Two-terpyridine • Cu(II) complexes-containing antisense systems for rapid and highly site-specific RNA cleavage

Hideo Inoue1, Takako Furukawa1, Takashi Tamura1, Yasuo Komatsu1 and Eiko Ohtsuka1
1Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan and 2Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan

ABSTRACT
In an approach toward artificial ribonucleases, novel RNA cleaving systems were constructed that contained two terpyridine•Cu(II) residues. The first antisense system used tandem Cu(II) complex-2'-O-methyloligonucleotide 5'- and 3'-conjugates to cleave an RNA substrate. The second system, which will be described in a future paper, contained two contiguous Cu(II) complex residues at an internal site of a 2'-O-methyloligonucleotide. We found that the first system rapidly cleaved RNA with high site-specificity. Based on these results, we expect the second system to also show efficient RNA cleavage.

INTRODUCTION
Chemical RNA cleavers with sequence-specificity can be good candidates for antisense chemotherapy and can also be useful for studies on the structure-function relationships of native RNAs (1). We recently found that an antisense 2'-O-methyloligonucleotide with a terpyridine•Cu(II) complex linked to the sugar-5'-oxygen of the 5'-end-nucleoside residue can cleave RNA site-specifically and in a moderate yield. In addition, less than 10 molar equivalents of the agent were sufficient for the reaction (2). We also carried out the synthesis of an antisense agent with the complex at an internal position and showed that the internal complex cleaved an RNA substrate in a similar manner (3). To create antisense systems with more efficient cleavage activity, we constructed RNA cleaving systems containing two Cu(II) complex residues, which we hoped would function cooperatively for phosphodiester cleavage.

RESULTS AND DISCUSSION
The structures of terpyridine-linked nucleoside building units for the construction of oligonucleotide agents used in this study are shown in Fig. 1. The syntheses of 1 and 2 have been reported previously (2,3) and the new unit 3 was easily synthesized from a 2'-O-(2-hydroxyethyl)uridine derivative described previously (4).

The sequences and structures of the oligonucleotides prepared using these units are shown in Fig. 2 which also indicates the results of RNA cleavage reactions. These reactions were carried out with 5'[^32P]-end-labeled RNA 24-mers (R24s with analogous sequences) in a buffer (pH 7.5) at 37 °C or 45 °C for 20 hr. Cleavage positions and yields, which were obtained by analyses of the cleavage products by polyacrylamide gel electrophoresis (data not shown), are also represented in Fig. 2.
The previous reactions (at 45 °C) of R24s(UUU, AUU) using the Cu(II) complex 5'-conjugate 12-mer (oligo-1) and the 18-mer (oligo-2) with the complex at an internal site are also shown as Entries 1 and 2 in Fig. 2, respectively. The present reactions of R24(UAA) were carried out at 37 °C with oligo-1 and/or the Cu(II) complex 3'-conjugate 12-mer (oligo-3). The reaction with combination of oligo-1 and oligo-3 proceeded rapidly and gave a high yield (92%; for 5 hr reaction, 63%) (Entry 3). In control experiments, oligo-1 cleaved the center site 5'U-A3' in the RNA (18% yield) as expected (Entry 4), but no cleavage was observed when only oligo-3 was used (Entry 5). Thus, the 3'-conjugate promoted RNA cleavage by the 5'-conjugate. The results suggest the involvement of cooperative action of the two complexes. This led us to construct 2'-O-methyloligonucleotide with contiguous two Cu(II) complex residues at the internal site by the use of units 2 and 3. Reactions of RNA with this agent are in progress in our laboratory.

REFERENCES