Pergamon

Tetrahedron Letters 40 (1999) 6835–6840

TETRAHEDRON LETTERS

Artificial oligonucleotides consisting of an analog of nucleoside antibiotics, carbocyclic oxetanocins[1]

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Received 16 June 1999; accepted 12 July 1999

Abstract

Modified oligonucleotides, hexadecamers, containing carbocyclic analogues of oxetanocin A and T, have been synthesized from the corresponding chiral carbocyclic nucleosides. The oligonucleotide derived from carbocyclic oxetanocin A forms a stable triple-helix with uridine oligoribonucleotide even under physiological conditions.

Keywords: artificial oligonucleotides; carbocyclic oxetanocin; triple-helix; RNA.

Oxetanocin A (**1**) was isolated from the culture filtrate of *Bacillus megaterium*, and is a nucleoside having an oxetanosyl *N*-glycoside.[2] Later, its carbocyclic analogue **2** was found to be a more potent antiviral reagent (Fig. 1).[3]

Artificial oligonucleotides which can bind to natural oligonucleotides are attracting much attention in relation to the development of antisense or antigene drugs.[4] Although various modifications of the natural nucleic acids have been attempted, their complexation abilities were generally lower than that of the natural complementary bases. A relatively small number of exceptions is known.[5] We would like to present here a novel approach which utilizes the oligonucleotides consisting of nucleoside antibiotics.[6] As a successful example of this methodology, described here is the synthesis and complexation ability of an oligonucleotide consisting of carbocyclic oxetanocin. The oligomer strongly bound to RNA by a triplex formation.

Phosphoramidites **9** and **13** were synthesized from optically active monobenzoyloxetanocin derivatives **5**[1] and **10**[1] by a standard method as shown in Scheme 1, and were converted to oligonucleotides $coxA_{15}dA$ (**3**)[7] and $coxT_{15}dT$ (**4**)[7], respectively, by solid-phase synthesis.[8] The structures of hexadecamers **3** and **4** were confirmed by MALDI-TOF-mass spectrometry.[9]

The hybridization of adenosine derivative **3** with complementary natural dT_{15} and rU_{15} was examined by the melting curve method under high-salt conditions (1 M NaCl), and the results are summarized in

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0040-4039/99/$ - see front matter
PII: S0040-4039(99)01410-0

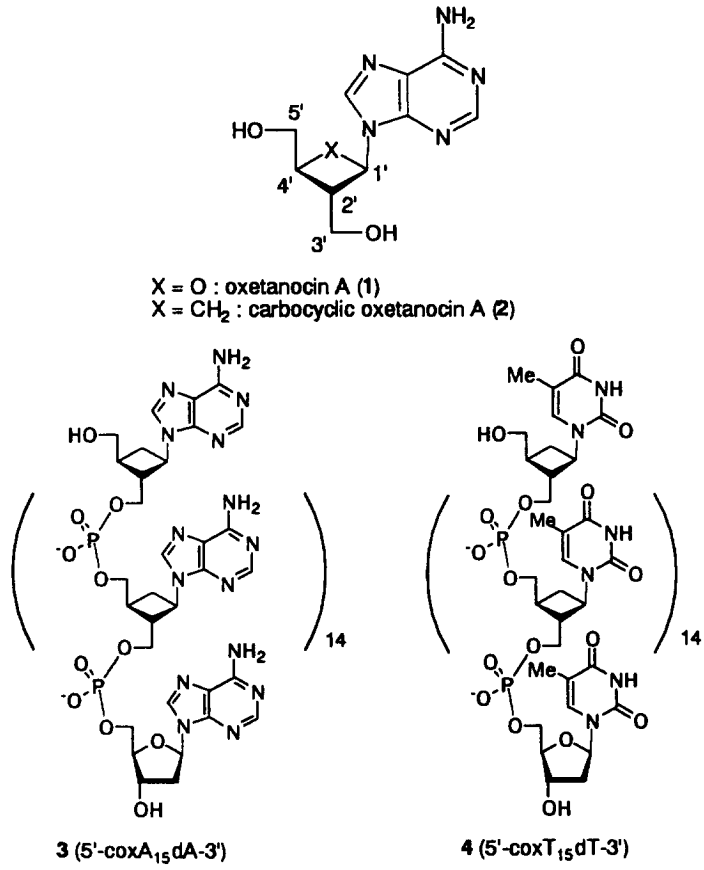
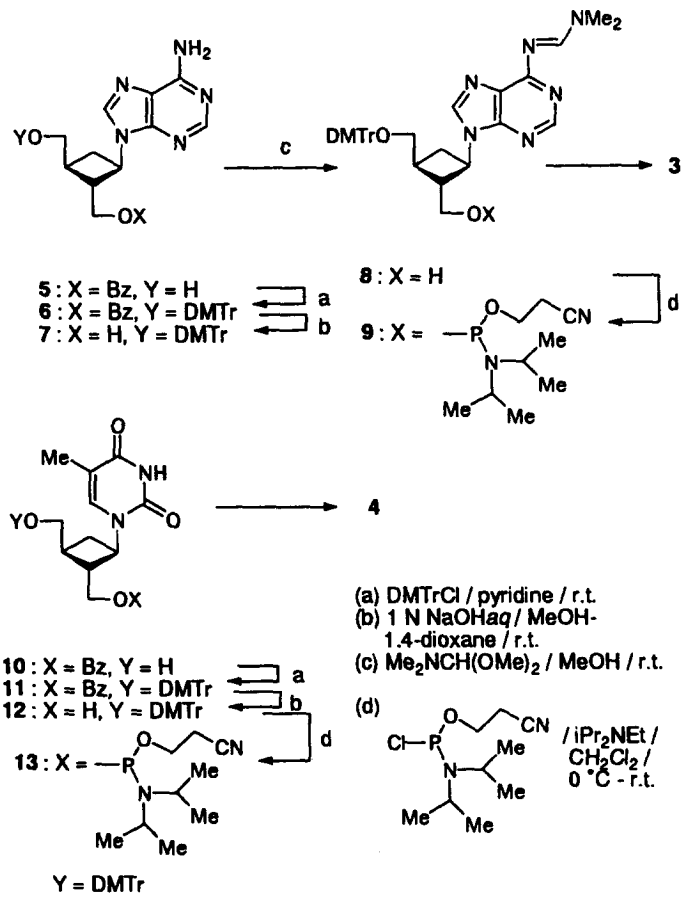


