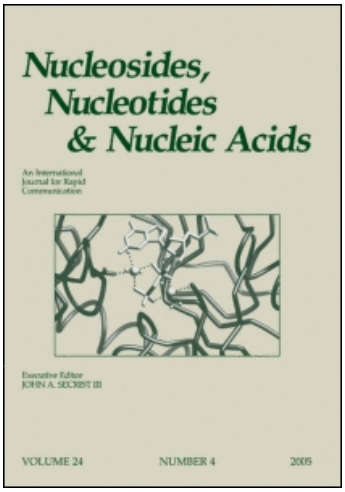

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[a] Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan [b] PRESTO, JST, Tokyo, Japan

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ACID-MEDIATED CLEAVAGE OF OLIGONUCLEOTIDE P3′ →N5′ PHOSPHORAMIDATES TRIGGERED BY SEQUENCE-SPECIFIC TRIPLEX FORMATION

Satoshi Obika □ *Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan and PRESTO, JST, Tokyo, Japan*

Masaharu Tomizu, Yoshinori Negoro, Tomohisa Osaki, Ayako Orita, Yuji Ueyama, Osamu Nakagawa, and Takeshi Imanishi □ *Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan*

□ *The P-N bond in oligonucleotide P3′ →N5′ phosphoramidates (5′-amino-DNA) is known to be chemoselectively cleaved under mild acidic conditions. We prepared homopyrimidine oligonucleotides containing 5′-amino-5′-deoxythymidine (5′-amino-DNA thymine monomer) or its conformationally locked congener, 5′-amino-2′,4′-BNA thymine monomer, at midpoint of the sequence. The effect of triplex formation with homopurineohomopyrimidine dsDNA targets on acid-mediated hydrolysis of the P3′ →N5′ phosphoramidate linkage was evaluated. Very interestingly, it was found that the triplex formation significantly accelerates the P-N bond cleavage.*

Keywords P-N bond; homopyrimidine oligonucleotides; bridged nucleic acids; DNA sensing

INTRODUCTION

The oligonucleotide P3′ →N5′ phosphoramidates (5′-amino-DNA, Figure 1) can be cleaved at the P-N linkage under mild acidic conditions.[1] This property of 5′-amino-DNA has attracted much attention and has been applied to a DNA-sequence determination.[2,3] However, the 5′-amino-DNA decreases the hybridizing ability with its DNA complement.[4] Recently, we developed 5′-amino-2′,4′-BNA, which possesses a P3′ →N5′ phosphoramidate linkage and a locked N-type sugar conformation (Figure 1).[5] The 5′-amino-2′,4′-BNA showed high ability to hybridize with its DNA and RNA complements. In addition, it formed a stable triplex with the homopurino·homopyrimidine tract of the double-stranded DNA (dsDNA) target. To examine the possibility for novel and innovative DNA detection by the 5′-amino-2′,4′-BNA, we investigated the effect of the triplex formation on

Address correspondence to Takeshi Imanishi, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: imanishi@phs.osaka-u.ac.jp

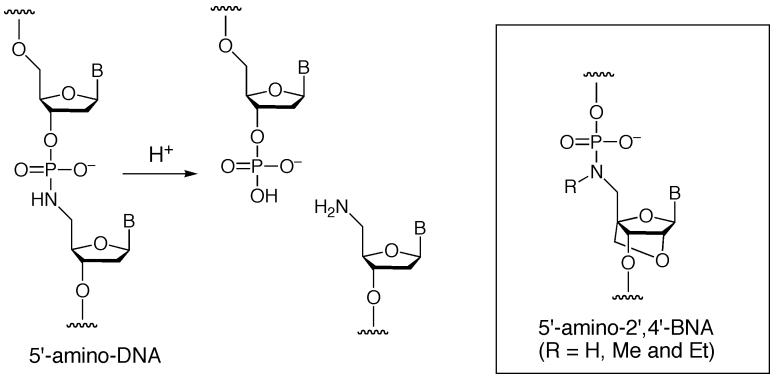

FIGURE 1 Acid-mediated hydrolysis of 5′-amino-DNA and structure of 5′-amino-2′,4′-BNA.

hydrolysis of 5′-amino-2′,4′-BNA. Here, we describe the significant acceleration of the acid-mediated P-N bond cleavage of 5′-amino-2′,4′-BNA by sequence-specific triplex formation. Its application to efficient DNA-sensing is also discussed.

RESULTS AND DISCUSSION

The 5′-amino-DNA and 5′-amino-2′,4′-BNA monomers were prepared and successfully incorporated into homopyrimidine oligonucleotides according to the previously reported procedure.[1,5] The sequences of the oligonucleotides and target dsDNA are shown in Figure 2.

At first, the oligonucleotides containing 5′-amino-DNA (**ON-1**) or 5′-amino-2′,4′-BNA (**ON-2**) were incubated with or without **dsDNA-1** and then treated under acidic conditions. The amount of intact oligonucleotides was determined by HPLC analysis. As shown in Figure 3a and 3b, the acid-mediated cleavage of **ON-1** and **ON-2** was accelerated by formation of

ON-1 – ON-4
5'-d(TTTTTCT**X**TCTCTCT)-3'
ON-1 [**X** = 5'-amino-DNA thymine monomer]
ON-2 [**X** = 5'-amino-2',4'-BNA thymine monomer (N-H)]
ON-3 [**X** = 5'-amino-2',4'-BNA thymine monomer (N-Me)]
ON-4 [**X** = 5'-amino-2',4'-BNA thymine monomer (N-Et)]
(C = 2'-deoxy-5-methylcytidine)

dsDNA-1
5'-d(GCTAAAAAGAAAGAGAGATCG)-3'
3'-d(CGATTTTTCTTTCTCTCTAGC)-5'

FIGURE 2 Sequences of the oligonucleotides (**ON-1 – ON-4**) and dsDNA target (**dsDNA-1**).

