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Convenient Synthesis of Oligodeoxyribonucleotides Bearing Arabinofuranosyl Pyrimidine Derivatives and Its Duplex Formation with Complementary DNA

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Abstract—The oligodeoxyribonucleotides bearing 2,2′-anhydro-β-D-arabinofuranosyluracil derivatives were synthesized and the modified residue was converted to β-D-arabinofuranosyluracil derivatives or β-D-arabinofuranosylisocytosine derivatives by post-synthetic modification method. The melting profiles of their ODNs with complementary DNA were studied.

Wide variety of modified oligonucleotides has been synthesized as antisense oligonucleotides (AON). One of the important properties for AON is the activation of RNase H. It was reported that arabinonucleic acid (ANA) formed duplex with RNA and was able to induce RNase H degradation of target RNA.[1] These properties of ANA seem to relate that the sugar puckering of an ANA strand in an ANA/RNA duplex adopt an O4′-endo conformation.[2] Also, mixed-backbone oligonucleotides, such as gapmer and altimer,[3] were designed and reported to elicit RNase H degradation of the target RNA when arabinonucleic acid was used as modified nucleosides. However, these useful arabinonucleic acid units were synthesized by the multi-step synthesis from ribonucleosides[1b] and a base-modified arabinonucleoside has not been incorporated into DNA or RNA. We undertook synthesis of oligodeoxyribonucleotides containing base-modified arabinonucleosides and studied the effect of these modification on a duplex formation.

Arabinofuranosyl pyrimidine nucleosides (araT, araU, and araC) have been prepared from the corresponding ribonucleoside via 2,2′-anhydro pyrimidine nucleoside derivatives. For example, araT was prepared by the reaction of ribothymidine derivative with diphenylcarbonate to give 2,2′-anhydro-β-D-arabinofuranosylthymine derivative and subsequent ring opening reaction with ethnolic potassium hydroxide.[1b] By use of the ring opening reaction of 2,2′-anhydro pyrimidine nucleoside derivative, 2′-substituted- or 2-substituted-pyrimidine derivatives, have been prepared.[4] For example, it was reported that 2,2′-anhydro-β-D-arabinofuranosyluracil (**1a**) reacted with methanolic ammonia to give β-D-arabinofuranosylisocytosine (**3a**) by Brown et al.[5] and 2,2′-anhydro-β-D-arabinofuranosylthymine (**1b**) reacted with ammonia to give β-D-arabinofuranosyl-5-methylisocytosine (**3b**) by Doerr et al.[6] Also, Legorburu et al.[4] reported that **1a** reacted with hydroxide ion to give β-D-arabinofuranosyluracil (**2a**). 5′-Protected-β-D-arabinofuranosylthymine was synthesized from 5′-protected-2,2′-anhydro-β-D-arabinofuranosylthymine by Noronha et al.[1b] In general, 'hard' nucleofile such as hydroxide ion or ammonia attacks at C-2 on the pyrimidine resulting in substitution on the C-2 and leave the hydroxyl on the 2′ position in the arabino configuration.[7] In this paper, we described the introduction of 2,2′-anhydro-β-D-arabinofuranosyluracil derivatives into ODN and conversion to the modified ODN containing arabinofuranosyl pyrimidine derivatives. Also the duplex stability of these modified ODN with complementary DNA was studied.

Syntheses and reactions of 2,2′-anhydropyrimidine nucleosides

2,2′-Anhydro-β-D-arabinofuranosyluracil (**1a**) was prepared by the previous reported methods.[8] 2,2′-Anhydro-β-D-arabinofuranosylthymine (**1b**) was synthesized by the method similar to our reported synthetic route of 2,2′-anhydro-α-D-ribofuranosylthymine.[9] 2,2′-Anhydro-β-D-arabinofuranosyl-5-methoxycarbonylmethyluracil

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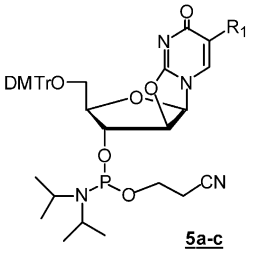
(**1c**) was synthesized from arabinoaminooxazoline and dimethyl α-bromomethylfumarate.[10]

