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Nucleosides and Nucleotides. 171. Thermal and Nuclease Stabilities of G-quadruplexes Consisting of Oligodeoxynucleotides Containing 5-[*N*-[2-[*N*,*N*-Bis(2-aminoethyl)amino]ethyl]carbamoyl]-2'-deoxyuridine or 5-[*N*-[3-[*N*,*N*-Bis(3-aminopropyl)amino]propyl]carbamoyl]-2'-deoxyuridine[1]

Yoshihito Ueno, Mai Mikawa, and Akira Matsuda*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

Abstract: The thermal and nuclease stabilities of G-quadruplexes consisting of oligodeoxynucleotides (ODNs) containing 5-[*N*-[2-[*N*,*N*-bis(2-aminoethyl)amino]ethyl]carbamoyl]-2'-deoxyuridine (^{BA}**E**) or 5-[*N*-[3-[*N*,*N*-bis(3-aminopropyl)amino]propyl]carbamoyl]-2'-deoxyuridine (^{BA}**P**) are described. We found that the modified nucleosides, ^{BA}**E** and ^{BA}**P**, stabilize quadruplex formation when positioned at the 3'-end of the ODN. Furthermore, ODNs containing the modified nucleosides were more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase than an unmodified ODN. © 1997 Elsevier Science Ltd.

Guanosine-rich oligodeoxynucleotides (ODNs), under physiological salt and pH conditions, form thermodynamically stable four-stranded helices (G-quadruplexes) containing a square-planar array of four guanosines that are hydrogen-bonded in the Hoogsteen manner and stabilized by a monovalent cation such as K^+ and Na^+.[2] G-quadruplexes are thought to participate in various biological processes, including the modulation of telomere activity, dimerization of HIV RNA and genetic recombination in immunoglobulin switch regions.[2,3] Furthermore, several biologically active quadruplexes have been discovered using a combinatorial screening method.[4] Recently, Rando and Bishop reported that an ODN composed of 2'-deoxyguanosine and thymidine, 5'-GTGGTGGGTGGGTGGGT-3', inhibited HIV-1 (human immunodeficiency virus type-1)-induced syncytium formation by 50% at 0.3 μM.[5] Under physiological conditions, these ODNs can form an intramolecular quadruplex as shown in Fig. 2a. They also reported that the ODN containing phosphorothioate linkages was more stable in serum and exhibited more potent antiviral activity than the corresponding unmodified phosphodiester ODN.[5]

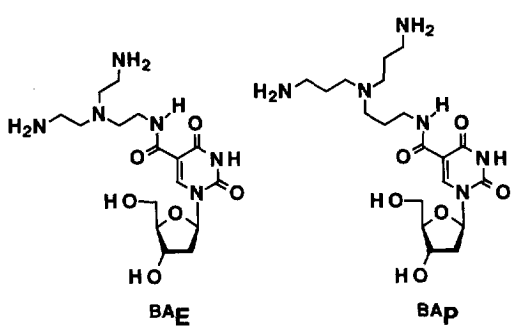
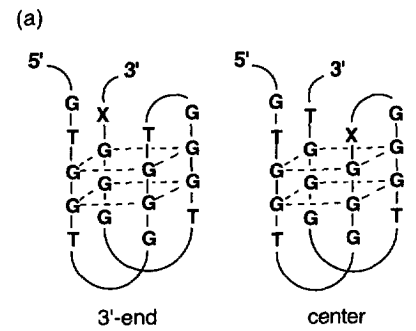


Fig. 1. Structures of the modified nucleosides.

On the other hand, we recently reported the synthesis of ODNs containing 5-(*N*-aminoethyl)-carbamoyl-2'-deoxyuridine (**E**) or 5-(*N*-aminohexyl)-carbamoyl-2'-deoxyuridine (**H**).[6] These ODNs stabilized duplex formation with a complementary DNA or RNA strand. The enhanced thermal stability of duplexes containing **E** or **H** is explained by a reduction of electrostatic repulsion between the phosphate moieties by the ammonium ions of the



(b)

1 5'-GTGGTGGGTGGGTGGGT-3'
2 5'-GTGGTGGGTGGGTGGG^{BA}**E**-3'
3 5'-GTGGTGGG^{BA}**E**GGGTGGGT-3'
4 5'-GTGGTGGGTGGGTGGG^{BA}**P**-3'
5 5'-GTGGTGGG^{BA}**P**GGGTGGGT-3'

Fig. 2. List of ODNs synthesized. (a) Schematic representation of the G-quadruplex structure. **X** = **T**, ^{BA}**E** or ^{BA}**P**. (b) Sequences of ODNs used in this study.