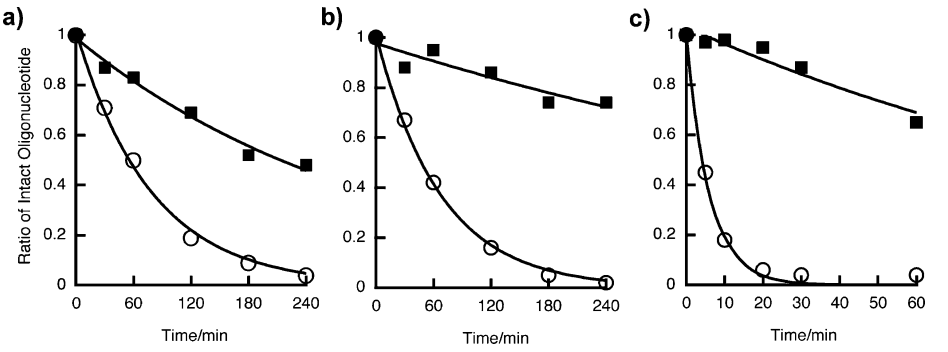

FIGURE 3 Acid-mediated P-N bond cleavage of (a) **ON-1**, (b) **ON-2,** and (c) **ON-3**. The reaction was carried out at pH 3.0 with (open circle) or without **dsDNA-1** (closed square).

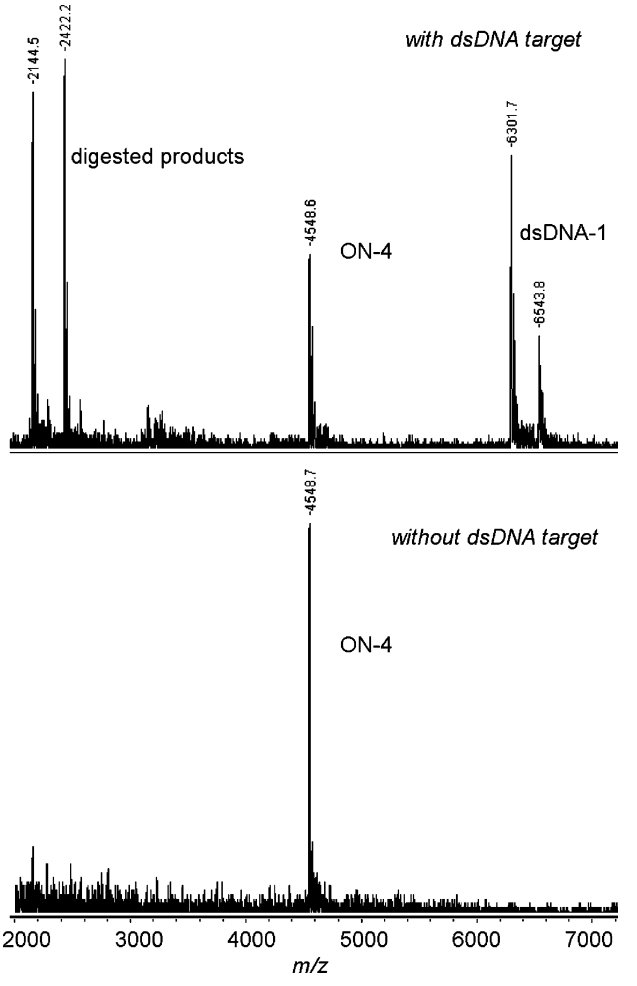

FIGURE 4 MALDI-TOF-MS spectra of the oligonucleotide (**ON-4**) with or without the dsDNA target (**dsDNA-1**).

triplexes with **dsDNA-1**. The dsDNAs that does not form a triplex with the oligonucleotides showed no effect on the cleavage of the P-N linkage (data not shown). Since the rate of the acid-mediated cleavage of a phosphoramidate linkage depends on basicity of the nitrogen atom in the phosphoramidate linkage,[6] the 5′-amino-2′,4′-BNA derivative bearing a methyl group on the 5′-nitrogen atom was prepared for more rapid cleavage of the P-N linkage.[7] As a result shown in Figure 3c, the P-N bond cleavage of the N-Me congener (**ON-3**) triggered by triplex formation was approximately 80% complete within 10 min at pH 3.0, while the digestion was scarcely detectable in the absence of the target strand.

For application to high-throughput DNA-sensing, MALDI-TOF-MS detection of the acid-mediated P-N bond cleavage was examined. Since **ON-1**, **ON-2**, and **ON-3** were so labile on the MALDI-TOF-MS target plate, we prepared the N-Et congener of 5′-amino-2′,4′-BNA as a more stable derivative.[7] The oligonucleotide containing 5′-amino-2′,4′-BNA (N-Et) (**ON-4**) was treated with or without **dsDNA-1** on the MALDI-TOF-MS target plate, where the matrix solution was spotted and dried prior to spotting the sample solution. In the presence of **dsDNA-1**, the digested product was clearly detected, while only the probe signal was observed in the absence of **dsDNA-1** (Figure 4).

CONCLUSION

We have found that the acid-mediated cleavage of the P3′ →N5′ phosphoramidate linkage incorporated into the third strand of the pyrimidine motif triplex is much faster than that in the single-stranded states, and we also showed that this attractive feature of the phosphoramidate linkage is applicable to a sequence-specific, rapid and convenient DNA-sensing.

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