Investigation of physical and physiological properties of 4'-thioribonucleotid e (4'-thioRNA)

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ABSTRACT
An efficient and practical synthesis of 4'-thioribonucleosides was accomplished via the Pummerer reaction. The resulting 4'-thioribonucleosides were converted into the corresponding phosphoramidite units, and 4'-thioribonucleic acids (4'-thioRNAs) of 15 mer were synthesized on a DNA synthesizer using a controlled pore glass (CPG) with 4'-thiouridine unit. The thermal stability of 4'-thioRNA:RNA duplex was higher than that of RNA:RNA duplex. Moreover, the thermal stability of 4'-thioRNA:4'-thioRNA duplex was the highest and stabilized 30 °C or more compared with RNA:RNA duplex (>99 °C vs 66 °C). Structural analysis by CD spectra indicates that 4'-thioRNA:4'-thioRNA duplex and 4'-thioRNA:RNA duplex adapt A-form conformation as well as RNA:RNA duplex.

INTRODUCTION
4'-Thioribonucleosides are nucleoside derivatives, in which the furanose ring oxygen is replaced by a sulfur atom (Figure 1). Imbach et al. have already reported the synthesis and some properties of 4'-thioRNA.1,2) The 4'-thioRNA forms thermally more stable duplex with natural RNA than natural RNA duplex (RNA:RNA duplex). In addition, the 4'-thioRNA showed high resistance not only toward exonucleases but also toward endonucleases. Moreover, they reported that the 4'-thioRNA exhibits high stability toward degradation in cell culture medium containing 10% heat-inactivated fetal calf serum. However, despite these favorable properties of 4'-thioRNA, no further work in this area has been reported thus far, probably due to the difficulty in efficiently synthesizing the 4'-thioribonucleosides. Recently, we succeeded an efficient stereoselective synthesis of 4'-thioribonucleosides.3,5) With the aim of developing 4'-thioRNA as an artificial RNA molecule including antisense, siRNA, and aptamer,6) synthesis and reinvestigation of physical and physiological properties of 4'-thioRNA were examined.

RESULTS AND DISCUSSION
Chemistry
According to our previous report,3,5) the Pummerer reaction of sulfoxide 1 with nucleobases gave 4'-thioribonucleoside derivatives 2-5 in good yield. The resulting 2-5 were then converted into the phosphoramidite units 6-9 and the controlled pore glass (CPG) unit with 4'-thiouridine 10 (Scheme 1). 4'-ThioRNAs along with natural RNAs and DNA were synthesized on a DNA synthesizer according to a standard phosphoramidite protocol. Sequences were shown in Figure 2.
Thermal denaturation study of 4'-thioRNA

Stability of the duplexes was studied by thermal denaturation in a buffer of 0.01 M Na cacodylate (pH 7.0) containing 0.1 M NaCl (Table 1). The thermal stability of 4'-thioRNA:RNA duplex was higher than that of RNA:RNA duplex (74 °C vs 66 °C). Moreover, the thermal stability of 4'-thioRNA:4'-thioRNA duplex was the highest and stabilized 30 °C or more compared with RNA:RNA duplex (>99 °C vs 66 °C). The thermal stability of 4'-thioRNA:DNA duplex was lower than RNA:DNA duplex (45 °C vs 51 °C). 4'-ThioRNA exhibited high affinity for RNA rather than DNA, as is reported by Imbach et al.

CD spectral study of 4'-thioRNA

CD spectra of the duplexes were measured in a buffer of 0.01 M Na cacodylate (pH 7.0) containing 0.1 M NaCl at 25 °C (Figure 3). Our result indicates that the structures of 4'-thioRNA:4'-thioRNA duplex and 4'-thioRNA:RNA duplex adapt A-form conformation. Thus, modification of RNA duplex by 4'-thioribonucleosides does not alter the A-form conformation.

Further investigations of 4'-thioRNA are now in progress.

REFERENCES