Thermal stability and nuclease-resistance properties of oligonucleotides having an aminoalkyl side chain at the nucleobase and sugar moieties

AKIRA MATSUDA¹, YOSHIHITO UENO², AND AKIO TAKENAKA³

¹Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan, ²Faculty of Engineering, Gifu University, Yanagido, Gifu 501-1193, Japan, and ³Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Midori-ku, Yokohama 226-8501, Japan.

Abstract

Recent studies concerning properties of oligonucleotides (ONs) with aminoalkyl side chains at the nucleobase and sugar moieties have been comprehensively reviewed in relation to the antisense, antigene, ribozyme, aptamer, decoy, and siRNA strategies. Thermal stabilities of ONs incorporating nucleoside analogs with a wide variety of aminoalkyl side chains at the 5- and 4-positions of pyrimidine bases, the 2-position of a purine base, the 1'-, 2'-, 4'- and 5'-positions of sugar moieties, and the 6'-position of a carbocyclic nucleoside have been compared. The resistant properties of these ONs to hydrolysis by endo- and exo-nucleases are also discussed.

Introduction

Several methods are available to modulate cell functions by relatively short synthetic oligonucleotides (ONs), such as antisense (Uhlmann and Peyman, 1990; Milligan et al., 1993), antigene (Faria and Ulrich, 2002; Guntaka et al., 2003), ribozyme (DNAzyme) (Khan and Lal, 2003; Emilsson et al., 2003), aptamer (Burgstaller et al., 2002; Sen, 2002), decoy (Tomita et al., 2003), and siRNA (Kurreck, 2003; Scherr et al., 2003; Shuey, 2002). These methods can be useful tools for elucidating a function of the gene product. Also, such ONs can be important not only as diagnostic probes and tools for drug-developments but also as therapeutic agents. Backbone-modified ONs such as phosphorothioate and methylphosphonate analogues, have been extensively used due to their nuclease-resistance properties, since a natural phosphodiester linkage in DNA and RNA is a good substrate for ubiquitious nucleases (Crooke and Lebleu, 1993). However, these backbone-modified analogues have several drawbacks for use in biological studies: 1) Phosphorothioate ONs have lower binding affinity to their complementary strands than phosphodiester ONs, due to their diastereomer formation (Cosstick and Eckstein, 1985; LaPlanche et al., 1986; Latimer et al., 1989; Hacia et al., 1994; Mesmaeker et al., 1995). 2) Although the role of RNase H cleavage in antisense strategy is not clearly understood, it has been reported that RNA is not a substrate for the enzyme when the methylphospho-
nate ON is the complementary strand (Tidd et al., 1988; Walder and Walder, 1988). 3) Nonspecific binding of phosphorothioate ONs to certain proteins was more frequent than that of the corresponding phosphodiester ONs, perhaps due to an inherent characteristic of the P=S bond (Stein, 1995; Guvakova et al., 1995; Agrawal, 1996; Rockwell et al., 1997). Several attempts have been made to overcome these problems. One of the most promising methods is the use of natural phosphodiester ONs, if they could be made resistant to nucleolytic digestion and had membrane permeability.

On the other hand, naturally occurring polyamines, such as spermidine and spermine (Figure 1), are known to bind strongly to DNA (Tabor and Tabor, 1976, 1984; Etter, 1990) and stabilize duplex (Tabor, 1962; Thomas and Bloomfield, 1984) and triplex formations (Hampel et al., 1991; Thomas and Thomas, 1993; Musso and van Dyke, 1995), although the precise mode of binding is not clear (Jain et al., 1989). Their enhanced thermal stability is anticipated by the reduction of the anionic electrostatic repulsion between the phosphate moieties by the cationic polyamines under physiological conditions. It is also known that the bacteriophage φW-14 DNA contains up to 50% of positively charged α-putrescylthymidine (1, Figure 1) in place of thymidine (one α-putrescylthymine every eight nucleotides on average) (Kropinski et al., 1973; Fleischman et al., 1976; Kaplan and Nierlich, 1975; Miller et al., 1985) and has been shown to be more resistant to a variety of endonucleases and some exonucleases than unmodified DNA (Warren, 1980). In addition, this modification results in a higher melting temperature ($T_m = 99.3 \, ^\circ C$) than that expected for unmodified DNA based on the GC content ($T_m = 90.3 \, ^\circ C$) (Gerhard and Warren, 1982). However, short synthetic ONs (12-mer) containing 1 were found to have reduced thermal stability compared with their parent ONs (Takeda et al., 1987). Based on the results of this study, we thought that the length of the amino side chain and the distal position of the amino group would be important factors in protecting against nuclease degradation and in stabilizing duplex and triplex formations. In this review, we describe the thermal stability and nuclease-resistance properties of ONs having an aminoalkyl side chain at the various positions of the nucleobase and the sugar moieties.

![Figure 1. Structures of polyamines and α-putrescylthymidine.](image-url)
1. Synthesis and properties of ONs containing an aminoalkyl side chain at the nucleobase moiety

1.1. Thermal stability of ONs having an amino linker at the 5-position of pyrimidine nucleosides

A classical method for the synthesis of ONs bearing various aminoalkyl side chains is to first prepare such nucleoside phosphoramidite units separately and then to incorporate them into ONs by an automated DNA synthesizer (Manoharan, 1993). However, this method sometimes involves tedious protection-deprotection processes and solubility problems in the synthesis of the phosphoramidite units. In particular, when the optimum length of the side chain for a desired function is not known, several nucleoside phosphoramidite units with side chains of various lengths have to be constructed separately, and these phosphoramidite units have to be incorporated independently. To avoid such time-consuming processes, post-synthetic modifications have been developed (MacMillan and Verdine, 1990, 1991; Gao et al., 1992; Kim et al., 1992; Xu et al., 1992; Coleman and Kesicki, 1994). In these methods, a nucleoside unit with a leaving group within a molecule (a convertible nucleoside unit), which should be stable under the conditions of DNA synthetic cycles, is initially introduced into an ON (a convertible ON unit), and the resin loaded with this ON should be divided into the required number. Each divided resin could be treated with an appropriately modified side chain molecule and/or further functionalized molecule to introduce it into the ONs. Thus, post-synthetic modifications would be suitable for identifying the proper kind and/or length of the side chains.

