"Decapitation" of a 5'-Capped Oligoribonucleotide by *o*-Phenanthroline:Cu(II)

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Cellular and viral messenger RNAs synthesized by RNA polymerase II have a unique chemical structure at their 5' termini (Figure 1)[1] which is required in varying degrees for processing and maturation of the transcript in the nucleus,[2] transport of the message from the nucleus to the cytoplasm,[3] mRNA stability,[4] and efficacy in translation of the message to the encoded protein.[5] Because of this pivotal role in mRNA metabolism and its inherent chemical properties, we are exploring the 5' cap as a target for antisense technologies. Antisense directed chemical alteration of the 5' cap may provide transcript specific inhibition of protein expression.[6] An understanding of its reactivity will provide the basis for the design and synthesis of antisense oligonucleotides equipped with 5' cap specific reactive moieties. This report describes the reaction of a select set of metal complexes upon the 5' cap structure of messenger RNA.[7,8] The most reactive metal complex assessed, *o*-phenanthroline:Cu(II), is shown to selectively decapitate a 5'-capped RNA hybridized to its DNA complement.

Cu(II) and Zn(II) metal ions at 300 μM and their complexes with *o*-phenanthroline (OP), bipyridine (Bipy), and terpyridine (Terpy), where L:M = 1.1:1.0, were assessed for their reactivity upon the capped monoribonucleotide m^{7}GpppG at 50 uM. The order of reactivity for the Cu(II) series was OP:Cu(II) > Cu(II) ≥ Bipy:Cu(II) ≫ Terpy:Cu(II).[9] Zn(II) metal ion and its complexes demonstrated negligible activity.[10] This order of reactivity differs from that observed for single-stranded RNA.[7c] The most reactive compound, OP:Cu(II), hydrolyzed 67% of the 5'-capped monoribonucleotide to m^{7}GMP, m^{7}GDP, GMP, and GDP after 24 h at 37 °C (Figure 2). Product assignment was performed by coinjection experiments utilizing commercially available standards. Approximately twice as much more of the product pair m^{7}GMP/GDP was produced over the m^{7}GDP/GMP pair. Disappearance of m^{7}GpppG at 50 μM in the presence of 300 μM OP:Cu(II) is described by a first-order rate process with a rate constant of 4.99×10^{-2} h^{-1} and $t_{1/2}$ = 14 h.

On the basis of the hydrolytic activity of OP:Cu(II) upon the model substrate, m^{7}GpppG, the metal complex was tested further for its reaction upon a 5'-capped radiolabeled 20-nt oligoribonucleotide hybridized to its DNA complement. The 5'-capped RNA substrate was incubated at 37 °C over a 48-h period in the presence and absence of 100 μM OP:Cu(II) under buffered conditions (pH 7.4). Analysis of the reactions by gel electrophoresis demonstrated that in the absence of the metal complex

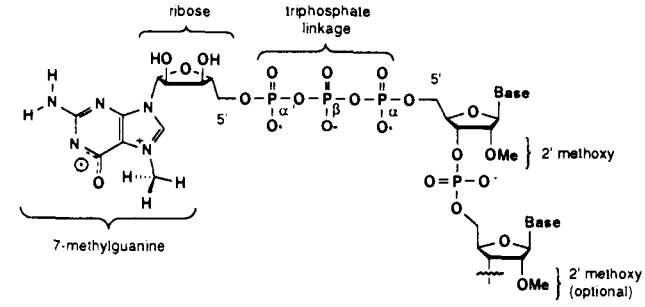

Figure 1. 5' cap structure of messenger RNA.

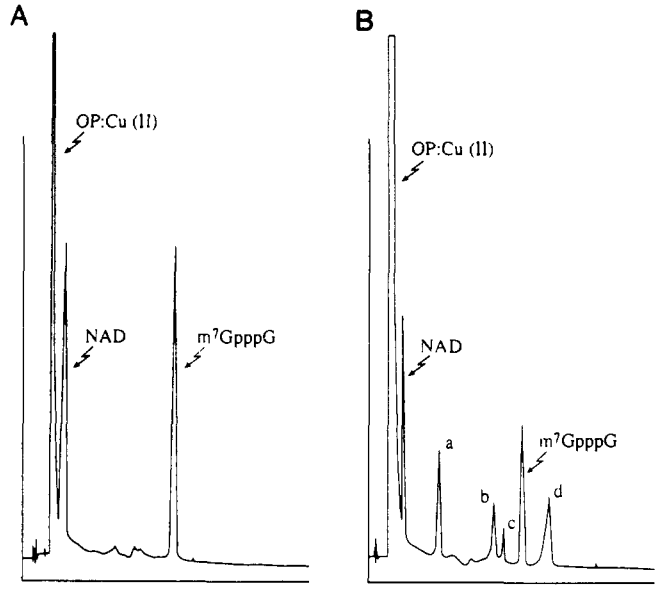

Figure 2. FPLC chromatograms[11] of the OP:Cu(II) + m^{7}GpppG reaction at (A) 0 h and (B) 24 h, where products have been labeled as (a) m^{7}GMP, (b) GMP, (c) m^{7}GDP, and (d) GDP. NAD (nicotinamide adenine dinucleotide) was the internal standard utilized to measure the rate of disappearance of the starting material. The standard was added immediately prior to chromatographic analysis of each reaction. Reaction conditions: 300 μM OP:Cu(II), 50 μM m^{7}GpppG, in 20 mM HEPES (pH 7.1) at 37 °C.

