DALTON COMMUNICATION

Dinuclear copper(II) complexes that promote hydrolysis of GpppG, a model for the 5′-cap of mRNA †

Kevin P. McCue, David A. Voss, Jr., Christian Marks and Janet R. Morrow *

Chemistry Department, State University of New York at Buffalo, Amherst, New York 14260-3000, USA

The hydrolysis of the monoribonucleotide GpppG, a model compound for the 5′-cap structure of mRNA, by dinuclear Cu(II) complexes of triazacyclononane was 100-fold more rapid than in the presense of the analogous mononuclear complex; a first-order or second-order dependence on the catalyst was observed for two different dinuclear complexes.

There is much interest in the development of metal ion complexes that catalyze the cleavage of RNA.[1–3] Such complexes when attached to a recognition agent such as an antisense oligonucleotide catalyze the sequence-specific cleavage of RNA,[4–9] and it is proposed that these catalysts may be useful in the selective inactivation of mRNA. The hydrolytic cleavage or transesterification of the RNA phosphate diester backbone[1–9] by metal ion complexes has been studied extensively. An alternate approach which may be useful for the inactivation of mRNA entails the hydrolysis of the 5′-cap structure of mRNA.[10] Baker has demonstrated that several Cu(II) complexes hydrolyze the 5′-cap structure both free in solution[11] and when attached to oligonucleotides.[12] Inert lanthanide(III) complexes are also effective in promoting cleavage of the 5′-cap structure.[13] Recently, we found that lanthanide(III) complexes promote cleavage more efficiently in the presence of an equivalent of a second metal ion.[14] This result has prompted us to study dinuclear metal ion complexes as catalysts for cleavage of GpppG, a model for the 5′-cap structure of mRNA (Fig. 1). Our studies here suggest that the mechanism of hydrolysis of GpppG is distinctly different from that of other phosphorus(V) substrates. Depending on the dinuclear complex employed as catalyst, a first-order or a second-order dependence on catalyst is observed in the hydrolysis of GpppG.

Cu(II) complexes of linked triazacyclononane ligands were chosen for our initial studies of dinuclear catalysts (see below). The triazacyclononane ligand L^1 binds strongly to transition metal ions[15] and recent studies have shown that similar ligands readily bind two Cu(II) ions.[16–18] In addition, Zn(II) and Cu(II) complexes of triazacyclononane catalyze the hydrolytic cleavage of RNA and phosphate diester hydrolysis.[19–21] The two dinucleating ligands were prepared ‡ by treatment of the linkers α,α′-dibromo-*m*-xylene or α,α′-dibromo-*p*-xylene with 2 equivalents of the *N*,*N*′-bis(*p*-tolylsulfonyl)-1,4,7-triazacyclononane[22] and standard deprotection conditions were used.[23] Potentiometric titrations of solutions containing a 2:1 ratio of $Cu(NO_3)_2$ to L^2 or L^3 (1.0 mM Cu^{2+}, 0.5 mM ligand, 0.1 M NaCl) showed well defined inflections at 6 equivalents and 8 equivalents of base (supplementary Figs. 1 and 2, SUP 57417), similar to data for other dinuclear Cu(II) complexes with two triazacyclononane ligands.[16,17] This data suggests that L^2 and L^3 both bind two Cu(II) ions and the predominant species at a 2:1 ratio of Cu(II) to ligand at pH 5 is a dinuclear complex $[Cu_2L]^{4+}$ while at neutral pH the predominant species is a bis-hydroxide dinuclear Cu(II) complex $[Cu_2L(OH)_2]^{2+}$.[16,17]

Hydrolysis of the capped monoribonucleotide, GpppG, by Cu(II) complexes of L^1–L^3 was examined. Disappearance of GpppG was monitored by use of an HPLC assay[13] and the sole products detected were GMP (guanosine 5′-monophosphate)

* E-mail: jmorrow@acsu.buffalo.edu

† Supplementary data available: potentiometric titrations, plots of the log of pseudo-first-order rate constants *versus* log[$(Cu_2L)^{4+}$] and the energy minimized structure of $[Cu_2L^3]^{4+}$ with a bridging pyrophosphate. For direct electronic access see http://www.rsc.org/suppdata/dt/1998/2961/, otherwise available from BLDSC (No. SUP 57417, 8 pp.) or the RSC Library. See Instructions for Authors, 1998, Issue 1 (http://www.rsc.org/dalton).

‡ The ligands L^2 and L^3 were prepared by treating α,α′-dibromo-*m*-xylene or -*p*-xylene with 2 equivalents of the *N*,*N*′-bis(*p*-tolylsulfonyl)-1,4,7-triazacyclononane in acetonitrile with a 2–3 fold excess of triethylamine. The mixture was refluxed under nitrogen for 24 h and the ligands were purified by use of silica gel chromatography (2% methanol in chloroform). The ligands were deprotected (ref. 23) and the HBr salts of L^1, L^2 and L^3 were analyzed by use of FAB-MS and ^{1}H NMR.