We have developed a new convertible nucleoside, 5-trifluoromethoxy carbonyl-2'-deoxyuridine (4), which reacts smoothly with alkyldiamines in MeOH under mild conditions, and developed a new post-synthetic modification method using 4, as shown in Scheme 1 (Nomura et al., 1997; Ueno et al., 1997a). Previously, we had used 5-methoxy carbonyl-2'-deoxyuridine as a convertible unit (Ono et al., 1994; Haginoya et al., 1997). However, the reactivity of the methyl ester was not enough for completion of the amine-substitution reactions. The nucleoside 4 was readily synthesized from the 2'-deoxy-5-iodouridine derivative 3 using a Pd-catalyzed carboxylation in the presence of CF$_3$CH$_2$OH and was converted into its phosphoramidite unit 5. ONs 6 containing the convertible nucleoside attached to a solid support were treated with 1,2-diaminoethane or 1,6-diaminohexane to give the ONs 7 containing various numbers of 5-N-(2-aminoethyl)- or N-(6-aminohexyl)carbamoyl-2'-deoxyuridines (8a or 8b) at various positions. Either tris(2-aminoethyl)amine or tris(3-aminopropyl)amine was also reacted with the ON 6 to afford ONs containing 5-[N-[2-[N,N-bis(2-aminoethyl)amino]ethyl] (8c) or 5-[N-[3-[N,N-bis(3-aminopropyl)amino]propyl]carbamoyl]-2'-deoxyuridine (8d) (Ueno et al., 1997b; Ueno et al., 1998a). The terminal amino group in these ONs can be further modified by various functional groups after purification of each ON.

This post-synthetic method was quite useful for 1) the synthesis of 5'-5'-linked ONs with the potential for triple-helix formation (Ueno et al., 1996), 2) the development of a method for site-specific introduction of a functional group with (N,N-dimethylamino)hexylcarbamoyl side chains at positions different from the functional group introduced by the DNA sequence (Nomura et al., 1997), and 3) the development
Scheme 1. Synthetic route of the convertible nucleoside and an outline of the post-synthetic modification method.
of a new method for chemical ligation (Ueno et al., 1998c). The synthesis of ONs containing 5-(N-aminoalkyl)carbamoyl-2'-deoxycytidines such as 10 was also achieved using 5-trifluoroethoxycarbonyl-2'-deoxycytidine (9) as a convertible unit (Nomura et al., 1996). However, for the incorporation of the aminoalkyl side chain at the 5-position of cytosine, a more reactive ester group than the trifluoroethoxycarbonyl group should be introduced into the 5-position to further facilitate the substitution reactions.

Thermal stability of the duplexes containing 8a–8d, which were prepared by our postsynthetic method, has been studied as a function of 50% melting temperature ($T_m$). When 3 residues of the analogue were incorporated into 17-mer DNA duplexes, increased $T_m$ values per modification under the conditions (0.01 M NaCl and pH 7.0) were: $+2.0 \sim +5.0 ^\circ C$ for 8a, $+2.6 \sim +6.0 ^\circ C$ for 8b, $+1.1 \sim +2.1 ^\circ C$ for 8c, and $+0.9 \sim +3.3 ^\circ C$ for 8d. Additionally, these values with a complementary RNA under the conditions (0.1 M NaCl and pH 7.0) were: $-1.0 \sim +0.67 ^\circ C$ for 8a, $0 \sim +1.5 ^\circ C$ for 8b, $+0.75 \sim +2.2 ^\circ C$ for 8c, and $+1.2 \sim +3.1 ^\circ C$ for 8d. The $\Delta T_m$ values per modification were not constant and depended on the number and the position where the modified nucleosides were introduced. Incorporation of 8b into ONs enhances the thermal stability of the DNA-DNA duplex and that of 8d stabilizes the RNA-DNA duplex most efficiently in the series. We next compared the $T_m$ values between higher (0.1 M NaCl) and lower (0.01 M NaCl) ionic strength conditions; $\Delta T_m = T_m (0.1 \text{ M NaCl}) - T_m (0.01 \text{ M NaCl})$. When the number of the analogues in one strand of the ON instead, the $\Delta T_m$ values for the DNA-DNA duplex decreased accordingly. We also observed that N-acetylation of the terminal amino group introduced in the DNA lowered its $T_m$ value in the duplex. Therefore, the effect of salt concentrations and the N-acetylation of the terminal amino group on duplex stability indicated that the terminal ammonium ion stabilized duplex formation by neutralizing phosphate anions to reduce the number of phosphate anions.

