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Synthesis and properties of cross-linkable DNA duplex using 4-amino-2-oxo-6-vinyl-1,3,5-triazine

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ABSTRACT

We synthesized the DNA oligonucleotide containing a new cross-linkable 4-amino-2-oxo-6-vinyltriazine (AOVT) nucleobase analogue (Et-AOVT) and evaluated these properties. Our results of the cross-link assay and thermal denaturing assay of duplexes containing AOVT demonstrated that the additional aza of AOVT has an impact on the duplex stability and crosslink properties. Our data suggests that the additional 5-aza of AOVT is involved in the hydrogen bonding with the complementary guanine, and this hydrogen bonding system successfully flipped the reactive vinyl group out to the major groove of the duplex demonstrating a new paradigm of a "cross-linkable duplex".

1. Introduction

Various RNAs, such as mRNAs, pre-mRNAs, and non-coding RNAs, are currently recognized as a potential drug target to regulate genetic diseases.[1–4] Synthetic oligonucleotides (ONs) are one of the promising druggable candidates in that the ONs can directly interact with gene-coding DNAs, mRNAs and functional non-coding RNAs in a sequence-specific manner.[5–9] Various potent ONs that perform the efficient inhibition of translation were developed by improving their chemical stability, binding affinity and selectivity toward the target DNA/RNAs.[10–13] In addition to single, double and higher-order structuring nucleic acids, nucleic acid binding proteins (NABPs) can also be targeted by using decoy-ONs and nucleic acid aptamers.[14,15] In all these techniques, the strong binding affinity with the targets is a pivotal factor to compete with other target-binding molecules. To further improve an affinity with the target nucleic acids and NABPs, cross-link formation is one of the robust approaches as it forms a stiff covalent linkage to targets.[16–22] Apart from the DNA-crosslinking natural products, such as cisplatin[23] and mitomycin,[24] Rokita's groups reported artificial inter-strand cross-linkers using a cross-linkable small molecule.[25,26] In addition, various crosslink-forming oligonucleotides (CFOs) have also been developed and various attractive approaches of stimulus-responsible CFOs by using photo,[27] UV[28–30], or oxidative[31,32] activation have been reported. We have focused on developing the methodology of ON-based gene regulations using cross-linkable artificial nucleobase, and have also reported several CFOs containing cross-linkable 2-amino-6-vinylpurine (2-AVP)[33–35] and 4-amino-6-oxo-2-vinylpyrimidine (AOVP)[36–38] targeting DNAs and RNAs. One of important benefits of our cross-linking nucleobase is *the requirement of no external stimuli* such as UV-irradiation which potentially is toxic to biological tissues,[39] but just requiring the *Michael acceptor (crosslinker) - donor (target) proximity effect* acquired by Watson-Crick base pairing. Furthermore, the crosslinking property was provided by minimal structural changes to the canonical nucleobases which is potentially compatible within natural nucleic acid-related intracellular biological reactions. In a context of efforts on studying our CFOs, we designed a new cross-linkable nucleobase, 4-amino-2-oxo-6-vinyl-1,3,5-triazine (AOVT), in which the structure contains an electron-deficient triazine ring.

We hypothesized for this molecule that (i) the electron-withdrawing effect of the triazine increases the reactivity of the 6-vinyl group of AOVT than that of the pyrimidine-type AOVP, and also that (ii) the additional aza moiety effectively reduces the reactivity of AOVT with guanine (G) by forming a stable base pairing via a three hydrogen bonding system (Fig. 1). Using such a unique molecular interaction with G, we assumed that our reactive cross-linking vinyl group can be encapsulated in the oligo-duplex remaining intact from the reaction with complementary bases,

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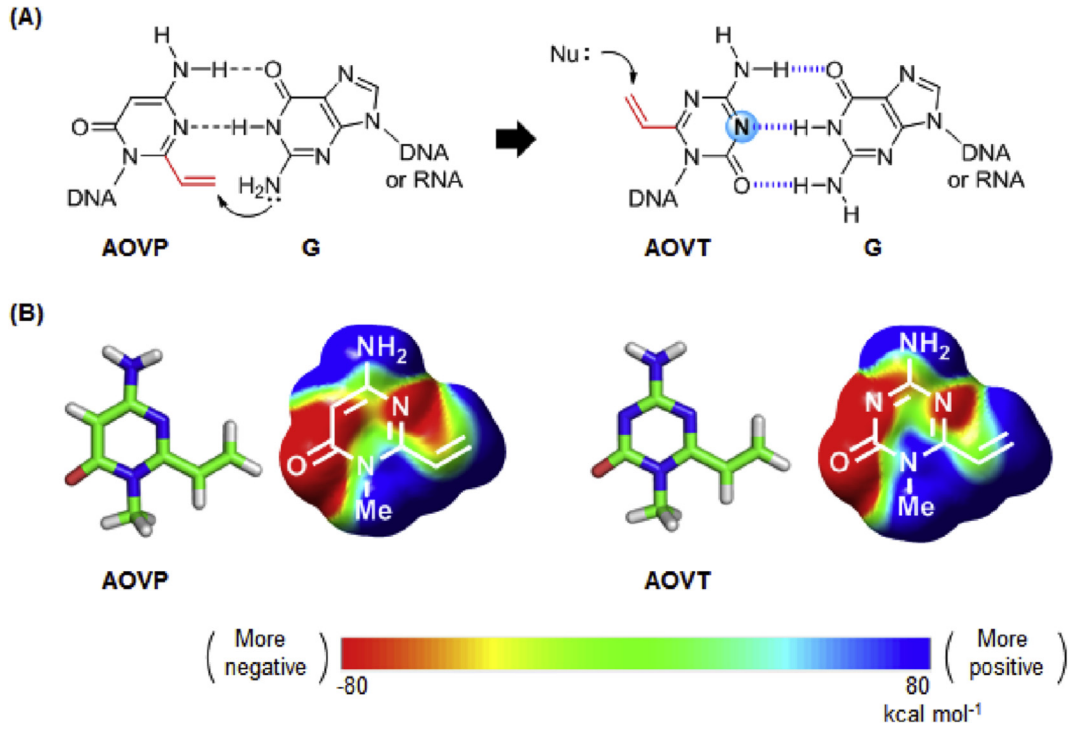