Figure 1. Structural formulas of oxetanocin A (**1**), carbocyclic oxetanocin A (**2**), and oligonucleotides (**3** and **4**) consisting of carbocyclic oxetanocins A and T

Fig. 2 and Table 1. The artificial **3** indeed formed a complex with both dT_{15} and rU_{15}. The melting point of the complex **3**/rU_{15} (Tm=54.0°C) was much higher than that of **3**/dT_{15} (Tm=30.0°C), indicating that **3** recognized the RNA model compound more strongly than the DNA derivative. Furthermore, the artificial adenyl **3** bound to rU_{15} much more strongly than the natural adenosyl dA_{15} and rA_{15}, the Tm of which was 33.7°C and 33.2°C, respectively.[10] The complexation of **3**/rU_{15} was so strong that the binding was observed even under low-salt conditions (0.1 M NaCl) with Tm=36.5°C (Fig. 2). Artificial nucleotides possessing strong and selective binding ability to RNA have been rare.[11] Notably, artificial thymidyl **4** did not bind with the complementary natural adenyl dA_{15} and rA_{15} even under high-salt conditions, although **4** did bind to the artificial adenyl **3**.

A mixing curve study (Job plots)[12] of the complex **3**/rU_{15} under high-salt conditions indicated the formation of a 1:2 complex probably with a triple helix structure (Fig. 3). The triplex formation took place in two steps as indicated by the melting profile (open circles in Fig. 2). Although the variable temperature CD spectrum (Fig. 4) did not indicate clearly the triplex formation via two steps, significant change of the spectrum between 49°C and 59°C corresponded to the Tm value (54.0°C). Notably, the triplex of **3**/rU_{15} was formed also under low-salt conditions. In this case, the triplex formation would occur in one step without any symptom of the double helix formation as indicated by the melting profile (triangles in Fig. 2).

While the natural dA_{10}/dT_{10} forms the triplex under high-salt conditions in two stages via a double



Scheme 1. Synthesis of oligonucleotides (**3** and **4**) containing carbocyclic oxetanocins A and T

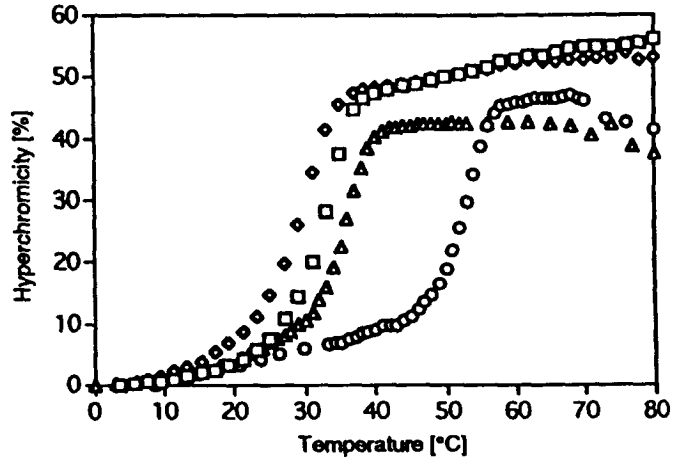


Figure 2. Melting profiles for $coxA_{15}dA/(coxT_{15}dT)_2$ (□), $coxA_{15}dA/(dT_{15})_2$ (◇), and $coxA_{15}dA/(rU_{15})_2$ (○) under high-salt conditions (1.0 M NaCl), and $coxA_{15}dA/(rU_{15})_2$ (△) under low-salt conditions (0.1 M NaCl). The buffer was 10 mM phosphate buffer (pH 7.0) and 1.0 M NaCl or 0.1 M NaCl in H_2O. The concentration of total strands was 2.1 μM. The profile was recorded at 260 nm with a temperature ramp of 0.5°C/min

Table 1
*T*m values (°C) from melting curves (260 nm)[a]

	coxT$_{15}$dT (4)	dT$_{15}$	rU$_{15}$
coxA$_{15}$dA (3)	33.6	30.0	54.0
dA$_{15}$	b	50.5	33.7
rA$_{15}$	b	49.0	33.2

[a]All experiments were carried out in 10 mM phosphate buffer (pH = 7.0) and 1 M NaCl. The conditions are the same as in Figure 2.
[b]No determinable *T*m value.