Hashimoto et al. reported that the 12-mer ONs containing various numbers of 5-(3-aminopropyl)-2'-deoxyuridine (11a) or 5-(6-aminohexyl)-2'-deoxyuridine (11b) at various positions destabilized the DNA-DNA duplexes by $-0.5 \sim -0.6 ^\circ C$ or $-0.1 \sim -0.4 ^\circ C$ per modification in a 0.05 M NaCl buffer, respectively, whereas the ONs containing 5-(6-aminohexyl)-2'-deoxycytidine (12) became more thermally stable ($+1.5 \sim +3.4 ^\circ C$ per modification). Since the ONs containing the 5-hexyl-2'-deoxyuridine (13) were destabilized more drastically ($-3.8 \sim -4.5 ^\circ C$ per modification), the terminal ammonium ion in 11 and 12 also increased the thermal stability of the duplex (Hashimoto et al., 1993a, b). On the other hand, Takeda et al. reported that incorporation of 5-[N-(4-aminobutyl)aminomethyl]-2'-deoxyuridine (1) ($\alpha$-putrescylthymidine) in 12-mer oligo dT (dT12) greatly reduced its $T_m$ values by $-3.9 \sim -2.8 ^\circ C$ with dA12. Although the $T_m$ values were further reduced ($-4.5 ^\circ C$ per modification) when 5-(N-methylaminomethyl)-2'-deoxyuridine (14) was introduced in T12, incorporation of 5-(methoxymethyl)-2'-deoxyuridine (15) in T12 only reduced the $T_m$ value by $-2.0 ^\circ C$ per modification. These experiments also revealed that the terminal ammonium ion in 1 contributed to the stabilization of the duplex formation, although the effects were insufficient to increase the apparent $T_m$ values. This might be in part due to the undesirable effect of the second ammonium ion near the 5-position of the uracil. Introduction of 5-[N-(2-aminoethyl)]carbamoylmethyl-2'-deoxyuridine (16a) and 5-[N-[2-[N,N-bis(2-
Figure 2. Structures of pyrimidine nucleoside analogs possessing aminoalkyl linkers at the 5-positions.
aminoethyl]aminoethyl]carbamoylmethyl-2’-deoxyuridine (16c) into DNA duplexes (25-mer) increased the thermal stability by +2.7 ~ +3.0 °C and +2.1 ~ +3.2 °C, respectively, in a 0.05 M NaCl buffer, whereas 3 residues of 5-[N-(6-aminohexyl)]carbamoylmethyl-2’-deoxyuridine (16b) in the same sequences reduced the stability by −0.9 °C (Ozaki et al 1995). DNA-DNA duplexes and triplexes containing 5-[4-[N,N-bis(3-aminopropyl)amino]butyl]-2’-deoxyuridine (17) were also stabilized by +1 ~ +5 °C and +1 ~ +4 °C, respectively, depending on the concentration of NaCl (Nara et al 1995). The thermal stability of 12-mer ONs containing 5-(3-aminopropynyl)- (18a), 5-(propynyl)- (18b), 5-(3-aminopropyl)- (11a), and 5-(3-hydroxypropyl)- (19) derivatives of 2’-deoxyuridine was compared in various concentrations of NaCl in a buffer (Heystek et al 1998). The ONs containing 18a increased the duplex Tm values of +1.1 ~ +4.5 °C per modification depending on the NaCl concentrations. Since it has been known to increase thermal stability of the ONs containing 18b by +2.0 ~ +3.2 °C due to the stacking ability of the nucleobases and hydrophobic interactions by the acetylene group, the effects of the terminal ammonium ion were not so great. The nucleosides 11a and 19 having flexible short side chains destabilized the duplexes. However, whether the ammonium ion in 18a is capable of forming a salt bridge with the nonbridging major groove oxygen on the phosphate of the 5’-nucleotide is not clear from the experiments described above. The fidelity of base pairing of the uracil having the aminopropynyl side chain was also measured, and the Tm decreased by −10 ~ −14 °C for 1 mismatch (12-mer), which is in the range of that observed for mismatches involving thymine residues. Quite recently, Roig and Asseline (2003) described the synthesis of monoguanidinium nucleoside 20, bisguanidinium nucleoside 22 and tris-amino derivative 21 from 18a and compared their Tm values of 17-mer DNA-DNA duplexes and also RNA-DNA duplexes in 0.1 M NaCl buffer at pH 7.0. For the Tm values of the DNA-DNA duplexes, the order of increasing thermal stability is: 20 (+4.0 °C per modification) > 22 (±3.25 ~ ±3.3 °C) > 21 (±2.5 ~ ±2.75 °C) > 18a (±1.75 ~ ±2.0 °C), whereas that of the RNA-DNA duplexes is: 22 (+4.0 °C) > 20 (+3.5 °C) > 21 (+3.0 °C) > 18a (+2.0 °C). ONs containing 18a, 20, 21, or 22 in the third strand also thermally stabilized triplex structure by +1.3 ~ +3.0 °C.

These results indicate that the reduction of the total negative charge number on one strand is a critical parameter for the thermal stability of duplexes and triplexes. It is also clear that the location of the ammonium ion determines the magnitude of the stabilization. Therefore, three-dimensional structures of these ONs should be solved in the crystal or in solution.

1.2. Nuclease resistant property of ONs having the 5-[N-(6-aminohexyl)]carbamoyl-2’-deoxyuridine and its 2’-OMe derivative.

Nuclease stability of ONs is one of the most important properties when ONs are applied to in vitro and in vivo systems for elucidation of certain gene functions. 3’-Exonuclease activity is predominantly responsible for enzymatic degradation in a serum-containing medium whereas endonuclease works mainly in cells. However, the nuclease resistant property (measured as the half-life of the full-length ON, t1/2) of aminooalkyl-conjugated ONs has not been studied in a systematic manner. We have reported endo- and exonuclease resistant properties of ONs containing 8b. The ONs were markedly more resistant (about 160 times more stable) to nuclease hydrolysis by snake venom phosphodi-
esterase (SVPD, a 3'-exonuclease) than unmodified ONs and were very stable in a medium containing 10% fetal calf serum (Haginoya et al., 1997). However, it was also found that the endonuclease-resistant property of the ONs containing 8b was insufficient to allow it to be applied to biological studies. The half-life of the 17-mer ON containing alternatively five 8b toward nucleolytic hydrolysis by nuclease S1 (an endonuclease) was only 7 times greater than that of the unmodified ON. However, when increased numbers of unmodified nucleosides were incorporated between 8b in the ONs, the nuclease S1-resistant property was greatly reduced (Ueno et al., 1997a).

On the other hand, it has been reported that a 2'-O-methyl modification of RNA increases the stability of the RNA to nucleolytic hydrolysis by nucleases (Inoue et al., 1987a, b; Lesnik et al., 1993). Thus, we envisioned that a 2'-O-methyl modification of 8b would further increase the stability of the ONs to nucleolytic hydrolysis especially by endonucleases.

5-[N-(6-Aminohexyl)carbamoyl]-2'-O-methyluridine (23a) was synthesized from the corresponding 5-iodo derivative in a similar manner, as depicted in Scheme 1 and was incorporated into the ONs (Itoh et al., 2003). The ONs containing 23a were found to be more resistant against nucleolytic hydrolysis not only by SVPD, but also by DNase I (an endonuclease). The half-life of the 17-mer ON containing 5 residues of 23a (ON-4, see Table 1) against nucleolytic hydrolysis by SVPD was 230 times larger than the unmodified ON-1, which is 1.8 and 200 times greater than ON-3 containing 5 residues of 8b and the ON-2 containing 5 residues of 2'-O-methyluridine (Um) in the same sequence, respectively. Acetylation of the terminal amino groups in the ON-3 and ON-4 can remove the positive charges to give ON-5 and ON-6 containing 23b, respectively. Although the SVPD resistant property of the ONs-5 and -6 was slightly reduced, these ONs were still 100 and 170 times more stable, respectively, than those of the unmodified ON-1. Moreover, the ON-2 containing 5 residues of Um showed almost the same stability as ON-1. Therefore, stability against SVPD would come from the bulkiness of the 5-substituent together with terminal positive charges.