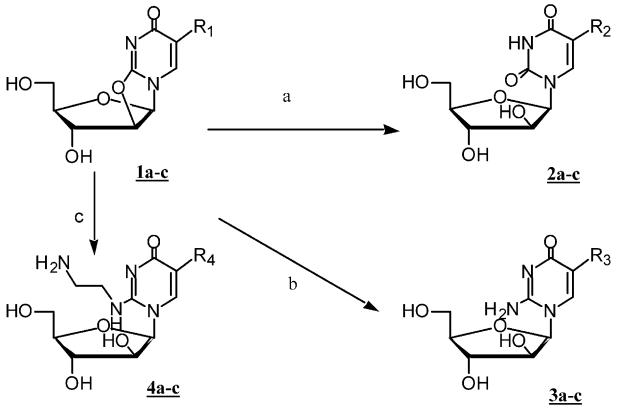
Preliminary we carried out the reactions of 2,2′-anhydronucleosides (**1a–c**) with OH^-, NH_3, and $NH_2CH_2CH_2NH_2$ (Scheme 1). The products were analyzed by ESI-MS and their found values were consistent with the calculated values for each compound.[11] As a result, an alkaline hydrolysis of **1a–c** with aqueous sodium hydroxide produced arabinofuranosyluracil derivative (**2a–c**). The reactions of **1a–c** with methanolic ammonia resulted in arabinofuranosylisocytosine derivatives (**3a–c**). Ethylenediamine in methanol reacted with **1a–c** to give arabinofuranosyl-N^2-aminoethyl-isocytosine derivatives (**4a–c**). These results suggested that these nucleofiles attacked to a C2 position of anhydonucleosides to give the arabinofuranosyluracil derivatives or arabinofuranosylisocytosine derivatives because these nucleofiles were a hard nucleofile.

Synthesis of ODN containing 2,2′-anhydropyrimidine nucleosides

5′-Dimethoxytrytilation and 3′-phosphatylation of compounds **1a–c** were carried out by the standard manner.[12] Then these nucleoside phosphoramidites (**5a–c**) were incorporated into ODN by an automated DNA synthesizer. The protected ODN on CPG allowed to react with nucleofiles for a post-synthetic modification method. The ODN was treated with sodium hydroxide aq solution, methanolic ammonia, or ethylenediamine/ethanol. Then, the suspension containing CPG was treated with concentrated ammonia solution in order to make sure the deprotection and cleavage of ODN from CPG. The 5′-DMTr-ODNs were purified by HPLC and then 5′-DMTr group of ODNs were removed by an acid treatment. The obtained modified ODNs were checked by HPLC and identified by ESI-MS. Under the condition of nuclease digestion, we found that β-D-arabinofuranosylisocytosine derivatives (**3a–c** and **4a–c**) were unstable and they were slowly converted to 2,2′-anhydro-β-D-arabinofuranosyluracil derivatives.[13] This is consistent with the previous report,[14] which suggested that β-D-arabinofuranosylisocytosine derivatives were converted to 2,2′-anhydro-β-D-arabinofuranosyluracil derivatives in presence of acid. Therefore, the characterization of these ODNs was only performed by ESI-MS. Yields and ESI-MS data are shown in Table 1. It was suggested that 2,2′-anhydro-β-D-arabinofuranosyluracil derivatives in ODNs were converted to the corresponding arabinofuranosyl pyrimidine derivatives from the ESI-MS data of ODN. **ODN1a** and **ODN1b** containing 2,2′-anhydronucleosides were prepared by use of this conversion in aqueous solution.



5a R_1=H
5b,R_1=CH_3
5c, R_1=CH_2COOCH_3



1a, **2a**, **3a**, and **4a**; R_1=R_2=R_3=R_4=H
1b, **2b**, **3b**, and **4b**, ; R_1=R_2=R_3=R_4=CH_3
1c; R_1=CH_2COOCH_3: **2c**; R_2=CH_2COOH
3c; R_3=CH_2CONH_2: **4c**; R_4=$CH_2CONHCH_2CH_2NH_2$

Scheme 1. (a) 1 M NaOH, 37 °C, 2–7 days; (b) NH_3/MeOH, 37 °C, 2–7 days; (c) ethylenediamine/EtOH, 37 °C, 2–7 days.

Duplex formation

The ability of duplex formation of these ODNs with a complementary DNA was studied by UV melting experiments. The results were summarized in Table 2. **ODN2b** which contained araT showed lower T_m than normal ODN (**N-ODN**) by 4 °C. This is consistent to previously reported results[1a] and other araU derivative-containing **ODN2a** and **ODN2c** showed a similar T_m to **ODN2b**. This destabilization of duplex is likely caused by the change of a sugar puckering compared with **N-ODN**, which consisted of all-deoxyribonucleoside. The *T*m of ODN duplex containing other nucleosides with

Table 1. Yields and ESI-MS data of ODN containing arabinofuranosylpyrimidine

Compd	X	Yield[a]/%	ESI-MS[b]	
			Calcd	Found
ODN1a	**1a**	24.8	4458.8(Na^+)	4458.7(Na^+)
ODN1b	**1b**	34.0	4450.8	4450.5
ODN2a	**2a**	21.3[c]	4476.8(Na^+)	4475.8(Na^+)
ODN2b	**2b**	16.1	4468.8	4468.3
ODN2c	**2c**	25.7	4512.8	4512.8
ODN3a	**3a**	16.3[c]	4453.8	4456.3
ODN3b	**3b**	65.0[d]	4467.8	4468.2
ODN3c	**3c**	16.4	4510.8	4510.2
ODN4a	**4a**	72.6[c]	4496.8	4498.1
ODN4b	**4b**	25.3[d]	4510.9	4511.0
ODN4c	**4c**	18.4	4596.9	4596.5