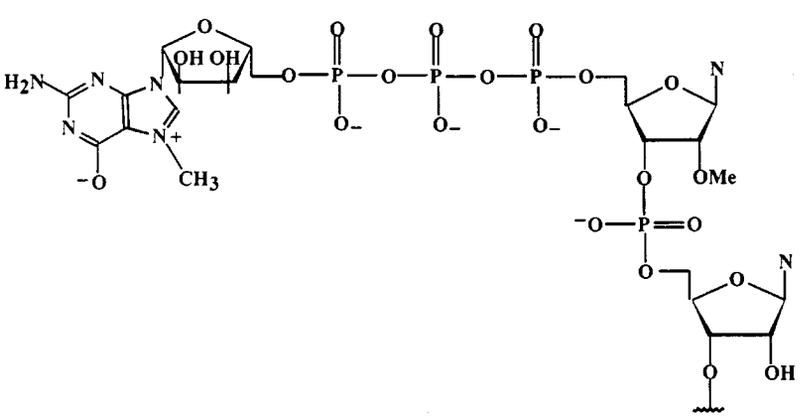
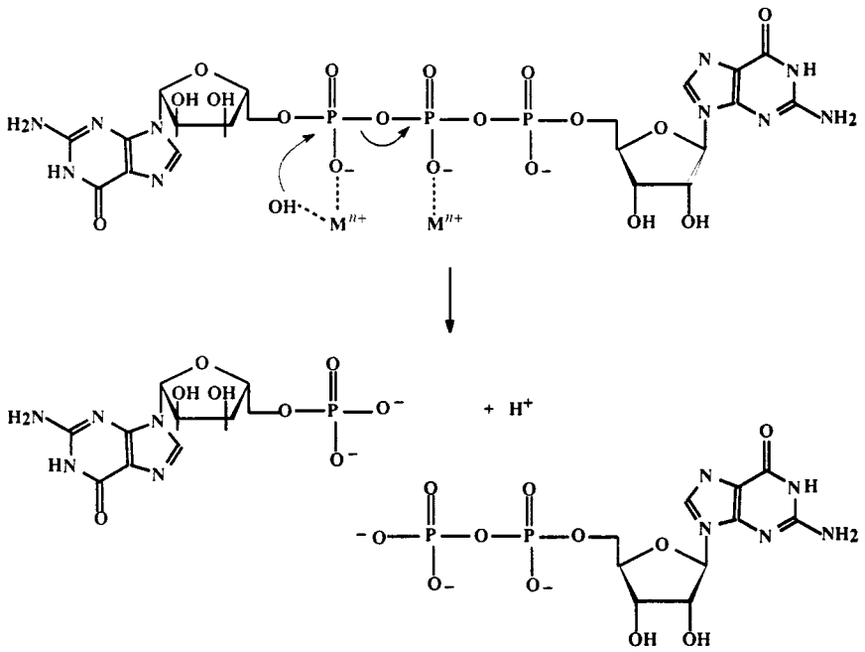
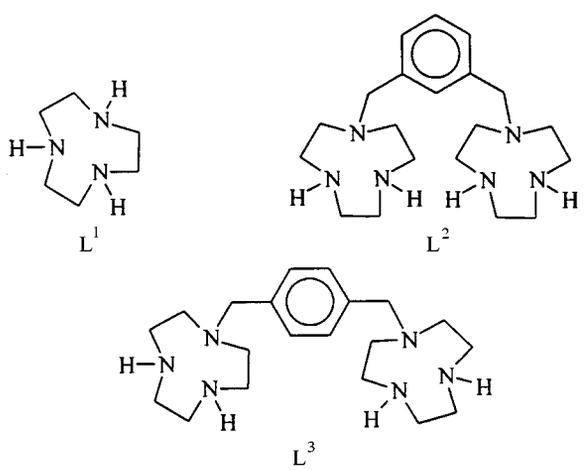


Fig. 1



Scheme 1



and GDP (guanosine 5′-diphosphate). A pseudo-first-order rate constant of 2.3×10^{-7} s^{-1} was determined (half-life of 840 h) for the hydrolysis of GpppG by 0.25 mM $[CuL^1]^{2+}$ at pH 7.3 and 37 °C, with 20 mM hepes buffer. Interestingly, this rate constant is approximately 60-fold lower than other mononuclear Cu(II) complexes that promote hydrolysis of GpppG under similar conditions.[11] Both dinuclear Cu(II) complexes hydrolyzed GpppG more rapidly than did $[CuL^1]^{2+}$. Pseudo-first-order rate constants of 3.6×10^{-5} and 2.2×10^{-5} s^{-1} (half-lives of 5.3 and 8.8 h) were obtained for the hydrolysis of GpppG in solutions containing 0.25 mM Cu(II) and 0.125 mM L^2 or L^3, respectively at pH 7.3 and 37 °C. Thus hydrolysis of GpppG is some 100-fold more rapid per Cu(II) ion in the dinuclear complexes than it is for the monomeric $[CuL^1]^{2+}$ complex under similar conditions. Under similar conditions but in the absence of catalyst, only 3% of the GpppG was hydrolyzed over a period of 5 d.