The phosphodiester linkages around the modified nucleoside 23a were also highly resistant to the endo-hydrolysis by DNase I. For the most part, the $t_{1/2}$s of the ONs containing 8b did not depend on the number of 8b (Ueno et al., 1997a), while those containing 23a were dependent on the number of 23a. The $t_{1/2}$s of the ONs containing 23a increased as the number of 23a increased. The $t_{1/2}$ of the ON-3 containing 5 molecules of 8b was almost equal to that of the control ON-1, while that of the ON-4 containing 5 molecules of 23a was 24 times greater than that of the control ON (see Table 2). From these results, it can be confirmed that the ONs containing 23a were also highly resistant against hydrolysis by the endonuclease. Thus, the high endonuclease resistant property of the ONs containing 23a was not due to the electrostatic interaction between the terminal ammonium ion of the aminoalkyl chain of 23a and the enzyme, but mainly due to the steric hindrance of the 2'-O-methyl group.
1.3. Crystal structures of Dickerson-Drew dodecamers containing 5-\textit{N}-(6-amino- hexyl)carbamoyluracil instead of thymine.

It is well known that the Dickerson-Drew dodecamer with a self-complementary sequence 5'-CGCGAATTCGG-3' forms a stable palindromic duplex. When the T residue at the 8-position is replaced with $8b$ or $23a$, the modified nucleotides appear at 8-position and the 20-position in the duplex. These duplexes were crystallized and analyzed by X-ray. Figure 3 shows their overall structures, which are similar to the unmod-

<table>
<thead>
<tr>
<th>ODN</th>
<th>Sequence</th>
<th>$t_{1/2}$ (min)</th>
<th>Relative $t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODN-1 (control)</td>
<td>5'-TATGTATTTTATCTGT-3'</td>
<td>4.9</td>
<td>1</td>
</tr>
<tr>
<td>ODN-2 (Umb)</td>
<td>5'-TAUmbGAUmbTTUmbTAUmbCTGUmb-3'</td>
<td>5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>ODN-3 ($8b$)</td>
<td>5'-TAbGTA8bTT8bTA8bCTG8b-3'</td>
<td>660</td>
<td>160</td>
</tr>
<tr>
<td>ODN-4 ($23$)</td>
<td>5'-TAT23GTA23TT23TA23CTG23-3'</td>
<td>1200</td>
<td>230</td>
</tr>
<tr>
<td>ODN-5 ($Ac8b$)</td>
<td>5'-TAAc8bGTAAc8bTTAc8bTAAc8bCTG Ac8b-3'</td>
<td>504</td>
<td>100</td>
</tr>
<tr>
<td>ODN-6 ($Ac23$)</td>
<td>5'-TAAc23GTAAc23TTAc23TAAc23CTG Ac23-3'</td>
<td>840</td>
<td>170</td>
</tr>
</tbody>
</table>

*Each ODN labeled with $^{32}$P at the 5'-end (10 pmol) was incubated with SVPD (20 ng) in the presence of Torula RNA (0.15 OD units at 260 nm) in a buffer containing 37.5 mM Tris-HCl (pH 8.0), 8 mM MgCl$_2$, and 5 mM DTT (total 20 mL) at 37 °C. At appropriate periods, aliquots of the reaction mixture were separated and added to a solution of EDTA (5mM, 10 mL), then mixture were heated for 3 min at 90 °C. The solutions were analyzed by electrophoresis on 20% polyacrylamide gel containing 7 M urea.

$^{2'}$O-Methyluridine.

### Table 1. Half-lives and relative half-lives of ODNs treated with SVPD.

<table>
<thead>
<tr>
<th>ODN</th>
<th>Sequence</th>
<th>$t_{1/2}$ (min)</th>
<th>Relative $t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODN-1 (control)</td>
<td>5'-TATGTATTTTATCTGT-3'</td>
<td>4.9</td>
<td>1</td>
</tr>
<tr>
<td>ODN-2 (Um)</td>
<td>5'-TAUmbGAUmbTTUmbTAUmbCTGUmb-3'</td>
<td>5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>ODN-3 ($8b$)</td>
<td>5'-TAbGTA8bTT8bTA8bCTG8b-3'</td>
<td>660</td>
<td>160</td>
</tr>
<tr>
<td>ODN-4 ($23$)</td>
<td>5'-TAT23GTA23TT23TA23CTG23-3'</td>
<td>1200</td>
<td>230</td>
</tr>
<tr>
<td>ODN-5 ($Ac8b$)</td>
<td>5'-TAAc8bGTAAc8bTTAc8bTAAc8bCTG Ac8b-3'</td>
<td>504</td>
<td>100</td>
</tr>
<tr>
<td>ODN-6 ($Ac23$)</td>
<td>5'-TAAc23GTAAc23TTAc23TAAc23CTG Ac23-3'</td>
<td>840</td>
<td>170</td>
</tr>
</tbody>
</table>

*Each ODN labeled with $^{32}$P at the 5'-end (10 pmol) was incubated with DNase I (10 or 5 units) in the presence of Torula RNA (0.26 OD units at 260 nm) in a buffer containing 40 mM Tris-HCl (pH 7.5) and 5 mM MgCl$_2$ (total 20 mL) at 37 °C. At appropriate periods, aliquots of the reaction mixture were separated and added to a solution of EDTA (5mM, 10 mL), then mixture were heated for 3 min at 90 °C. The solutions were analyzed by gel electrophoresis as described for Table 1.

$^{2'}$O-Methyluridine.

### Table 2. Half-lives and relative half-lives of ODNs treated with DNase I.
ified duplex except for the presence of the aminohexylcarbamoyl side chains. In the structure of the 23a-duplex, the terminal ammonium ion at the 8-position interacts with the phosphate anions of the neighboring duplex. However, another terminal ammonium ion at the 20-position interacts with the phosphate anions at the 19-position in the same strand of the duplex, the hydrogen bond distance being 2.6 Å, as shown in Figures 4 and 5. In the 8b-duplex, the ammonium ion at the 8-position interacts with the neighboring duplex in a similar way, but the ammonium nitrogen atom and the following three methylene carbon atoms at the 20-position can not be seen on electron density map. In this case, the terminal half of the aminohexylcarbamoyl side chain may be disordered to interact electrostatically around the phosphate group of the 19-position (see broken lines in Figures 4 and 5). Here it is interesting to note that the only differences are the sugar puckers found at the modified positions between the two duplexes. In the 23a-duplex, the modified nucleotide contains a methoxyl group attached to the C2' atom. This substitution induces the 3'-endo conformation on the ribose ring, as shown in Figure 5. Therefore, it is reasonable that the long chain of the aminohexylcarbamoyl group is stabilized in conformation to interact with the phosphate group at the terminal ammonium ion. The base-pair structures containing the modified residues are presented in Figure 5. It is clear that the carbamoyl moieties in both duplexes are fixed to form a 6-membered ring through a hydrogen bond between the carbamoyl N-H and the 4-carbonyl group. The sugar puckers of 8b and 23a in the duplexes are C2'-endo and C3'-endo conformations, respectively, as expected. Therefore, the minor groove of the 23a-duplex is slightly wider at the 8- and 20-positions than that of the 8b-duplex, as seen in Figure 3. Water molecules surrounding A-5: 8b-20 and A-5: 23a-20 base-pairs are similar to those found in the minor groove of A:T pairs (see Figure 5). We are now solving the crystal structures composed of DNA containing 23a and RNA, the structures of which will reveal how the terminal ammonium ion interacts with the phosphate anions of the RNA strand.

