}^{2+}$ complex (MW_{cal} = 510.1). This is additionally confirmed by the greater stability of the $Cu(NH_2dmp)_2{}^{2+}$ complex, which is easily detected by MS. It is also noteworthy

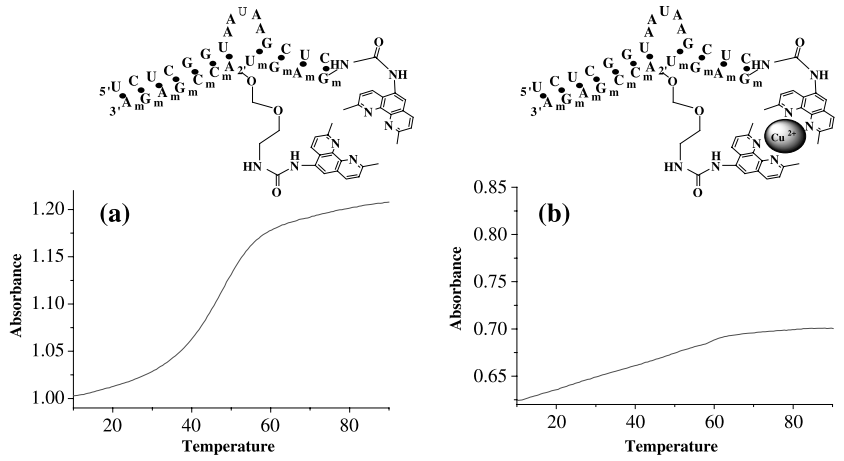

FIGURE 3

that the UV/Vis absorption spectra of the isolated **VI** · Cu^{2+} adduct is characterized by the same MLCT band centered at 460 nm as $Cu(NH_2dmp)_2^{2+}$ complex (Figure 2c), supporting that the Cu^{2+} ion in **VI** · Cu^{2+} is coordinated by both dmp ligands.

Coordination of the two dmp units of OBAN **VI** to the same metal ion leads to cyclization in a part of the oligonucleotide. This could then reduce its ability to hybridize to a target RNA and diminish the nuclease activity of the OBAN. To evaluate this assumption, we performed thermal denaturation experiments. With mono-dmp conjugate **V** and a bulge forming 16-mer RNA (4 μM of each strand; 0.1 M NaCl, 10 mM phosphate buffer (pH 7.0), the melting temperatures with and without 4 μM Cu^{2+} were identical (47.3°C). This confirms that the presence of metal ion does not disturb the hybridization of conjugate **V**. Interestingly, the melting profile of the mixture of *bis*-dmp conjugate **VI** and 16mer RNA in a buffer containing Cu^{2+} was also identical to that obtained in a metal-free buffer (T_m = 47.4°C, Figure 3a). However, when the preformed and isolated **VI** · Cu^{2+} adduct was used in the corresponding melting experiment in a metal-free buffer, no clear transition was detected (Figure 3b).

This result can be explained if we assume the metal comlexation to be the rate-limiting event. The hybridization of OBAN **VI** to the RNA appear to be favored kinetically compared to the formation of **VI** · Cu^{2+}, while when the **VI** · Cu^{2+} species is preformed the decoordination of the Cu^{2+} ion is slow and hybridization does not occur readily.

To our knowledge, this is a first example of metal ion induced conformational reorganization in a single-stranded oligonucleotide carrying two unnatural metal binding residues. The cooperative binding of the two dmp ligands to Cu^{2+} leads to cyclization of a part of the oligonucleotide which in turn puts out of action the ability of the oligonucleotide to hybridize to the RNA target. Further studies on these constructs will be pursued to investigate their properties in more detail.

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