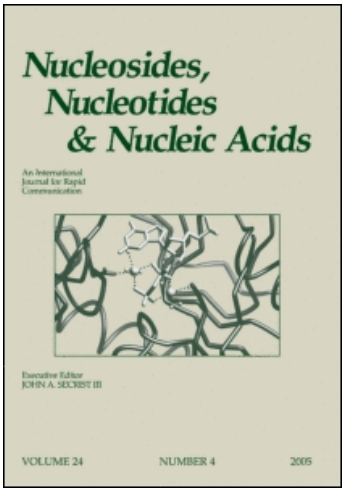

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Synthesis of DNA Conjugates by Solid Phase Fragment Condensation

Yosuke Anno[ab]; Takanori Kubo[a]; Ryoji Ueki[a]; Mayuka Yano[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] Kyusyi School of Engineering, 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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Synthesis of DNA Conjugates by Solid Phase Fragment Condensation

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

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

ABSTRACT

Development of a novel method for the synthesis of DNA conjugates is described. Oligonucleotides were successfully conjugated with a variety of functional molecules on a solid phase (Solid Phase Fragment Condensation) using an amino, a hydroxyl, a thiol, and a carboxyl group. DNA-peptide conjugate was obtained as a pure from by a single RPHPLC purification approximately in 20% yield. Moreover, it was demonstrated that the present method was effective for the preparation of conjugate molecules, DNA-sugar, DNA-polyamine, DNA-lipid and so on. The study to create new intelligent DNAs by accumulation various biofunctions on the molecule by SPFC is now in progress in our laboratory.

*Correspondence: Yosuke Anno, Kyusyi School of Engineering, Kinki University, 11-6 Kayanomori, Iizuka, 820-8555 Fukuoka, Japan; Fax: +81 94 823 0536; E-mail: yanno@iron.chem.fuk.kindai.ac.jp.

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270 Madison Avenue, New York, New York 10016

Key Words: Genetic medicines; Solid phase fragment condensation; DNA-peptide conjugate.

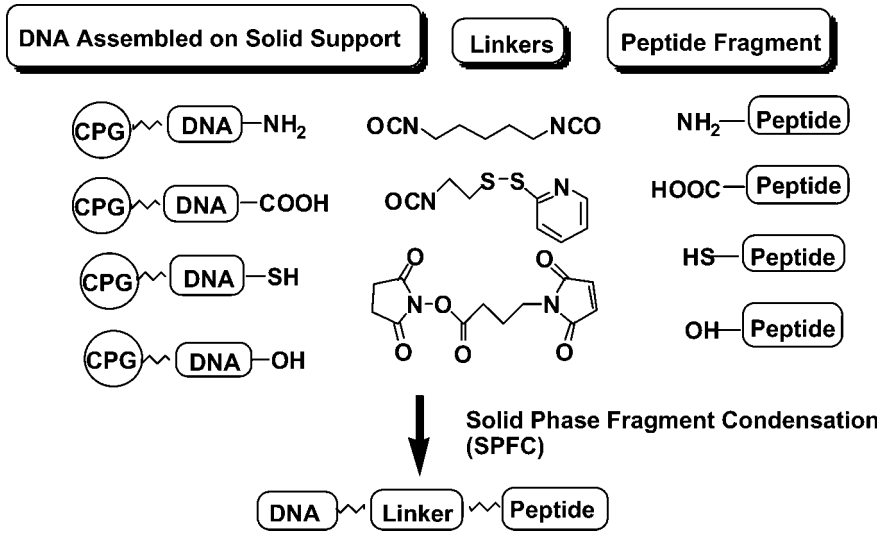
INTRODUCTION

Conjugation of oligonucleotides with functional peptides[1] is a fascinating way to improve the properties of native antisense and triple-helix forming oligonucleotides, for example, enhanced membrane permeability, improved stability against cellular nucleases and increased affinity and specificity.

RESULTS AND DISCUSSIONS

In this paper development of a novel method for the synthesis of DNA conjugates was studied. Oligonucleotides were successfully conjugated with a variety of functional molecules on a solid phase (Solid Phase Fragment Condensation)[2] using an amino, a hydroxyl, a thiol, and a carboxyl group. (Sch. 1)

For example, an oligonucleotide fragment bearing an amino group on its 5′-terminus assembled on CPG support by automated DNA synthesizer was first reacted with hexamethylenediisocyanate and then with a protected peptide fragment bearing an amino group on solid phase. After the completion of the reaction, cleavage from the solid support and deprotection of nucleobases and peptide side chains was performed by aqueous ammonia at 50°C for 5 h. DNA-peptide conjugate was obtained as a pure form by a single RPHPLC purification approximately in 20% yield. Because there is no limitation in peptide components and sequences in principle, this



Scheme 1. Synthesis of DNA conjugate by solid phase fragment condensation.

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C1:
3'-GCTAGAGAGAGAGAAAATCG-$CH_2CH_2OCH_2CH_2NHCONH(CH_2)_6NHCONH$-$CH_2CH_2CO$-(LAKL)$_3$-OH
Yield 24.4% (based on $\mathbf{A}_{260}$, after HPLC purification)

C2:
3'-TCTCTCTCTCTTTTT-OCONH$CH_2CH_2NHCONH(CH_2)_6NHCONH$-$CH_2CH_2CO$-(LRAL)$_3$-OH
Yield 18.8% (based on $\mathbf{A}_{260}$, after HPLC purification)

C3: 5'-TTTTTCTCUCTCTCT-3'
OCONH$CH_2CH_2NHCONH(CH_2)_6NHCONH$-$CH_2CH_2CO$-(LRAL)$_3$-OH

Yield 18.2% (based on $\mathbf{A}_{260}$, after HPLC purification)

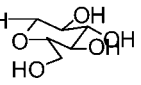
C4: 5'-TTTTTCTCUCTCTCT-3'
OCONH$CH_2CH_2NHCONH(CH_2)_6NHCONH$-$CH_2CH_2CO$-GGGYGRKKRRQRRRG-OH (**HIV1-Tat**)

Yield 15.5% (based on $\mathbf{A}_{260}$, after HPLC purification)

C5: 5'-TTTTTCTCUCTCTCT-3'
OCONH$CH_2CH_2NHCONH(CH_2)_6NHCONH$-$CH_2CH_2CO$-RQIKIWFQNRRMKWKK-OH (*Drosophila* **Ant**)

Yield 14.8% (based on $\mathbf{A}_{260}$, after HPLC purification)

C6:3'-AAAAAGAGAGAGAGA-OCONH$CH_2CH_2NHCONH(CH_2)_6NHCO$-NH-(D-Glucosamine)
Yield 28.6% (based on $\mathbf{A}_{260}$, after HPLC purification)



Scheme 2. Sequences of DNA conjugates.

method is useful for the general synthesis of DNA-peptide conjugates. Moreover, it was demonstrated that the present method was effective for the preparation of conjugate molecules, DNA-sugar, DNA-polyamine, DNA-lipid and so on. (Sch. 2)

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