Metal ion promoted cleavage of RNA phosphodiester bonds: from Zn(II) aqua ion to artificial ribonucleases

Qi Wang, Teija Nittymäki, Pasi Virta, Kaisa Ketomäki, Satu Mikkola and Harri Lönnberg*

Department of Chemistry, University of Turku, FIN-20014 Turku, Finland

ABSTRACT

The potential of Zn$^{2+}$ azacrown chelates as constituents of artificial ribonucleases is discussed.

INTRODUCTION

Many of the enzymes catalyzing phosphoryl transfer reactions contain Zn$^{2+}$ in their catalytic center. For this reason, Zn$^{2+}$-based cleaving agents have received exceptionally wide interest among researchers attempting to create chemical models for the enzyme action and developing artificial restriction enzymes. Since azacrowns are known to bind Zn$^{2+}$ very tightly and the resulting chelates cleave phosphoesters, such complexes form a reasonable starting point for development of artificial nucleases.

RESULTS AND DISCUSSION

Among various Zn$^{2+}$ azacrown chelates, the complex of 1,5,9-triazacyclododecane (1; Zn$^{2+}$[12]aneN$_3$) has turned out to be the most efficient cleaving agents of RNA phosphodiester bonds, evidently for the reason that the tridentate binding acidifies Zn$^{2+}$ aqua ion more markedly than the more common tetra-dentate coordination and allows the phosphate oxygen and an aqua ligand to take a cis position upon phosphate binding. The $pK_a$ value of the Zn$^{2+}$-promoted cleavage of uridine 3'-alkylphosphates is only slightly negative, -0.32 ± 0.04, suggesting that the leaving group departs rather as an alcohol than an alkoxide ion. In other words, the reaction probably proceeds by intra-complex general acid-catalyzed breakdown of the phosphonate intermediate, an aqua ligand of the phosphonate-bound Zn$^{2+}$ protonating the leaving group concerted with the rupture of PO bond. The zinc ion may additionally facilitate the formation of the phosphonate intermediate by increasing the electrophilicity of the phosphate group and possibly by facilitating the deprotonation of the attacking 2'-OH, but real kinetic evidence for this kind of participation does not exist. The Zn$^{2+}$ promoted cleavage, hence, rather resembles the pH-independent than hydroxide ion catalyzed cleavage of RNA. This pH-independent reaction, which at pH 7 is about 20 times slower than the base catalyzed cleavage, proceeds by rate-limiting intramolecular proton transfer from the phosphorane hydroxyl ligand to the departing oxygen atom.

Zn$^{2+}$[12]aneN$_3$ cleaves poly(U) twice as readily as uncomplexed Zn$^{2+}$. The depolymerization takes place by the cleavage of the non-terminal phosphodiester bonds rather than by stepwise release of monomeric nucleotides. The hydrolysis of the resulting terminal 2',3'-cyclic phosphates to 2'- and 3'-phosphates is also accelerated and, hence, cyclic phosphates are not markedly accumulated. Phosphodiester bonds within hairpin loops and bulges are cleaved approximately as rapidly as those within linear single strands. Only the bonds next to a double helical stem are cleaved somewhat less readily. It is, however, worth noting that even a single nucleotide bulge allows cleavage, while no cleavage within a double helical stem has been observed, evidently for the reason that stacking of the base-pairs prevents the attacking 2'-O and the departing 5'-O to adopt collinear “in-line” geometry.

