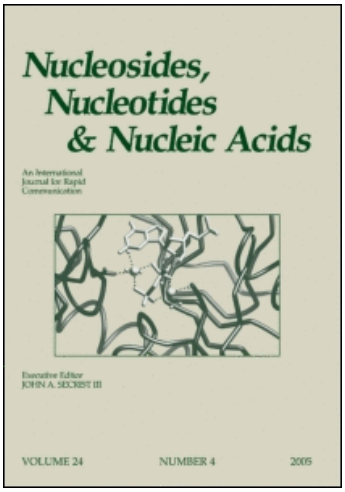

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Cu^{2+}TerPy COMPLEXES AS CATALYSTS OF THE CLEAVAGE OF THE 5′-*cap* STRUCTURE OF mRNA

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□ *Cu^{2+} TerPy is a fairly good catalyst of the cleavage of dinucleoside triphosphates, but its efficiency is not suffiecient for the use in artificial RNA cleaving enzymes. The present work is aimed at improving the catalysis by Cu^{2+} TerPy with additional catalysts. Electrophilic and general acid catalysis have been studied and bifunctional catalysts have been synthesized. The most efficient catalysis was achieved with a Cu^{2+} TerPy-dimer.*

Keywords 5′-*cap* structure; mRNA; Cu^{2+} TerPy-dimer

INTRODUCTION

5′-*cap* structure found at the 5′-terminus of mRNA molecules synthesized by RNA polymerase II has been identified as a potential target of artificial enzymes that cleave RNA molecules.[1] Metal ion complexes have been studied as catalytic groups required in the design of an artificial enzyme, and complexes of Cu^{2+} and trivalent lanthanide ions are the most promising candidates.[1] Cu^{2+} complexes generally are stable enough, but the rate-enhancement is not sufficient for practical applications. We, therefore, have studied different catalytic strategies as means to improve the catalysis by Cu^{2+}TerPy (TerPy = 2,2′,2″-terpyridine). 5′,5′-ApppA (**1a**), m^7GpppG (**1b**), and 5′,5′-UpppU (**1c**) were used as 5′-*cap* models the cleavage of their triphosphate bridge was studied kinetically.

RESULTS AND DISCUSSION

Electrophilic catalysis is one of the strategies employed by triphosphate hydrolysing enzymes. In this work hexacyclen (1,4,7,10,13,16-hexaazacyclooctadecane; HX) and its Cu^{2+} complexes were tested as

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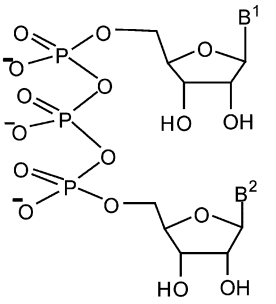
electrophilic catalysts. At pH 7.5, hexacyclen exists in a tricationic form[2] and it could be expected to bind to the trianionic phosphate group, and thus electrophilically make it more susceptible towards a nucleophilic attack. An increasing concentration (0.2–2 mM) of hexacyclen did not, however, enhance the catalysis by 5 mM Cu^{2+}TerPy at pH 7.5. There is a possibility that there are two opposite effects that compensate each other; hexacyclen ligand may bind the Cu^{2+}TerPy complex and inactivate an equivalent concentration of the catalyst. In order to avoid this, [HX] was kept lower than [Cu^{2+}TerPy] and hence it is also possible that despite the opposite charges, only a small fraction of the substrate exists as a hexacyclen complex under the experimental conditions (K_{eq} values are not known). The fact that no rate enhancing effect was observed, in any case suggests that should there be any additional effect by hexacyclen, the effect is modest.

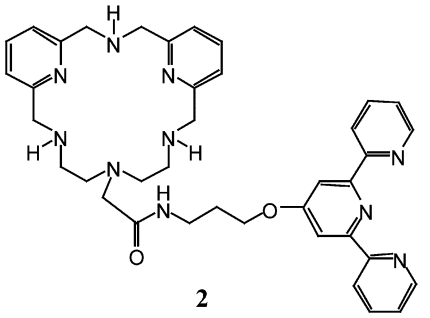
Cu^{2+} complexes of hexacyclen had a larger effect on the catalytic activity of Cu^{2+}TerPy (2 mM, pH 7.5, 25°C). The catalytic activity of a 2:1 Cu^{2+}-HX complex levelled of at a concentration of 0.5 mM and the maximal rate constant of the cleavage of **1a** obtained is 15-fold larger than that in the absence of the hexacyclen complex. In the presence of the 1:1—complex the rate constants increase as [Cu^{2+}-HX] increases and it can be estimated that they eventually reach the same maximal value as in the presence of the 2:1 Cu^{2+}-HX complex.