Fig. 1. Alteration of AOVP to a new cross-linkable AOVT having a reduced reactivity with guanine. (A) Additional 3-aza of AOVT enables forming of a stable AOVT-G base pair and to crosslink to endogenous nucleophiles. (B) Optimized structure and difference in electron density maps of me-AOVP and me-AOVT.

which may be able to target nucleophiles of the oligo-duplex binding species, such as NABPs. We now report the synthesis of CFOs having AOVT and its crosslink properties to complementary base and also report structural preferences of AOVT in the form of AOVT-G base pair.

2. Results and discussion

2.1. Synthesis of 6-(octylthio)ethyl 5-azacytidine derivative

We initially designed 6-vinyl-decitabine derivative (**1**) as a cross-linkable AOVT precursor (Fig. 2). Our previous study suggested that the direct glycosylation of the 6-octylthioethyl-substituted 5-azacytosine derivative with the ribose-sugar was difficult to obtain the desired compound. We planned to synthesize the C6-substituted 5-azacytosine by ring opening of the 5-azacytosine moiety forming guanylurea and subsequent ring closing reaction[40] using the orthoester, 1,1,1-trimethoxy-3-(octylthio) propane (**6**), which was synthesized in 3 steps from 3-bromopropionitrile (**3**) (Scheme S-1).[41–43] The glycosylation of the silylated 5-azacytosine (**8**) with 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribose was carried out to yield the α/β anomeric mixture of 3′,5′-O-acetyl-5-azacytidine (**9**) (Scheme 1).[44] The 5-azacytosine moiety of **9** was transiently converted to the guanylurea (**10**) by ammonia treatment, and then **10** was condensed with orthoester **6**. However, the desired ring-closed product **11** was not obtained. We next modified the scheme of which the 3′,5′-O-TIPDS -protected intermediate (**12**) was prepared from **10**, then carried out the condensation of **12** with the orthoester (**6**). As a result, the C-6 substituted 5-azacytidine derivative (**13**) was obtained as an anomeric mixture in 43% yield. However, rapid cleavage of the glycosidic bond of **13** occurred in 10 min by the treatment with 3% TCA/CH_2Cl_2, which is one of the typical deprotection conditions of the DMTr group during solid-phase oligonucleotide synthesis (data not shown). These results suggested the difficulty in the incorporation of the AOVT nucleoside into oligonucleotides by a general solid-phase synthesis.

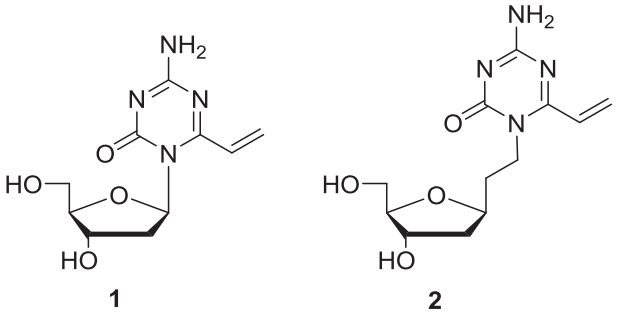

Fig. 2. Structures of cross-linkable decitabine analogues **1** and **2.**

2.2. Synthesis of phosphoramidite 25 and oligonucleotide modified with Et-AOVT nucleoside

The ethyl-bridged compound (Et-AOVT nucleoside, **2**) was next designed to avoid the glycosidic bond cleavage (Fig. 2), and the ethyl spacer between the pentose sugar and 5-azacytosine moiety allows decoupling the 1-*N*-neighboring 4′-O group participation and to avoid release of the nucleobase.[45–47] The sugar part (**16**) was synthesized in several steps from 5-*O*-Tr-2-deoxy-D-ribose (**15**) according to a previously reported method (Scheme 2).[48] After the reduction of the ethylester of **16**, the mesyl group was introduced to the hydroxyl group of **17** to yield **18**. For the glycosylation, the sodium salt of the commercially available 5-azacytosine (**7**) was coupled with **18** in the presence of $CsCO_3$ and the desired 5-azacytidine analogue (**19**) was successfully synthesized in 55% yield. The formation of the glycosidic bond at the N1 position of 5-azacytosine was confirmed by HMQC and HMBC analyses (Figs. S1,