1.4. Modification at other positions of the nucleobases

Schmid and Behr developed a new post-synthetic method using 9-(2-fluoro-O-6-p-nitrophenylethoxy)purine 2'-deoxyriboside (24) (Figure 6) as a convertible nucleoside unit (Schmid and Behr, 1995; Potier et al., 2000). Using this convertible ON, the 11-mer ON containing 2'-deoxyinosine (25) (Figure 6) bearing a spermine at the 2-position, which would be accommodated in the minor groove in the duplex, was prepared. Thermal stability of the duplex consisting of 11-mer ON containing 2 residues of 25 was examined to increase its stability by +3.0 °C per modification (0.1 M NaCl, pH 7.4). Using the same method, Shinozuka et al. prepared 15-mer ONs containing 2-[N-2-[N,N-bis(2-aminoethyl)amino]ethyl]-2'-deoxyinosine (26a) and 2-[N-3-[N,N-bis(3-aminopropyl)amino]propyl]-2'-deoxyinosine (26b) (Shinozuka et al., 2002) (Figure 6). The ONs containing 26a or 26b also stabilized DNA-DNA duplexes by +2.7 ~ +3.5 °C (0.1 M NaCl, pH 7.4). The 5'-side phosphodiester bonds of 26ab were shown to be resistant to the hydrolysis by nuclease P1 (an endonuclease). Ganesh et al. prepared 18-mer ONs containing spermine conjugated to 2'-deoxy-5-methylcytidine (27) (Figure 6) at the N4-position (Prakash et al., 1994; Barawkar et al., 1994). The ON containing 27 showed stable formation of a triplex with a duplex ON under physiological conditions (Tm = 25 ~ 40 °C, 0.1 M NaCl, pH 7.3), while its duplex formation was destabilized by −4.5 ~ −5.0 °C per modification (0.1 M NaCl, pH 7.0).
Thermal stability and nuclease-resistance of oligonucleotides

Figure 3. Sequence numbering (a), overall structures of the 8b-duplex (b) and the 23a-duplex (c). X is 8b or 23a.

Figure 4. Interaction between the terminal ammonium ion of 23a at the 20th position and the phosphate group at the 19th position in the same strand of the duplex. The hydrogen bond distance is in Å.
A tricyclic cytosine analogue 28b (Figure 6) bearing a 2-aminoethoxy group at the 9-position of the phenoxazine ("G-clamp") greatly increased thermal stability of the ONs to their complementary target DNA or RNA strands. The $\Delta Tm$ value relative to 5-methylcytosine in 10-mer 5'-TCTC$X$CTCTC-3', where $X$ is 28b, was +18.0 °C, while that of a duplex containing phenoxazine 2'-deoxyriboside 28a was +6.5 °C in a 0.14 M KCl buffer at pH 7.2 (Lin and Matteucci, 1998; Lin et al., 1995). This increased affinity of 28b is presumably considered to be due to the combination of extended base stacking and an additional specific hydrogen bond. Introduction of one residue of 28b at the 3’-terminus completely protected the ONs by 3’-exonuclease, such as SVPD and bovine intestinal mucosal phosphodiesterase (BIPD) (Maier et al., 2002). Moreover, incorporation of a single 28b into a previously optimized 20-mer phosphorothioate (PS) antisense ON targeting c-raf increased the potency of the unmodified PS ON 25-fold (Flanagan et al., 1999). Therefore, a detailed structural analysis of an ON containing 28b is required before further developments can be made.

2. Synthesis and properties of ONs containing an aminoalkyl side chain at the sugar moiety

2.1. Aminoalkyl-conjugation at the 1’-C-position

Since the 1’-position of the sugar moiety in a duplex is in the center of the minor groove, the aminoalkyl side chains may not sterically disorder the duplex formation, especially in the minor groove of DNA-RNA heteroduplexes since the minor grooves of the heteroduplexes are wider than those of DNA-DNA duplexes (Saenger, 1984). The synthesis of the amidite unit 34 is shown in Scheme 2 (Dan et al., 1993; Ono et al., 1993). Tri-O-benzoyl-1’-hydroxymethyl-O2,2’-anhydouridine 29 (Holy, 1974) was converted into its 2’-deoxy derivative 31. The 1’-hydroxymethyl group in 31 was further converted into a carbonylimidazoyl derivative, which was conjugated with diaminobutane or diaminohexane to give deoxyuridine analogues carrying aminoalkyl side chains at the 1’-position. After the amine function was protected with an Fmoc group, the TIPDS group was
deprotected, and the nucleosides were converted into the corresponding nucleoside 3’-phosphoramidites \textit{35a} and \textit{35b}.

Duplexes consisting of the ONs (T16) carrying aminoalkyl side chains and a complementary ribonucleotide (poly rA) were slightly less stable (−1 °C per modification, 0.05 M NaCl, pH 7) than an unmodified parent duplex, but the duplexes consisting of the ONs and a complementary dA16 were much more unstable (−7.5 ~ −8.5 °C per modification). The ONs carrying the aminoalkyl side chains were more resistant to nuclease P1 and SVPD than an unmodified ON. The susceptibility of the 3’-side phosphodiester bond of the modified nucleoside by the action of SVPD and both 3’- and 5’-side phosphodiester bonds by that of nuclease P1 was suppressed.

2.2. Aminoalkyl-conjugation at the 2’-O-position

As alternative forms of zwitterionic ONs where a cationic group is introduced into the minor groove of the duplex, derivatization of the ribose moiety at the 2’-O-position has been studied extensively and systematically (Figure 7).