Further kinetic studies were conducted to study the mechanism of hydrolysis of GpppG by the dinuclear complexes. Hydrolysis of GpppG by both dinuclear Cu(II) complexes was first order in GpppG.§ Hydrolysis of GpppG by the dinuclear Cu(II) complex of L^3 was first-order in complex in the concentration range 0.10 to 0.50 mM with a second-order rate constant of 0.10 M^{-1} s^{-1}.¶ In contrast, the hydrolysis of GpppG by the dinuclear Cu(II) complex of L^2 was second-order in complex for concentrations ranging from 0.030 to 0.210 mM with an apparent third-order rate constant of 730 M^{-2} s^{-1}.‖

How might the two Cu(II) centers in the dinuclear complexes of L^2 and L^3 cooperatively promote hydrolysis of GpppG? Dinuclear metal ion complexes hydrolyze phosphate esters and RNA by double Lewis acid activation[24–35] with one metal ion binding to the incoming nucleophile, the second metal ion binding to the leaving group and both metal ions binding to the phosphate diester. Hydrolysis of GpppG by two metal ions more likely proceeds through interaction of the two metal ions (Scheme 1) at two different phosphate groups similar to the mechanism proposed for nucleoside triphosphate hydrolysis by two metal ions.[36,37] In this scheme, one metal ion delivers the nucleophile and the second metal ion binds to the GDP leaving group through one or both phosphates. Modeling studies suggest that the two dinuclear Cu(II) complexes bind differently to GpppG. Molecular mechanics calculations** were carried out using five-coordinate Cu(II) complexes with three nitrogen donors and two water molecules.[38] Bridging ligands were incorporated by replacement of water molecules. These structures were minimized and energies of the dinuclear complexes with and without bridging ligands were compared.[39] Strain

§ With a ten-fold excess of the dinuclear Cu(II) complexes of L^2 or L^3, plots of log of the concentration of GpppG *versus* time were linear for greater than four half-lives.

¶ Supplementary data (Fig. 3) contains a plot of log *k versus* log of the concentration of the dinuclear Cu(II) complex of L^3 giving a slope of 1.1.

‖ Supplementary data (Fig. 4) contains a plot of log *k versus* log of the concentration of the dinuclear Cu(II) complex of L^2 giving a slope of 2.1.

** All calculations were done with Hyperchem 3.0 (Autodesk Inc, Sausalito, CA). The starting structure for the five-coordinate Cu(II) triazacyclononane complexes was obtained by replacing the two bromide ligands in the CuL^1Br_2 complex (ref. 38) with water ligands. Ideal Cu–N bond distances were obtained from the crystal structure and the Cu–O distance was set to 2.00 Å. Stretching parameters were set to 5.0 mdyne $Å^{-1}$ in order to maintain the macrocycle dimensions. M–L bending parameters were added to maintain the square pyramidal geometry. Torsional parameters about the metal–ligand bond were set to zero so ligand interactions would dictate the overall structure except for torsional interactions where the metal was the terminal atom. For these cases a C4 type atom replaced the metal. After energy minimization, final Cu–ligand bond lengths, bond angles and torsional angles were all within 0.03 Å, 1.3 and 2.0°, respectively of those in the crystal structure.

energy increased only slightly (less than 7%) when phosphate or pyrophosphate are incorporated as bridging ligands into the dinuclear Cu(II) complex of L^2. In contrast, the dinuclear Cu(II) complex of L^3 could not bind a bridging phosphate ligand without large increases in strain energy due to distortions induced in the aromatic linker. However, a pyrophosphate ligand bridged the two Cu(II) ions without substantial strain being introduced (14%) into the complex.†† This result and the first-order dependence on the dinuclear complex are consistent with the dinuclear Cu(II) complex of L^3 promoting GpppG hydrolysis by the mechanism shown in Scheme 1. In contrast, the dinuclear Cu(II) complex of L^2 may promote hydrolysis through either binding to a single or to two different phosphate groups of GpppG. One possible mechanism which is consistent with the rate law has the dinuclear Cu(II) complex of L^2 binding through a single phosphate of GpppG. Thus 2 equivalents of dinuclear complex are required, one to activate the phosphate group undergoing nucleophilic attack and one to bind to the leaving group. This mechanism is consistent with the manner in which $[Cu_2L^2]^{4+}$ binds to small molecules. For example $[Cu_2L^2(OH)_2]^{2+}$ contains two briding hydroxide ligands and the complex has a short Cu–Cu distance.[18] Other mechanisms are possible and studies are underway to further characterize binding of the dinuclear complexes to GpppG.

In summary, we have shown for the first time that dinuclear metal ion complexes efficiently hydrolyze GpppG, a model substrate for the 5′-cap structure of RNA. Catalytic properties of the complexes vary dramatically with different linkers for the triazacyclononane ligands. Future studies will focus on further delineating the mechanism of hydrolysis of phosphoric anhydrides with dinuclear metal ion complexes and the design of new linkers to more precisely position the two metal ion centers for the hydrolysis of phosphoric anhydrides.

†† See supplementary data for an example of a minimized structure of a dinuclear complex with a bridging pyrophosphate.

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