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(9) The percentages of m^{7}GpppG hydrolyzed after 24 h at 37 °C measured as 67%, 32%, 29%, and 5%, respectively. Percentage values for the metal reactions are based upon the amount of remaining substrate measured against an internal standard (NAD) and that of the control (buffer only, no metal complex under the same reaction times and temperature). Average percent error = ±2%.

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(11) Chromatographic analyses of the reactions were performed on a Pharmacia LKB FPLC system utilizing a Mono Q HR 5/5 column. Solvent A: H_2O. Solvent B: 1 M NaCl + 5 mM sodium phosphate (pH 7.0). Program gradient: 0–40% B in 30 min, 40% B for 1 min, 40–100% B in 1 min, 100% B for 1 min, 100–0% B in 0.1 min, and 0% B for 10 min. Flow rate = 1 mL/min. Compounds were detected by UV at 260 nm in the 0.1-AU range.

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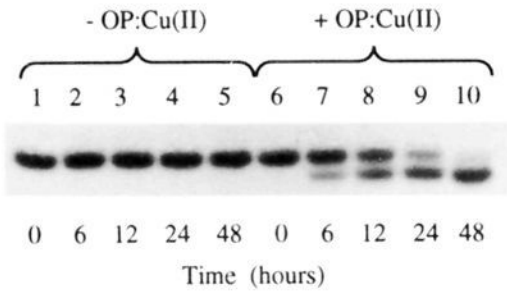

Figure 3. Decapitation of a 5′-capped oligoribonucleotide (antisense duplex) by OP:Cu(II). Autoradiogram of the analytical gel (20% acrylamide, 1:20 cross-linkage, 40% urea). Lanes 1–5: m^7GpppRNA control reaction at 0, 6, 12, 24, and 48 h. Lanes 6–10: m^7GpppRNA + 100 μM OP:Cu(II) at 0, 6, 12, 24, and 48 h. Both reactions included 10 μM antisense DNA (5′ TCTGAGTAGCAGAGGAGCTC 3′) preannealed to the internal ^{32}P-radiolabeled RNA (m^7Gppp-GAGCUCCUCUGCUACUCAGA)[12] by a heat denaturation, slow cool step. Solutions were buffered in 20 mM Hepes (pH 7.4), 50 mM KCl, 100 mM NaCl. Reaction temperature was 37 °C.

the 5′-capped RNA was stable and nonreactive (Figure 3, lanes 1–5), but in the presence of OP:Cu(II) a reaction occurred which resulted in the appearance of a single RNA band, with faster mobility,[13] in increasing amounts over time (lanes 6–10). The resulting OP:Cu(II) RNA product(s) was not a substrate for the human recombinant 5′ cap specific binding protein, eIF-4E (Figure 4, lane 9).[14] Additional experiments showed that the OP:Cu(II) + m^7GpppRNA:DNA reaction occurred under argon and was not dependent upon the amine sulfonate buffer (pH 6.8).[15] No reaction occurred upon the m^7GpppRNA:DNA substrate in the presence of Cu(II) or *o*-phenanthroline alone at 10 and 100 μM, respectively. The uncapped RNA equivalent, where the 5′ terminus was a hydroxyl group in lieu of m^7Gppp–, was not a substrate for the OP:Cu(II) complex.

These studies demonstrate that the 5′ cap structure of mRNA is effectively hydrolyzed by complexes of copper under the given conditions. Further investigations will include the analysis of other metal complexes which are 5′ cap specific, yet better suited for in vivo therapeutic applications, along with the synthesis and analysis of antisense oligonucleotides equipped with such reactive moieties.

(13) Reaction of OP:Cu(II) with the 3′-end-labeled 5′-capped oligoribonucleotide confirmed that the cleavage reaction occurs at the 5′ terminus of the target RNA. Resolution of the expected products, e.g., the 5′ mono- and diphosphate oligoribonucleotides, was not achievable by the analytical procedure utilized in these studies.

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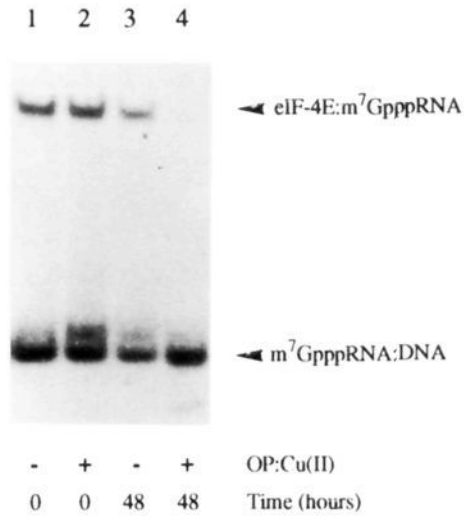

Figure 4. eIF-4E:m^7GpppRNA gel shift assay: a 5′ cap specific product analysis. Autoradiogram of the 6% (1:50) nondenaturing acrylamide gel. Lane 1: m^7GpppRNA control reaction at 0 h. Lane 2: m^7GpppRNA + 100 μM OP:Cu(II) at 0 h. Lane 3: m^7GpppRNA control reaction at 48 h. Lane 4: m^7GpppRNA + 100 μM OP:Cu(II) reaction after 48 h. This analysis represents a portion of the 0- and 48-h reaction samples analyzed in Figure 3. Each sample was ethanol precipitated and resolubilized in buffer (40 mM HEPES, 100 mM KCl, 0.5 mM EDTA, pH 7.4) prior to incubation with 1 μM eIF-4E.[14]

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Supplementary Material Available: Details of data and experimental results (11 pages). Ordering information is given on any current masthead page.