ONs containing the 2’-O-(3-aminopropyl)(AP)-substituted ribonucleosides \textit{36}, which were directly synthesized via alkylation of ribonucleosides with N-(3-bromopropyl)phthalimide in the presence of NaH, have been prepared using conventional phosphoramidite chemistry (Griffey \textit{et al.}, 1996). Thermal stability of DNA-RNA duplexes, where \textit{AP}-nucleosides are incorporated into the DNA strand, did not show clear results. \( \Delta Tm \) values per modification varied −1.78 ~ +1.13 °C depending on the sequences, lengths, and numbers incorporated and are very similar to those of duplexes containing the corresponding 2’-O-propyl-substituted ribonucleosides (\( \Delta Tm \) values were −1.14 ~ +0.84 °C). However, fully \textit{AP}-substituted ONs (11- and 19-mer RNA) were
A Matsuda, et al.

Scheme 2. Synthetic route of the nucleoside analog possessing an amino linker at the 1'-position.

1. N,N'-thiocarbonyldiimidazole, DMF
2. Bu₃SnH, AIBN, toluene, 92% (2 steps)
3. NaOMe, MeOH
4. TIPDSCl₂, 57% (2 steps)

1. Bu₄NF, THF, 98%
2. DMTrCl, pyridine, 83%
3. NaOMe, MeOH
4. TIPDSCl₂, 57% (2 steps)

a series : n = 4, b series : n = 6

33: R₁ = R₂ = H
34: R₁ = DMTr, R₂ = Fmoc
35: R₁ = Fmoc
Thermal stability and nuclease-resistance of oligonucleotides

563

slightly stabilized by +0.4 °C per modification compared to DNA, but slightly destabilized compared to ONs containing 2'-O-methyl and 2'-O-propyl modified nucleosides. Sugar puckering of an AP-uridine moiety (P) in 5-mer ON, 5'-ACTPC-3' was characterized by NMR to be a C3'-endo conformation. The 3-aminopropyl group rotates freely and is not bound electrostatically to any phosphate anion. ONs containing 2 continuous AP-uridine at the 3'-terminus showed a 100-fold increase in resistance to SVPD digestion, which is greater than that observed for phosphorothioate ONs.

Triple helix formation (T·AT triplex) of ONs containing 2'-O-(2-aminoethyl (AE)-5-methyluridine (37) as a third strand was compared to ONs containing 2'-O-AP and several other 2'-O-alkyl congeners to determine the proper length of the alkyl chain at the 2'-O-position (Cuenoud et al., 1998). The rank order of the stabilizing effect of the 2'-O-substituents to increase ΔTm values (°C/modification) for the triple helix formation at pH 7.0 in 5'-tttttCTCTCTCT-3' as the third strand, where t is 2'-O-substituted 5-methyluridine derivatives, was: 2'-O-(2-AE) (+3.5 °C) > 2'-O-(N-methyl-2-AE) (+3.4 °C) > 2'-O-(3-AP) (+2.1 °C) > 2'-O-(2-hydroxyethyl) (+1.9 °C) > 2'-O-methyl (+0.6 °C) > 2'-O-(2-methoxyethyl) (+0.5 °C) derivatives. This data together with a solution conformation analysis by NMR, indicated that the protonated aminoethyl side chain is ideally positioned by gauche effect to interact specifically with the pro-R oxygen of the nearby phosphate group (Blommers et al., 1998). Measurement of hybridization kinetics by real-time surface plasmon resonance revealed that the association rate constant of the fully AE-modified 5-methyluridine and 5-methylcytidine containing ON was more than a 1000 times greater than that of the unmodified ON control. The equilibrium dissociation constants for the above modified ON is 46000-fold smaller than that obtained with the unmodified control. Using AE-modified triplex forming ONs (TFOs), the hypoxanthine-guanine phosphoribosyltransferase gene knockout activity in CHO cells resulted in TFOs with lesser amounts of the AE-modification forming the most stable triplexes in cells and displaying the highest activity (Puri et al., 2001).

As antisense molecules, the properties of the 2'-O-(2-aminoxy)ethyl ribonucleosides 38 (Salo et al., 1999) and their N,N-dimethyl and N,N-diethyl derivatives 39 and 40, respectively, containing ONs (Prakash et al., 2000; Prakash et al., 2002) have been studied to improve upon the 2'-O-(2-methoxyethyl)-modified ONs. Thermal stability of the ONs containing 39 or 40 with complementary RNA showed a duplex stabilization of +1.1 ~ +1.5 °C per modification or +1.0 ~ +1.5 °C per modification, respectively, as compared to the unmodified DNA. In contrast, hybridization of the above ONs bearing

---

**Figure 7.** Structures of nucleoside analogs possessing amino linkers at the 2'-OH positions.

- 36: R = (CH$_2$)$_3$NH$_2$
- 37: R = (CH$_2$)$_2$NH$_2$
- 38: R = (CH$_2$)$_2$ONH$_2$
- 39: R = (CH$_2$)$_2$ONMe$_2$
- 40: R = (CH$_2$)$_2$ONEt$_2$
- 41: R = CH$_2$C(O)NH$_2$
- 42: R = CH$_2$C(O)NHMe
- 43: R = CH$_2$C(O)NMe$_2$
- 44: R = CH$_2$C(O)NH(CH$_2$)$_2$NMe$_2$
39 or 40 with complementary DNA led to duplexes less stable than those formed with unmodified DNA (–0.9 and –1.1 °C per modification, respectively). Half-lives of T19 in which 4 continuous Ts at the 3'-terminus were replaced by 39 or 40, to SVPD digestion were more than 24 h, and the rank order of the resistance was: ONs containing 38 > 40 > 39 > 2'-O-(2-methoxyethoxy)-substituted ribonucleosides >> 2'-O-propyl-substituted ribonucleosides > unmodified 2'-deoxyribonucleosides.

Furthermore, the 2'-O-modified 5-methyluridine derivatives, including 2'-O-carbamoylmethyl 41, 2'-O-(N-methyl) carbamoylmethyl 42, 2'-O-(N,N-dimethyl) carbamoylmethyl 43, and 2'-O-[(N,N-dimethylamino)ethyl] carbamoylmethyl 44 derivatives, were designed to improve the antisense properties of 2'-O-(2-methoxyethyl)-modified ONs (Prakash et al., 2003). These modified ONs were found to have a high binding affinity to complementary RNA (+0.1 ~ +1.8 °C per modification), but not to DNA (–0.8 ~ –0.3 °C per modification). Thermal stabilizing effects of the 2'-modified ribonucleosides 42–44 for the 16-mer ONs containing 4-modified nucleosides separately as compared to the unmodified DNA were in the following order (per modification): 41 (+1.8 °C) > 42 (+1.7 °C) > 44 (+1.4 °C) > 43 (+1.2 °C). SVPD stability of T19, in which 4 continuous Ts at the 3'-terminus were replaced by the modified nucleosides decreased in the following order 44 > 42 > 43 > 41 > 2'-O-(2-methoxyethoxy)-substituted ribonucleosides > unmodified 2'-deoxyribonucleosides.

