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Inhibition of Deoxyribooligonucleotides on the Circle Opening Reaction of the Intervening Sequence from Tetrahymena thermophila

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The opening reaction of the circular form of the intervening sequence from Tetrahymena thermophila by the dinucleotide CU has been studied in the presence of some deoxyoligonucleotides. The results indicate that d(AGAG) does not inhibit the reaction, but d(CTCT) does. The oligomer, d(TGCA) also inhibits the reaction and the inhibition largely depends on the Mg^{2+} concentration.

The circular form of the self-splicing intervening sequence from the rRNA precursor of Tetrahymena thermophila (CIVS) is linearized by addition of oligonucleotides in the absence of any protein.[1-3] One of the linear products has been found to have catalytic activity and is known as a ribozyme.[4] Our kinetic study[2] on the opening reaction of CIVS by oligonucleotides CU, UCU, CUCU, and CUCUCU has indicated the presence of intermediates that are generated by separate binding steps for both oligomer and Mg^{2+}. Furthermore, the result has also indicated the transition state for the rate-determining step is less ordered than the intermediate. This raises the possibility that a partial unfolding of the CIVS structure occurs during reaction.

In this paper, we report inhibition studies with deoxyribooligonucleotides d(AGAG), d(CTCT), and d(TGCA) on the circle opening reaction of CIVS by CU in 4.5 and 20 mmol dm^{-3} Mg^{2+} buffers at 30 °C. The d(AGAG), d(CTCT), and d(TGCA) molecules have the possibility to bind the substrate CU, the internal guide sequence, and some hairpin loops of CIVS, respectively.

CIVS was obtained and purified by the method described previously.[2,3] The oligomers CU, d(AGAG), and d(CTCT) were obtained from Sigma and Pharmacia, and purity was confirmed by high-performance liquid chromatography (HPLC). The d(TGCA) molecule was synthesized on solid support with

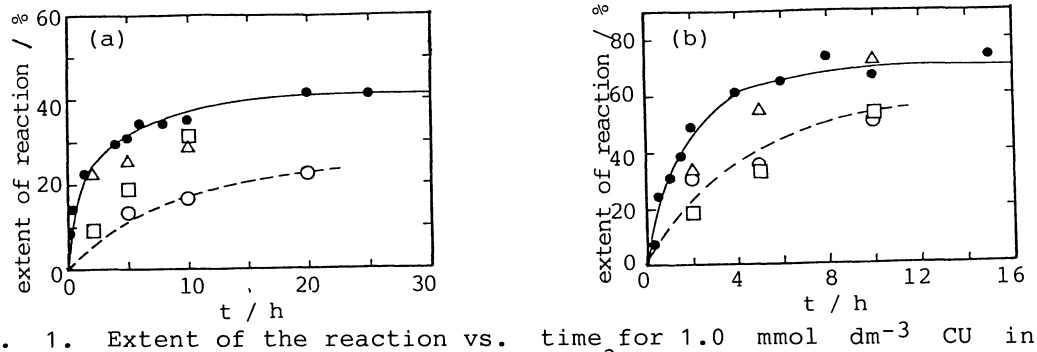

Fig. 1. Extent of the reaction vs. time for 1.0 mmol dm^{-3} CU in the absecence (●) and presence of 1.0 mmol dm^{-3} d(AGAG) (Δ), d(CTCT) (□),and d(TGCA) (○) in (a) 4.5 and (b) 20 mmol dm^{-3} Mg^{2+} buffers. Solid and dotted curves are single exponential functions for the reaction without and with d(TGCA), respectively.

a phosphoramidite method,[5] and purified by HPLC. The buffer was 10 mmol dm^{-3} NaCl, 0.5 mmol dm^{-3} EDTA, 10 mmol dm^{-3} PIPES, pH 6.5 containing 4.5 or 20 mmol dm^{-3} Mg^{2+}. Reaction mixtures were analyzed by running on 4% polyacrylamide/8 M urea gels, cutting out bands from dried gels, and quantifying radioactivity by scintillation counting.

Figure 1 shows typical examples of the time courses of the reaction of CIVS with CU in the absence and presence of the deoxyribooligonucleotides in 4.5 and 20 mmol dm^{-3} Mg^{2+} buffers at 30 °C. Here, the extent of reaction was defined as [CU-L'IVS]/([CU-L'IVS]+[CIVS]), where CU-L'IVS represents CIVS opened by CU. The results show that d(AGAG) does not inhibit the reaction, but d(CTCT) does in both low and high Mg^{2+} concentrations. Deoxy-C_5 is known to inhibit the cleavage of rC_5 by a linear IVS.[6] The inhibition by d(CTCT) in this work suggests the deoxyoligonucleotide is a competitive inhibitor for substrate binding to the internal guide sequence (IGS) of CIVS. The oligomer, d(TGCA) inhibits largely the reaction and the inhibition appears to be less at the higher Mg^{2+} concentration. The CIVS has the sequence UGCA in several hairpin loops that could interact with themselves and/or d(TGCA). The result of the large inhibition by d(TGCA) may suggest that the unfolding of the tertiary interaction of the UGCA sequence in the hairpin loops plays some role during reaction and Mg^{2+} affects the interaction.

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