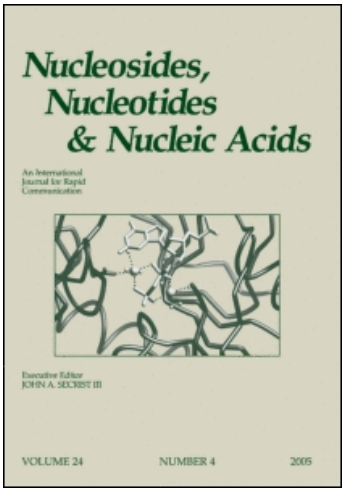

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Hideo Inoue[a]; Takako Furukawa[b]; Takashi Tamura[b]; Akihiro Kamada[b]; Eiko Ohtsuka[b]
[a] Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Osaka, Japan [b] Graduate School of Pharmaceutical Sciences, Hokkaido University, 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 Ohtsuka[2]

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

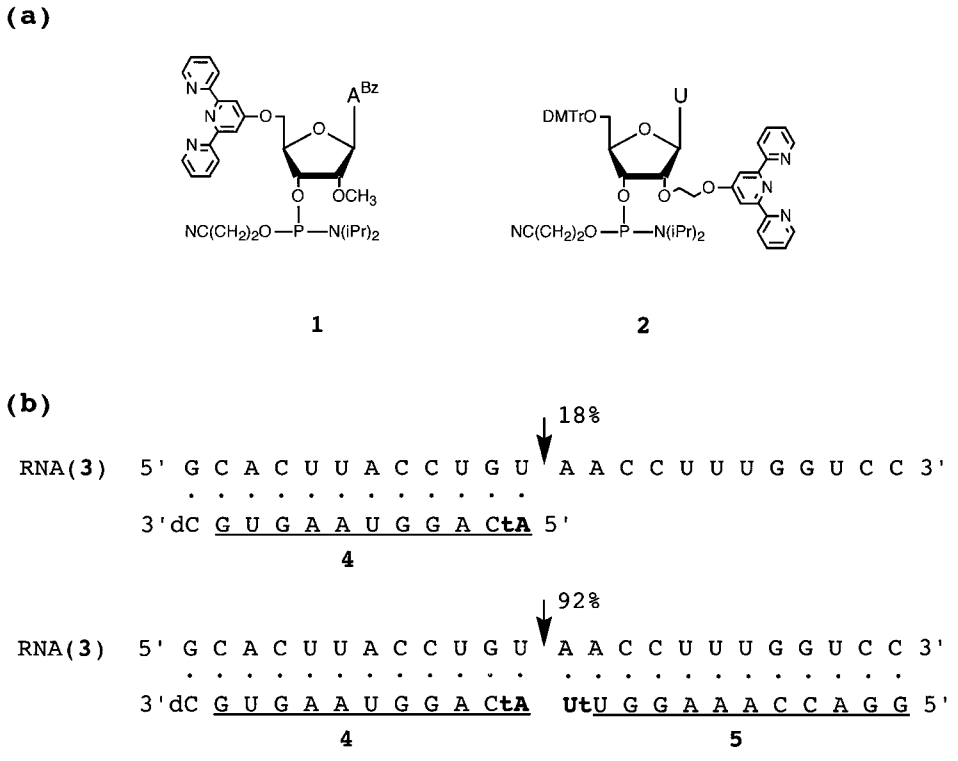

Figure 1. (a) Structures of terpyridine-linked nucleoside 3′-phosphoramidits. (b) The sequences of the terpyridine-linked oligonucleotide agents and the RNA substrate, and the positions and yields for the RNA cleavage reaction. N indicates the position of the 2′-*O*-methylnucleotide residue. **tA** and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

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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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