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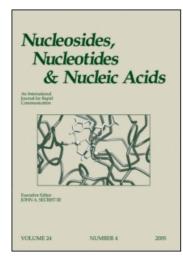
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Properties of Conjugate DNA Enzymes

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Properties of Conjugate DNA Enzymes

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INTRODUCTION

DNA enzymes which can cleave RNA in a sequence specific manner in the presence of metal ions have been genetically engineered by SELEX.^[1] Although DNA enzymes have some advantages over ribozymes in the application to cellular system because they are more resistant against degradation by cellular nucleases, they still have some problems to be improved. If one should create a DNA enzyme which

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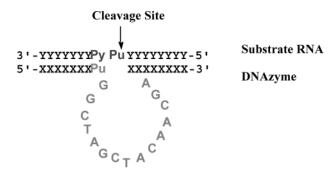
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is stable in vivo, taken up into cells and localized in cytoplasm, ^[2] and has high catalytic activity and sequence specificity to the target, the DNA enzyme would be a promising candidate as a therapeutic agent and a powerful biological tool.

In the present study, we tried to improve the properties of genetically engineered DNA enzymes by conjugation with other functional molecules.

RESULTS AND DISCUSSIONS

Oligodeoxynucleotides with RNA cleavage activity1) were conjugated with amines and peptides by solid phase fragment condensation (SPFC). [3] (Sch. 1) It was found that 29 mer DNA enzyme conjugated with spermine at its 5'-end showed higher affinity to the target RNA sequence and 40 times higher activity of cleavage than native DNA enzyme. It is to be noted that conjugate DNA enzymes showed increased resistance against nuclease digestion. Studies on pecific intracellular delivery and localization of DNA enzymes are now in progress in our laboratory.



DNAzyme; 5'-GCACCCAGGCTAGCTACAACGACTCTCTC-3' RNA; FL-5'-GAGAGAGAUGGGUGC-3'

cleavage site

C-DNAzyme 1; DNAzyme-5'-OCONH(CH₂)₃NH(CH₂)₄NH(CH₂)₃NH₂ **C-DNAzyme 2**; DNAzyme-5'-OCONH(CH₂)₂NH(CH₂CH₂NH₂)₂

C-DNAzyme 3; DNAzyme-5'-OCONH(CH₂)₂NH(CH₂)₂NH(CH₂)₂NH(CH₂)₂NH₂

C-DNAzyme 4; DNAzyme-5'-OCONH(CH₂)₁₁CH₃

C-DNAzyme 5;

 $\label{eq:decomposition} DNAzyme-5'-OPO_3(CH_2)_2O(CH_2)_2NHCONH(CH_2)_6NHCONH(CH_2)_2CO-ALPPLERLTL-OH~(HIV1~NES)$

C-DNAzyme 6;

DNAzyme-5'-OPO $_3$ (CH $_2$) $_2$ O(CH $_2$) $_2$ NHCONH(CH $_2$) $_6$ NHCONH(CH $_2$) $_2$ CO-PKKKRKV-O H (SV40T-antigen NLS)

Scheme 1. Conjugate DNAzymes.

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