The potential of general acid catalysis was studied using a 1,5,9-triazacyclododecane complex of Zn^{2+} (Zn^{2+}[12]aneN_3) as an additional catalyst. No additional catalysis was, however, observed in this case, even though Zn^{2+} aquo ions have been proposed to enhance the catalysis by a Eu^{3+} complex as a general acid catalyst.[3] The problem with such studies is that anything that binds the phosphate group also may prevent the binding of the catalyst, and, hence, a net effect of two opposite factors is observed. It can be concluded, however, that even if there was a favorable effect by Zn^{2+}[12]aneN_3, it could not be large.

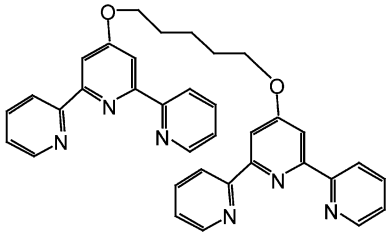
Polyazacyclophane ligand **2** carrying a terpyridine side arm was synthesized to achieve a catalyst that combines the electrophilic assistance by a Cu^{2+} complex of a macrocyclic amine and the catalysis by Cu^{2+}TerPy complex. Cu^{2+} complexes of this ligand did not, however, show enhanced catalytic activity in comparison to Cu^{2+}TerPy. The rate constants of the cleavage of **1b** obtained in the presence of 1:1, 2:1, and 3:1 Cu^{2+}-**2** complexes (5 mM, pH 7.2, 60°C) were 1.5×10^{-5} s^{-1}, 6.3×10^{-5} s^{-1}, and 4.2×10^{-5} s^{-1}, whereas that obtained in the presence of 2 mM Cu^{2+}TerPy[4] under the same conditions is 1.8×10^{-5} s^{-1}. The reason that a catalytic advantage of an intramolecular catalysis was not observed may result from the design of the ligand. Polyazacyclophane ring with aromatic amines may not bind as strongly as an aliphatic amine,[5] and the linker may be too short and/or rigid to allow the interaction between the Cu^{2+}TerPy and the triphosphate substrate.



1a: $B^1=B^2$=Ade
1b: B^1= Gua, $B^2=m^7$Gua
1c: $B^1=B^2$=Ura



2



3

In contrast to the Cu^{2+} complexes of **2,** efficient co-operativity was achieved with a Cu^{2+}TerPy dimer **3**. A 2:1 Cu^{2+}-**3** complex (1 mM, pH 7.5, 60°C) promotes the cleavage of **1a** 100 times as efficiently as 2 mM Cu^{2+}TerPy[4] under the same conditions. In comparison to the uncatalyzed reaction,[6] the complex can be estimated to enhance the reaction by a factor of 3×10.[5] This result is consistent with the fact that previous studies[4] have shown that two Cu^{2+}TerPy complexes are involved in the catalysis of the cleavage of **1a**. Similar results have been reported previously[7] also with other Cu^{2+} complexes. The cleavage of UpppU (**1c**) by $2Cu^{2+}$-**3** was 7 times as fast as that of **1a,** and **1b** seemed to disappear immediately, but the reason is not clear.

EXPERIMENTAL

Substrates **1b** and **1c** were synthesized by imidazolide activated nucleotide coupling[8] in dry DMF. Ligand **2** was prepared by converting first a selectively protected polyazacyclophane[5] into an *N*-carboxymethyl *tert*-butyl ester. The ester group was hydrolyzed with trifluoroacetic acid and a conventional peptide coupling reaction[9] was utilized to introduce the terpyridine unit. The amino-functionalized terpyridine required was

prepared from 3-amino-1-propanol and 4′-chloro-2, 2′:6,2″-terpyridine.[10] TerPy–dimer **3** was prepared by two consecutive Mitsunobu reactions[11] from 1,4-butanediol and 2,6-di(pyridin-2-yl)pyridin-4(*H*)-one. Step-wise ligation of the terpyridine moieties was required to prevent a formation of a complex product mixture and was achieved by protecting first one of the alcohol groups of the diol by monomethoxytrityl function. Substrate **1a** as well as hexacyclen and [12]aneN_3 ligands are commercially available. The details of the kinetic experiments have been described before.[2,4]

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