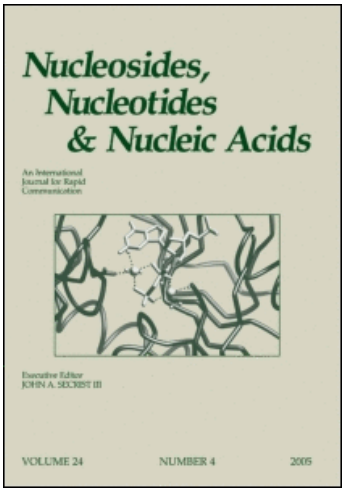

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Takanori Kubo[ab]; Kinuko Yokoyama[a]; Ryoji Ueki[a]; Mayuka Yano[a]; Yosuke Anno[a]; Kotomi Sasaki[a]; Hideki Ohba[c]; Masayuki Fujii[ad]
[a] Department of Biological and Environmental Chemistry, Kyushu School of Engineering, Kinki University, Iizuka, Fukuoka, Japan [b] Kinki University, Iizuka, Fukuoka, Japan [c] Kyushu National Industrial Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tosu, Saga, Japan [d] Molecular Engineering Institute, Kinki University, Iizuka, Fukuoka, Japan

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DNA Conjugates as Novel Functional Oligonucleotides

Takanori Kubo,[1,*] Kinuko Yokoyama,[1] Ryoji Ueki,[1] Mayuka Yano,[1] Yosuke Anno,[1] Kotomi Sasaki,[1] Hideki Ohba,[3] and Masayuki Fujii[1,2]

[1]Department of Biological and Environmental Chemistry, Kyushu School of Engineering, and [2]Molecular Engineering Institute, Kinki University, Iizuka, Fukuoka, Japan
[3]Kyushu National Industrial Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tosu, Saga, Japan

ABSTRACT

Oligodeoxynucleotides with RNA cleavage activity 1) were conjugated with amines and peptides by solid phase fragment condensation (SPFC). 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 also to be noted that conjugate DNA enzymes showed increased resistance against nuclease digestion

Key Words: Conjugate DNA enzyme; Soild phase fragment condensation.

*Correspondence: Takanori Kubo, Kinki University, 11-6 Kayanomori, 820-8555 Iizuka, Fukuoka, Japan; Fax: +81 94 823 0536; E-mail: takanorikinki@hotmail.com.

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INTRODUCTION

Conjugation of oligonucleotides with intelligent biomolecules is a fascinating way to produce novel functional DNAs and RNAs.[1] Accumulation of a variety of functions possessed by naturally occurring molecules and artificially designed molecules on a scaffold of oligonucleotides may open the door to create "super oligonucleotides" that will never happen in nature.

RESULTS AND DISCUSSIONS

First of all, our effort has been focussed on the development of a general synthetic method of DNA conjugates which can allow ones to prepare oligonucleotides covalently linked to a variety of intelligent molecules bearing a variety of reactive groups without any complicated reaction procedures. We have got successful to connect an oligonucleotide fragment bearing an amino group on its 5′-terminus assembled on CPG support with amino derivatives by using diisocyanatoalkane as a linker on solid phase. (Solid Phase Fragment Condensation, SPFC) After cleavage,

Table 1. Stabilization of DNA by DNA conjugates.

N1 : 5'-AGAGAGAGAGAAAAA-3'
N2 : 3'-TCTCTCTCTCTTTTT-5'
N3 : 5'-S-(AGAGAGAGAGAAAAA)-3'

	Tm (°C)		ΔTm (°C)	
Hybrids	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$
N1/N2	51.0	44.0	–	–
N1/N2 + $(LRAL)_3$(12 eq.)	51.5	48.5	+0.5	+4.5
N1/C2 (N2-5′-$(LRAL)_3$)	55.5	47.5	+4.5	+3.5
N1/C3 (N2-U^9-$(LRAL)_3$)	45.0	40.5	−6.0	−3.5
N1/C4 (N2-U^9-Tat)	46.5	38.0	−4.5	−6.0
N1/C5 (N2-U^9-Ant)	40.5	39.5	−10.5	−5.5
N2/N3	47.0	41.0	–	–
N2/N3 + $(LRAL)_3$(12 eq.)	48.0	43.0	+1.0	+2.0
C2/N3	52.5	44.5	+5.5	+3.5
N2/C6 (N3-5′-glucosamine)	61.0	54.5	+10.0	+10.5
N1/C7 (N4-U^9-ph)	43.5	38.0	−7.5	−6.0
N1/C8 (N4-U^9-propyl)	45.0	41.5	−6.0	−2.5
N1/C9 (N4-U^9-propargyl)	46.0	43.0	−5.0	−1.0
N1/C10 (N4-U^9-CH_2CH_2OH)	46.0	44.0	−5.0	±0
N1/C11 (N4-U^9-$CH_2CH_2NMe_2$)	40.0	38.5	−10.0	−5.5

Conditions: 50 mM Tris buffer, pH 7.0, [NaCl] = 100 mM, [OligoDNA] = 3 mM, [Mg^{2+}] = 20 mM or none.

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deprotection and RPHPLC purification the desired products were obtained approximately in 20% yield. It was demonstrated that SPFC was effective for the preparation of conjugate molecules, DNA-peptide, DNA-sugar, DNA-polyamine, DNA-lipid and so on.

Oligonucleotide conjugates have preferable properties as antisense agents such as an increased affinity to complementary RNA and dsDNA (Table 1), and enhanced stability against nuclease digestion. Delivery and intracellular localization of oligonucleotides could be also controlled by conjugation with signal peptides. It was also found that DNA enzymes conjugated with amines and peptides had higher catalytic activity.

REFERENCE

1. Kubo, T.; Fujii, M. A novel approach for the solid phase synthesis of DNA-peptide conjugates. Nucleosides, Nucleotides and Nucleic Acids **2001**, *20*, 1321–1324.

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