From these results, it can be seen that ONs containing an ammonium group tethered at the 2'-O-position with a ribo-configuration thermally stabilize DNA-RNA duplexes, but destabilize DNA-DNA duplexes. These differences would be related to the shape of the minor groove. Furthermore, incorporation of several residues of these modified ribonucleosides into the 3'-terminus of the ONs makes them resistant to hydrolysis by 3'-exonucleases such as SVPD.

2.3. Aminoalkyl-conjugation at the 4'-C-position

The structure of a complex between DNase I (an endonuclease) and d(GCGATCGC)2 has been solved (Lahm and Suck, 1991). DNase I is bound in the minor groove of the B-DNA duplex forming contacts in and along both sides of the minor groove extending over a total of six base-pairs. The structures of restriction endonuclease, such as Eco RI and Bam HI, have also been solved and reveal that the minor groove of a DNA duplex interacts with the enzymes (Newman et al., 1994). Therefore, the ability to prevent hydrolysis by endonucleases is necessary to introduce an aminoalkyl side chain at the 4'-position of the deoxyribose moiety. (Figure 8 and Scheme 3)

Wang and Seifert reported that ONs containing 4'-C-(aminomethyl)thymidine (45a) were more resistant to nucleolytic hydrolysis by SVPD than unmodified ONs, although the endonuclease-resistance property was not examined (Wang and Seifert, 1996). It would also be important to determine the proper length of the aminoalkyl side chain at the 4'-position to maximize potency. However, effective synthetic methods to introduce a 2-aminoethyl group at the 4'-position of thymidine have not been explored. We have developed new, radical chemistry with a vinylsilyl or an allylsilyl group as a temporary
radical acceptor tether to synthesize 4'-C-(2-aminoethyl)thymidine (45b) and 4'-C-(3-aminopropyl)thymidine (45c) (Shuto et al., 1998; Kanazaki et al., 2000; Shuto et al., 2002). As shown in Scheme 3, an intramolecular radical cyclization of the 4'-phenylseleno nucleosides 48 and 49, which have a dimethylvinylsilyl and a dimethylallylsilyl group at the 3'-hydroxy group respectively, with Bu3SnH/AIBN treatment and subsequent Tamao oxidation provided 5'-O-[dimethoxytrityl (DMTr)]-4'-C-(2-hydroxyethyl)thymidine (52) and 5'-O-DMTr-4'-C-(3-hydroxypropyl)thymidine (53). Compounds 52 and 53 were then converted into the 4'-C-(2-trifluoroacetamidoethyl)thymidine derivative 58 and the 4'-C-(3-trifluoroacetamidopropyl)thymidine derivative 59, which were phosphorylated to give the phosphoramidite units 60 and 61. A thymidine analogue 45d bearing a longer side chain length than 45b was also prepared (Ueno et al., 1998b).

The nucleosides 45a, 45b, 45c and 45d were incorporated into the 18-mer ONs. The ONs containing the modified nucleosides formed slightly more stable duplexes with complementary DNA than the corresponding unmodified ON. These ONs also formed stable duplexes with the complementary RNA with slightly reduced Tms. For the ∆Tm values for the DNA-DNA duplexes, the order of increasing thermal stability is: 45b (+0.6 ~ +1.1 °C per modification) > 45c (0 ~ +0.98 °C) > 45a (–0.4 ~ +0.8 °C) > 45d (–3.6 ~ +0.48), whereas the ∆Tm values for the DNA-RNA duplexes are: 45a (–0.55 ~ –0.3 °C) > 45b = 45c (–0.68 ~ –0.5 °C) > 45d (–1.1 ~ –0.5). 4'α-C-[N-(Palmitoyl), N-(oleoyl), and N-(cholesteryloxycarbonyl)aminoethyl]thymidine analogs were incorporated into ONs and their Tm values were measured. It was found that the lipophilic groups introduced thermally destabilize the DNA/DNA duplexes, but they do not influence the thermal stability of the DNA/RNA duplexes (Ueno et al., 2000a). ONs containing the above modified nucleosides 45b and 45c and also 46 in the third strand also thermally stabilized the triplex structure (Atsumi et al., 2002). The ONs containing the modified nucleosides were significantly resistant to nuclease hydrolysis by both SVPD and DNase I and were also very stable in PBS containing 50% human serum (Kanazaki et al., 2000). Half-lives of the hydrolysis by SVPD for 18-mer ONs containing 5 residues of the modified nucleosides were: the ONs containing 45a (2.9 h), 45b (14.4 h), 45c (17.8 h) and 45d (5.2 h), while the half-
Scheme 3. Synthetic route of nucleoside analogs possessing amino linkers at the 4'-positions.

47 48: n = 0; 95%
49: n = 1; 92%
50: n = 2; 87%
51: n = 3; 66%
52: n = 2; 70%
53: n = 3; 54%
54: n = 2; 83%
55: n = 3; 90%
56: n = 2; 86%
57: n = 3; 79%
58: n = 2; 73%
59: n = 3; 79%
60: n = 2; 91%
61: n = 3; 62%

Thermal stability and nuclease-resistance of oligonucleotides

life of the unmodified control ON was 2 min. Moreover, half-lives of the endo-hydrolysis by DNase I were: the ONs containing 45a (27 h), 45b (29 h), 45c (28 h), and 45d (13 h), while the half-life of the control was 20 min. On the other hand, acetylation of the terminal aminoethyl group in 45b to remove the positive charge increased the susceptibility by about 10-fold against DNase I. Therefore, this suggested that the effects of the terminal ammonium ions of the aminoalkyl side chains played an important role in nuclease resistance of the ONs, as expected.

