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Hideo Inoue^a; Takako Furukawa^b; Takashi Tamura^b; Akihiro Kamada^b; Eiko Ohtsuka^b

^a Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Osaka, Japan ^b Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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RAPID RNA CLEAVAGE USING AN ANTISENSE SYSTEM WITH TWO TERPYRIDINE • Cu(II) COMPLEXES

Hideo Inoue,^{1,*} Takako Furukawa,² Takashi Tamura,²
Akihiro Kamada,² and Eiko Ohtsuka²

¹Department of Bioapplied Chemistry, Faculty of Engineering,
Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku,
Osaka 558-8585, Japan

²Graduate School of Pharmaceutical Sciences, Hokkaido University,
Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

ABSTRACT

An effective approach to promote sequence-specific RNA cleavage by an anti-sense 2'-*O*-methyloligonucleotide with a terpyridine • Cu(II) complex attached at the 5'-end was developed. We have synthesized a Cu(II) complex 3'-conjugate, which when used in a tandem fashion, greatly enhanced the RNA cleavage efficiency.

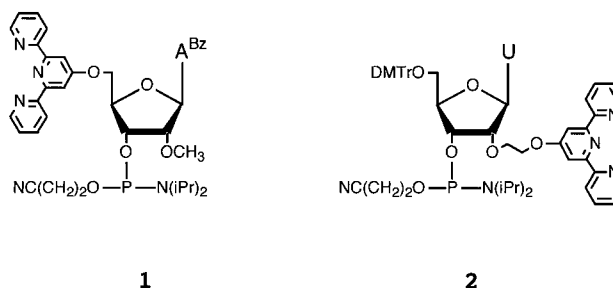
In recent years, there has been growing interest in the design of molecules that can cleave RNA in a sequence-specific manner (1–5). The antisense cleavers may be useful for basic studies of molecular biology and have potential applications for anti-sense chemotherapy. However, practical RNA cleavers with high activity have remained elusive. Recently, we showed that an antisense 2'-*O*-methyloligonucleotide, with a terpyridine • Cu(II) complex directly attached to the 5'-oxygen of the 5'-end-nucleoside, cleaves RNA predominantly at the site opposite the 5'-end (6,7). Although the cleavage yield was low (ca. 20%), 10 molar equivalents of the reagent were sufficient for the reaction, and this amount is comparable to the large excess required for cleavage of DNA derivatives.

*Corresponding author.

In this paper we report a novel system using the above 5'-conjugate and a 3'-conjugate described below for rapid RNA cleavage. When designing the system, we envisioned that the 3'-conjugate may assist in the cleavage by the 5'-conjugate, because both of the complex residues could be close to each other on an RNA substrate. We performed the novel synthesis of the 3'-conjugate, which was a 2'-*O*-methyl-oligonucleotide with the terpyridine residue linked to the 2'-oxygen of the 3'-end-uridine *via* a short linker arm. The terpyridine derivative of uridine was easily prepared *via* a series of reactions starting from a protected derivative of 2'-*O*-(2-hydroxyethyl)uridine (8), and was converted to a 3'-phosphoramidite derivative. The structures of this synthetic unit (**2**) as well as the unit (**1**) for the 5'-conjugate for automated oligonucleotide synthesis are shown in Figure 1 (a). The 3'-conjugate was synthesized on a universal support (Glen Research). Figure 1 (b) shows the sequences of the RNA substrate (**3**), the 5'-conjugate (**4**), and the 3'-conjugate (**5**), and also the results of the RNA cleavage reactions.

The reactions of the RNA (5'-end-labeled with ^{32}P) with the Cu(II) complex-agent(s) **4** and/or **5** were carried out at pH 7.5 and 37°C for 20 hr. Products were analyzed by denaturing polyacrylamide gel electrophoresis. As expected, **4** cleaved

(a)



(b)

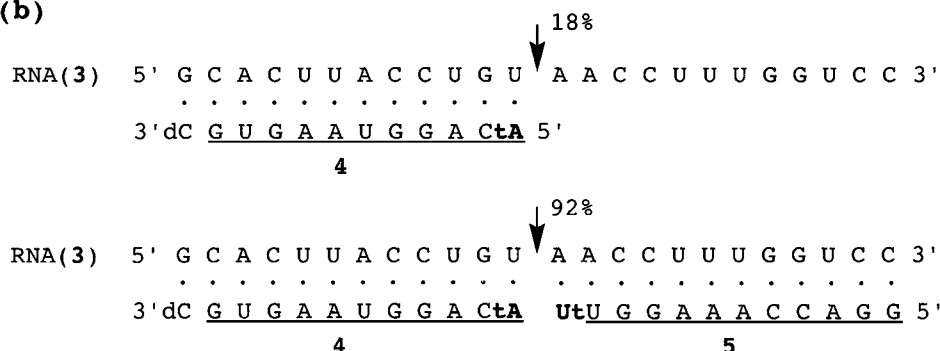


Figure 1. (a) Structures of terpyridine-linked nucleoside 3'-phosphoramidites. (b) The sequences of the terpyridine-linked oligonucleotide agents and the RNA substrate, and the positions and yields for the RNA cleavage reaction. N indicates the position of the 2'-*O*-methylnucleotide residue. **tA** and **Ut** indicate terpyridine complex-attached residues.



the center site 5'U-A3' in the RNA (18% yield), as shown in Figure 1 (b). Although no cleavage was observed when only **5** was used, the reaction with **4** and **5** proceeded rapidly to give a high yield (92%; for a 5 hr reaction, 63%). Thus, the 3'-conjugate promoted RNA cleavage by the 5'-conjugate. The results suggest cooperative action between the two complexes. Based on this finding, we are now investigating an alternative system for RNA cleavage, which involves the use of a 2'-*O*-methyl-oligonucleotide with two contiguous Cu(II) complex residues at the internal site.

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