ODN: 5′-d(CGC TTC TXC CTG CCA)-3′, which X is a modified nucleoside.
[a]Yields from ODN attached on CPG except for marked ones.
[b]Data derived for parent ODN molecule. (Na^+) indicate that one proton replaced with Na^+.
[c]Yield from **ODN1a**.
[d]Yield from **ODN1b**.

Table 2. Melting temperatures of the modified ODN/DNA

ODN	X	cDNA1(dA)		cDNA2(d*iso*G)	
		*T*m/°C	ΔT/°C(vs ODN2b)	*T*m/°C	ΔT/°C(vs cDNA1)
N-ODN	**T**	61.6	4.0	—	—
ODN1a	**1a**	49.0	−8.6	—	—
ODN1b	**1b**	49.3	−8.3	47.1	−2.2
ODN2a	**2a**	57.4	−0.2	—	—
ODN2b	**2b**	57.6	0	—	—
ODN2c	**2c**	57.5	−0.1	—	—
ODN3a	**3a**	49.6	−8.0	—	—
ODN3b	**3b**	49.3	−8.3	63.6	14.3
ODN3c	**3c**	47.9	−9.7	62.7	14.8
ODN4a	**4a**	48.9	−8.7	—	—
ODN4b	**4b**	44.5	−13.1	48.5	4.0
ODN4c	**4c**	48.8	−8.8	49.4	0.6

ODN: 5′-d(CGC TTC TXC CTG CCA)-3′, which X is a modified nucleoside. cDNA1: 5′-d(TGG CAG GAA GAA GCG)-3′ cDNA2: 5′-d(TGG CAG G*iso*GA GAA GAC)-3′, which is *iso*G is deoxyisoguanosine.

Condition: Conc. of DNA, 2 μM; buffer, 150 mM sodium chloride/10 mM sodium phosphate (pH 7.0).

cDNA1 were lower than araU derivatives-containing ODNs (**ODN2a–c**) by >8 °C. This may be because these anhydroU derivatives (**1a–c**) and arabinofuranosylisocytosine derivatives (**3a–c** and **4a–c**) don't have an imino proton at N^3 position, which is required for the Watson–Crick base-pairing with adenine residue on the complementary DNA. Also, 2,2′-anhydrouridine derivatives may have rigid conformation due to a 2,2′-ether bond. Isocytosine is known to form a base-pair with isoguanine.[15] To confirm the formation of base pair between arabinofuranosylisocytosine derivatives and isoganine, a complementary DNA bearing deoxyisoguanosine (**cDNA2**) was synthesized and melting experiments of **ODN**/**cDNA2** were carried out. The results for several ODN were listed in Table 2. T_m of **ODN1b** containing 2,2′-anhydrothymidine with **cDNA2** is lower than that with **cDNA1** by −2.2 °C (ΔT vs **cDNA1**). This is due to no amino group at C2. On the other hand, T_m's of ODN containing arabinofuranosylisocytosine derivatives (**ODN3b**, **ODN3c**, **ODN4b**, and **ODN4c**) with **cDNA2** is higher than that with **cDNA1**. Especially, the duplex of **ODN3b** containing arabinofuranosyl-5-methylisocytosine or **ODN3c** containing arabinofuranosyl-5-carboxymethylisocytosine with **cDNA2** showed higher T_m which was comparative to that of normal DNA duplex **N-ODN**/**cDNA1** (T_m 61.6 °C). These results suggest that arabinofuranosylisocytosine derivatives formed a base pair with isoguanine residue in **cDNA2**. Also, a substitution to ethylenediamine at N^2 amino group (**ODN4b** and **ODN4c**) caused a destabilization of duplex due to the steric hindrance by the substituent compared with **ODN3b** and **ODN3c**, respectively.

In this paper, we indicated that ODN containing araU, araT, or ara(isoC) derivatives were easily prepared by a post-synthetic modification. This method will provide a convenient synthesis of the ODNs containing arabinonucleosides. The ODNs containing arabinofuranosylisocytosine or arabinofuranosyl-5-methylisocytosine formed a stable duplex with complementary DNA containing isoguanosine and also the substitution of amino group to 2-aminoethylamino group at C-2 position led to the destabilization of the duplex. Also, C5 substitution of arabinofuranosyluracil residue (**ODN2c**) did not so much affect on duplex formation with a complementary DNA. The study on the synthesis of a novel modified ODN containing arabinonucleosides is in progress using our developed method.

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