2.4. Aminoalkyl-conjugation at the other position in the sugar moiety

Altmann and co-workers reported that thermal stability of DNA-RNA duplexes in which the DNA strands have the 6'-α-(5-aminobutoxy) derivative of the carbocyclic-thymidine 62 (Figure 9) was reduced by –1.1 ~ –1.9 °C per modification in a 0.1 M NaCl buffer at pH 7, although the ONs bearing 3 continuous 62 units at the 3'-endo position showed more than 24-fold increase in resistance than an unmodified ON in 10% heat-inactivated fetal calf serum (Altmann et al., 1995). On the other hand, since ONs containing carbocyclic-thymidine without further modifications thermally stabilized the duplexes with poly dA although the 3'-exonuclease resistance property was not enough for biological applications (Perbost et al., 1989; Sági et al., 1990), we thought that the reason for the decrease in the thermal stability of the duplexes containing 62 was due to the terminal ammonium ion not being in the proper position to interact with a phosphate anion in the back-bone. Therefore, we envisioned introducing an aminoalkyl side chain with a suitable length at the 6'-α-position of a carbocyclic-thymidine such as 6'-α-[N-(aminoalkyl)carbamoyloxy]carbocyclic-thymidines (63a, b, c) (Ueno et al., 2000b). If successful, we would then be able to synthesize the ONs that enhance the thermal stability of DNA-RNA duplexes and that are also resistant to both exo- and endonucleases. However, the ONs that contain the 6'-α-N-(aminoethyl) derivative 63a were found to enhance the thermal stability of the duplexes with the complementary DNA by +0.75 ~ +2.0 °C per modification in a 0.01 M NaCl buffer, while those containing the 6'-α-N-(aminobutyl) derivative 63b or the 6'-α-N-(aminohexyl) derivative 63c decreased the thermal stability of the DNA-DNA duplexes by –1.9 ~ –3.7 °C per modification. On the other hand, all DNAs that contained 63a, 63b, or 63c decreased the thermal stability of the DNA-RNA duplexes by –1.9 ~ –3.7 °C per modification in a 0.1 M NaCl buffer. The stability of these ONs bearing 63a-63c to nuclease hydrolysis by using SVPD and nuclease S1 was much more enhanced to both enzymes than to the unmodified ON.

As the third strand, the covalently linked spermine and the other polyamines through the phosphate group at the 5'-hydroxyl group of thymidine, such as 64, were attached at the 5' terminus of the 21-mer ONs to stabilize triplex formation in the major groove of the duplex DNA (Tung et al., 1993). The Tm value of this polyamine linked ON as the third strand for the triplex formation was 42 °C in 0.1 M NaCl at pH 6.5 (38 °C at pH 7.0 and 37 °C at pH 7.5) while no triplex formation was observed with an unmodified ON under the same conditions. Since mM levels of spermine are typically used for triplex formation, this conjugation was quite effective for a homopyrimidine-homopurine-homopyrimidine triplex formation.

The designed C-branched spermine was attached at the 2'-hydroxyl group or the 5'-hydroxyl group of arabinofuranosyluracil via a phosphodiester bond to give 65 and 66. ONs containing 65 and 66 were synthesized using standard phosphoramidite chemistry.
and their ability to stabilize DNA duplexes and triplexes was studied (Sund et al., 1996). When 1 residue of 65 or 66 was introduced into the 3'-terminus or the 5'-terminus of dT13, the duplex with dA26 was stabilized by +0.5 or +2.5 °C per modification, respectively, and the triplex with dT26 and dA26 was also stabilized by +6.5 or +2.5 °C per modification, respectively, at pH 7.0 over unmodified DNA. However, 65 at the center of dT13 decreased the Tm drastically (−28.5 °C per modification) and did not give triplex formation.

Conclusion
ONs bearing an aminoalkyl side chain have been extensively synthesized in order to study their effects on the thermal stability of duplexes (DNA-DNA and DNA-RNA) and triplexes, and their resistance properties against endo- and exonucleases, which are required for in vitro and in vivo applications of ONs not only as antisense and antigen, but also as ribozyme, aptamer, decoy, and siRNA. Although we cannot compare directly these effects of each of these modifications at the same level, since they have been studied under different conditions, we can nevertheless draw certain general conclusions. When an aminoalkyl side chain is introduced at the 5-position of uracil and cytosine bases but not at the N4-position of cytosine, in which the amino group is accommodated in the major groove of duplexes, the thermal stability of both DNA-DNA and DNA-RNA duplexes is increased because the terminal ammonium ion directly interacts with the nearby phosphate anions to neutralize the anion and to reduce phosphate anion-anion repulsion. To maximize the effect, fine-tuning of the length and the spatial position of the terminal amino group is necessary. If the stacking effect is added to a hydrogen-bonding effect,
Thermal stability and nuclease-resistance of oligonucleotides

569

Thermal stability is further increased as in the case of “G-clamp”. These modified ONs showed an enhanced resistance property against 3'-exonucleases such as SVPD and BIPD, but not endonucleases, such as DNase I and nuclease S1. On the other hand, when an aminoalkyl side chain is introduced, especially at the 1’- and 4’-positions of the 2'-deoxyribose, and the 6’α-position of the carbocyclic-nucleosides, in which the aminoalkyl group is accommodated in the minor groove, the thermal stability is decreased for the DNA-RNA duplexes, whereas the thermal stability of the DNA-DNA duplexes is dependent on the position; the 4’-modifications increase the thermal stability, but not the 1’-modifications. The C2-position-modification of hypoxanthine, which also faces the minor groove, increases the thermal stability of DNA-DNA duplexes, but DNA-RNA data have not been reported. However, the 2’-O-modifications show somewhat different results from those described above although the aminoalkyl group is also accommodated in the minor groove; the DNA-RNA duplexes are more thermally stabilized while the DNA-DNA duplexes are destabilized. These differences would be related to the shape and size of the minor groove between DNA-DNA and DNA-RNA duplexes. Introduction of the aminoalkyl side chains in the minor groove usually enhances nuclease stability towards both 3’-exo- and endonucleases. These data are consistent with the fact that endonucleases such as DNase I, RcoRI, and BamHI, are known to interact with a DNA duplex from its minor groove. However, the mechanism of 3’-exonuclease, such as SVPD is not yet fully understood.

Figure 10 shows the thermal stability and nuclease-resistance property of the ONs containing aminoalkyl-conjugated nucleosides at various positions, which have been prepared in our group. These data would be helpful to design further conjugations. Elucidations of a precise mode of the interaction between an ammonium group and a phosphate anion in duplexes and triplexes both in crystals and solutions are necessary to obtain metabolically stable and biologically active ONs.

![Chemical structures and tables showing thermal stability and nuclease-resistance properties of oligonucleotides containing nucleoside analogs with amino linkers.](image)

**Figure 10.** Thermal stability and nuclease-resistance properties of the oligonucleotides containing the nucleoside analogs with the amino linkers. +: high. -: low.
References


Thermal stability and nuclease-resistance of oligonucleotides


Thermal stability and nuclease-resistance of oligonucleotides