The Zn$^{2+}$[12]aneN$_3$ promoted cleavage of RNA can be made sequence selective by tethering the chelate to an oligo(2'-O-methylribonucleotide) that is not cleaved by metal ions. The results obtained so far suggest that the 3'-terminal conjugates (2) are more efficient nucleases than their 5'-terminal or intra-chain tethered counterparts. Independently of the site of tethering, the cleavage shows turnover without any indication of product inhibition. The rate-acceleration obtained by incorporation of a single chelate to the 3'-terminus is about 100-fold compared to free monomeric Zn$^{2+}$[12]aneN$_3$. Attachment of a chelate to both the 1'- and 3'-hydroxy groups of a 3'-terminal abasic β-D-ribofuranoside unit via dialkyl phosphodiester bridges (3) still increases the cleaving activity by more than one order of magnitude. Accordingly, the cleaving activity is similar to that of 2,9-dimethyl-5-aminophenanthroline-
oligonucleotide conjugates, the most efficient Zn$^{2+}$-based artificial nucleases described, but the maximal cleavage rate is achieved at a lower Zn$^{2+}$ ion concentration (<100 μmol L$^{-1}$). The cleavage takes place at the 5'-side of the nucleoside opposite to the abasic sugar bearing the azacrown ligands. The half-life at 18 μmol L$^{-1}$ concentration of the target and nuclease (pH 7.3, 35 °C) is 9 h.

In addition to the ability of cleaving phosphodiester bonds, Zn$^{2+}$[12]aneN$_7$ exhibits high affinity to uracil and thymine bases, since the Zn$^{2+}$-N3 interaction is enforced by simultaneous hydrogen bonding of the secondary amino groups of the azacrown ligand to the carbonyl oxygens of the nucleobase. Expression of these two characteristics in concert allows development of small molecule cleaving agents that are selective toward uracil base. Dinucleating ligands incorporating two azacrown moieties, such as 1,3-bis{(1,5,9-triazacyclodecan-3-yl)oxo}methyl]benzene (4), cleave as dinuclear Zn$^{2+}$ complexes ApU and UpA up to two orders of magnitude more readily than UpU or ApA. The trinuclear complex of 1,3,5-tris{(1,5,9-triazacyclodecan-3-yl)oxo}methyl]benzene (5), in turn, cleaves UpU as readily as ApU and UpA, while the cleavage of ApA remains slow. With ApU and UpA, one of the Zn$^{2+}$-azacrown moieties in all likelihood anchors the cleaving agent to the uracil base of the substrate, while the other azacrown moiety serves as a catalyst for the phosphodiester transesterification. UV spectrophotometric and NMR spectroscopic titrations lend support for the assumed high affinity binding. With UpU, two azacrown moieties are engaged in the base moiety binding. The catalytic activity of the di(azacrown) complex is, hence, lost, but it can be restored by addition of a third azacrown group. A similar selectivity operates at oligonucleotide level as long as the concentration of the cleaving agent remains below 0.5 mmol L$^{-1}$. The di(azacrown)-based cleaving agents discussed above contain on extra oxygen at C3, not present in the parent [12]aneN$_7$ monomer. This oxygen atom seems to afford the cleaving agents with additional selectivity: in addition to high affinity binding to uracil, a moderate affinity to guanine base is observed. Since unsubstituted Zn$^{2+}$[12]aneN$_7$ does not exhibit a similar tendency to recognize guanine base, the enhanced affinity to guanine may be attributed to hydrogen bonding of the 2-amino group with the ether oxygen. In other words, one of the hydrogen bonds between the secondary amino groups of [12]aneN$_7$ and carbonyl oxygens of uracil is replaced with a weaker hydrogen bond between the amino group of guanine and the ether oxygen.

A comparable selectivity towards the base moiety is observed with dinuclear complexes of a spiro-di(azacrown) ligand. 2,6,10,14,18,22-hexaaazaspiro[11,11]tricosane (6). The dinuclear Zn$^{2+}$ complex cleaves UpU, ApU and UpA much faster than ApA. Evidently one of the azacrown moieties is again engaged in uracil binding, while the other has a catalytic function. In this case, simultaneous binding to two uracil bases does not seem to be preferred, since UpU is cleaved even faster than UpA or ApA. Interestingly, a heterodinuclear Zn$^{2+}$, Ni$^{2+}$-complex is even more efficient catalyst than the homodinuclear Zn$^{2+}$ complex.

REFERENCES


*Corresponding Author. E-mail: harlon@utu.fi