aminoalkyl-linker. More recently, we also reported the synthesis of ODNs containing 5-[*N*-[2-[*N*,*N*-bis(2-aminoethyl)amino]ethyl]carbamoyl]-2'-deoxyuridine (^{BA}**E**) or 5-[*N*-[3-[*N*,*N*-bis(3-aminopropyl)amino]-propyl]carbamoyl]-2'-deoxyuridine (^{BA}**P**).[1] The ODNs containing ^{BA}**P** stabilized duplex formation more efficiently with a complementary DNA or RNA strand than the ODNs containing **E** or **H**, but destabilized triplex formation with a target duplex. Moreover, the ODNs containing ^{BA}**E** or ^{BA}**P** were more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase (a 3'-exonuclease) and nuclease S1 (an endonuclease) than the unmodified ODNs.

These results led us to investigate the thermal and nuclease stabilities of G-quadruplexes when ^{BA}**E** and ^{BA}**P** are introduced into the ODN, 5'-GTGGTGGGTGGGTGGGT-3', in place of thymidine. The ODN analogues synthesized are listed in Fig. 2b.[7] In this paper, we report the thermal stabilities of G-quadruplexes consisting of the ODNs **1**-**5**. The stability of these ODNs to nucleolytic hydrolysis by snake venom phosphodiesterase was also studied.

In this study, melting curves were obtained by measuring thermal changes of CD spectra. The CD spectra of ODNs **1**-**5** were measured in 0.01 M sodium cacodylate (pH 7.0) buffer containing 0.1 M KCl.[8] As shown in Fig. 3, each ODN has a positive CD band around 260 nm and a negative CD band around 240 nm at 5 °C (solid lines). These spectra are similar to that of the G-quadruplex of d(TTGGGG)$_4$ reported previously.[9]

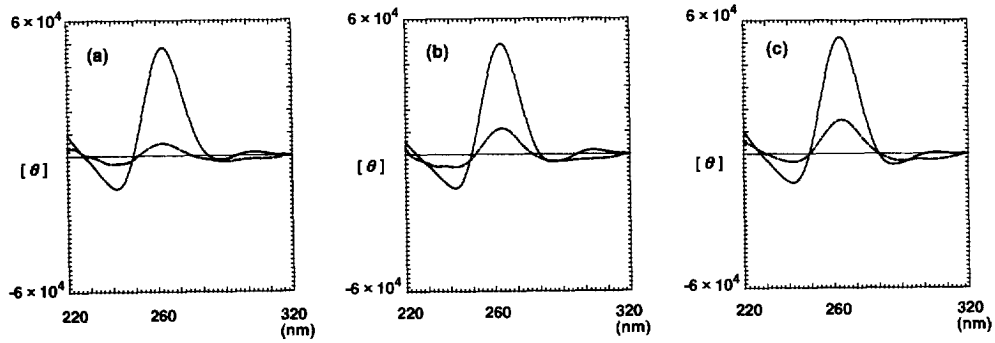


Fig. 3. CD spectra for ODNs. (a) ODN **1**; (b) ODN **2**; (c) ODN **4**. Solid line: spectra at 5 °C; dotted line: spectra at 90 °C. See References and Notes for the conditions.[8]

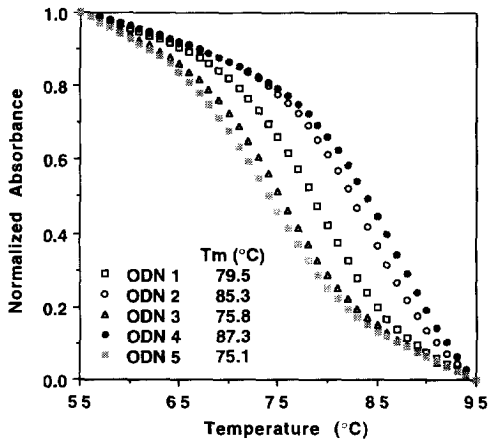


Fig. 4. Thermal stability of G-quadruplexes. See References and Notes for the conditions.[8]

Furthermore, the intensity of both bands was reduced when measured at 90 °C (dotted lines). Therefore, we concluded that these bands around 240 and 260 nm were typical of G-quadruplex formation.

The stability of quadruplexes consisting of ODNs containing ^{BA}**E** or ^{BA}**P** was studied by thermal denaturation. Melting curves of the quadruplexes were obtained by measuring the thermal changes of CD spectral intensity at 260 nm.[8] One transition was observed in the melting profile of each quadruplex (Fig. 4). The Tms were determined using the derivative of the melting curves and are summarized in Fig. 4. The stability of the quadruplexes was dependent on the position of the modified nucleosides. When positioned at the center of the ODNs the modified nucleosides, ^{BA}**E** and ^{BA}**P**, destabilized quadruplex formation. However, placing the modified nucleosides at the 3'-end of the ODNs largely stabilized quadruplex formation. As compared with ^{BA}**E**, ^{BA}**P** more efficiently stabilized quadruplex formation. A 7.8 °C increase of Tm was observed for ODN **4**.