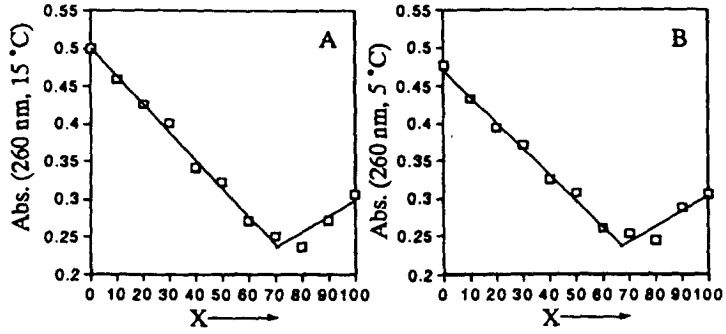


Figure 3. UV mixing curves for reactions between coxA$_{15}$dA and rU$_{15}$ at either 1 M NaCl (A) or 0.1 M NaCl (B). Mixing experiments were done in 10 mM phosphate buffer (pH 7.0), and the total strand concentration (C_T) was 2.1 μM. X=Concentration of rU$_{15}$ in mol%

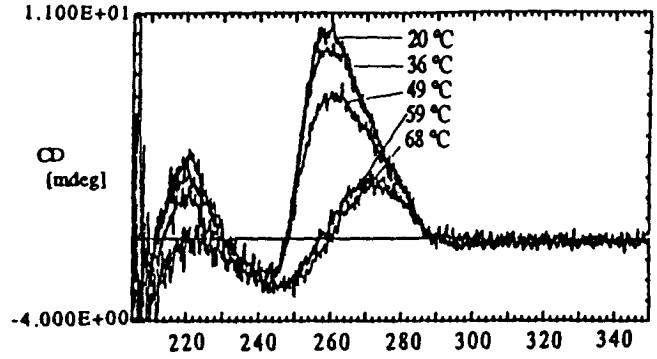


Figure 4. CD spectra of coxA$_{15}$dA/(rU$_{15}$)$_2$ triplex under high-salt conditions at differing temperatures. The conditions are the same as in Fig. 2

helix,[13] duplex formation predominates under low-salt conditions. The one-step triplex formation under low-salt conditions, therefore, is a characteristic aspect of the carbocyclic oxetanocin oligonucleotides. That the complex **3**/**4** and **3**/dT$_{15}$ formed a triplex in 1 M NaCl without the intermedicacy of a duplex is also in accordance with this view.

We also examined the susceptibility of the modified oligonucleotides to enzymatic hydrolysis. As a typical example, 5′-coxA$_5$dA-3′ (0.256 OD) synthesized from **9** was treated with venom phosphodiest-

erase (0.1 units) in 0.1 M phosphate buffer (pH 7.0) at 36°C for 5 h to give 2′-deoxyadenosine 5′-monophosphate (pdA) and $coxA_5$ in quantitative yields. On the contrary, 5′-$coxA_5$dA-3′ was completely inert to the hydrolysis by spleen phosphodiesterase. These results satisfy one of the requirements for antisense oligonucleotides.

In conclusion, we have achieved the first synthesis of oligonucleotide analogues containing carbocyclic oxetanocin A and T,[14] and have found that $coxA_{15}$dA binds strongly to RNA rather than DNA to form a triple helix even under physiological conditions.[15] Clearly, the structural basis for the strong stability of complexes of $coxA_{15}$dA with RNA and the triple-helix formation requires further investigation. However, the observed binding selectivity, combined with the stability to common nucleases, makes carbocyclic oxetanocin oligonucleotide an excellent candidate for diagnostic and therapeutic antisense applications targetting mRNA. Studies on the synthesis of oligonucleotides containing carbocyclic oxetanocin G and C and their properties are in progress, and the results will be reported in due course.

Acknowledgements

This work was supported in part by grants from the Japan Society of Promotion of Science (RFTF 97P00302), the Ministry of Education, Science, Sports, and Culture, Japan (no. 08672405), and Terumo Life Science Foundation.

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7. coxA and coxT represent carbocyclic oxetanocin A and T, respectively.
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9. The spectrum was measured by PerSeptive Biosystems Voyager™ DE SI 2. **3**: calcd for $^{12}C_{172}{}^{13}C_3H_{223}N_{80}O_{63}P_{15}$: 4920.3. Found: 4920.2.
10. Previous study on the stability of triplexes reveals that R–DR hybrids are as stable as D–DD and D–RD hybrids are unstable (R and D designate RNA and DNA, respectively). Unusual binding results for our unmodified controls may be attributable to the measurement of *T*m values under high-salt conditions. Han, H.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3806–3810.
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