We next examined the stability of ODNs **1**, **2**, and **4** against nucleolytic digestion using snake venom phosphodiesterase (a 3'-exonuclease). Each ODN was labeled at the 5'-end with ^{32}P, incubated with the enzyme and analyzed by 20% polyacrylamide gel electrophoresis (PAGE) under denaturation conditions. As shown in Fig. 5, the phosphodiester linkages at the 5'-sides of the modified nucleosides were more resistant to the nuclease than the phosphodiester linkage at the 5'-side of thymidine. The half-lives of ODNs **1**, **2**, and **4** were 43 min, 5.6 h, and 4.6 h, respectively.

In conclusion, we studied the thermal and nuclease stabilities of G-quadruplexes consisting of ODNs containing ^{BA}**E** or ^{BA}**P**. We found that the modified nucleosides, ^{BA}**E** and ^{BA}**P**, stabilize quadruplex formation when positioned at the 3'-end of ODNs. Additionally, ODNs containing the modified nucleosides were more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase than the unmodified ODN. The biological activity of ODNs **2-5** is now under investigation. These results will be reported elsewhere.

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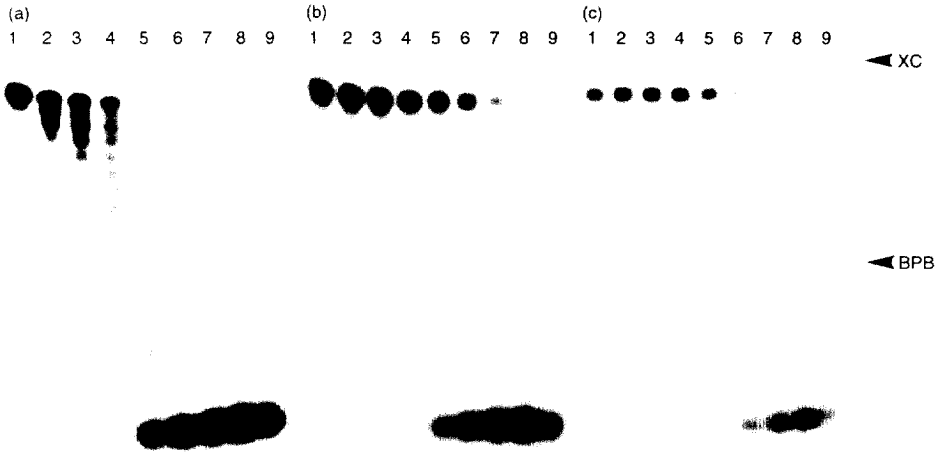

Fig. 5. Polyacrylamide gel electrophoresis of ODNs hydrolyzed by snake venom phosphodiesterase: (a) ODN **1**; (b) ODN **2**; (c) ODN **4**. ODNs were incubated with snake venom phosphodiesterase at 37 °C for 0 h (lane 1), 0.25 h (lane 2), 0.5 h (lane 3), 1 h (lane 4), 3 h (lane 5), 6 h (lane 6), 12 h (lane 7), 24 h (lane 8), and 48 h (lane 9).

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7. The ODNs were synthesized on a DNA/RNA synthesizer (Applied Biosystem Model 392) using a suitably protected 5-[*N*-[2-[*N,N*-bis(2-aminoethyl)amino]ethyl]carbamoyl]-2'-deoxyuridine or 5-[*N*-[3-[*N,N*-bis(3-aminopropyl)amino]propyl]carbamoyl]-2'-deoxyuridine phosphoramidite.[1]
8. CD spectra and thermal CD melting curves were obtained on a JASCO J720 Spectropolarimeter equipped with the temperature controller and interfaced with an NEC PC 9801 computer. Each sample contained appropriate ODN (3 μM) in a buffer of 0.01 M sodium cacodylate (pH 7.0) containing 0.1 M KCl. The ellipticities of quadruplexes were recorded from 220 nm to 320 nm in a cuvette with a path length 1 mm. CD data were converted into mdeg · mol of residues^{-1}cm^{-1}. Thermal chages of CD spectral intensity were monitored at 260 nm. The temperature of the solution was rised from 5 °C to 95 °C at the rate of 1.0 °C/min. The melting temperatures were determined by use of the derivatives of the melting curves.
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