Artificial Nucleic Acids

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Control of the Chirality and Helicity of Oligomers of Serinol Nucleic Acid (SNA) by Sequence Design**

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The construction of an artificial double helix that mimics natural DNA or RNA has been one of the most challenging endeavors in chemistry.[1–9] Recently, Meggers and co-workers showed that even a simple acyclic propylene glycol with two carbon atoms in the main chain (see (*S*)-GNA in Figure 1) as a nucleobase tether could form a more stable duplex than that of native DNA or RNA.[10–13] This pioneering work prompted us to synthesize a new foldamer with three carbon atoms in the main chain, acyclic threoninol nucleic acid (*a*TNA), from D-threoninol.[14] We found that *a*TNA has the following properties: 1) duplex formation involves complementary pairing in an antiparallel fashion, as for natural DNA or RNA; 2) owing to the flexibility of the backbone, the single-stranded state does not adopt a characteristic preorganized structure; 3) the thermal stability of the duplex is far greater than that of the natural DNA or RNA duplex and even higher than that of the GNA duplex. Studies on *a*TNA as well as GNA and peptide nucleic acid (PNA)[15,16] have confirmed that scaffold rigidity is not a prerequisite for stable duplex formation as previously thought. However, unlike PNA, with an acyclic scaffold, *a*TNA can not cross-hybridize with either natural DNA or natural RNA. Although A_{15} of (*S*)-GNA can hybridize with U_{15}, the incorporation of several GC pairs severely destabilizes the duplex with RNA.[11] Thus, there are no artificial nucleic acids comprising a fully acyclic backbone with a phosphodiester linkage that can cross-hybridize with DNA or RNA without sequence limitation. We hypothesize that the threoninol scaffold is still not flexible enough to form a duplex with natural DNA or RNA.

Herein, we propose a new artificial nucleic acid, serinol nucleic acid (SNA, see Figure 1a), with a 2-amino-1,3-propanediol (serinol) scaffold, which is even more flexible than threoninol. In comparison with *a*TNA (Figure 1a), the only structural difference is the lack of a methyl group next to the amino group. However, this small change affords the SNA oligomer a unique stereochemical property: since this methyl group provides chirality, its absence makes the scaffold achiral as well as flexible. Accordingly, the chirality of the "pure" SNA oligomer synthesized from four SNA monomers (or the helicity of its duplex) depends only on its sequence (see below). This property is specific to the SNA oligomer; DNA, RNA, and previously synthesized *a*TNA all have chirality (or helicity) that is inherently determined by the chirality of the scaffold.[17] In the present study, we first demonstrated this unique stereochemical property and then cross-hybridized the SNA oligomer, which was found to recognize both DNA and RNA sequence specifically.

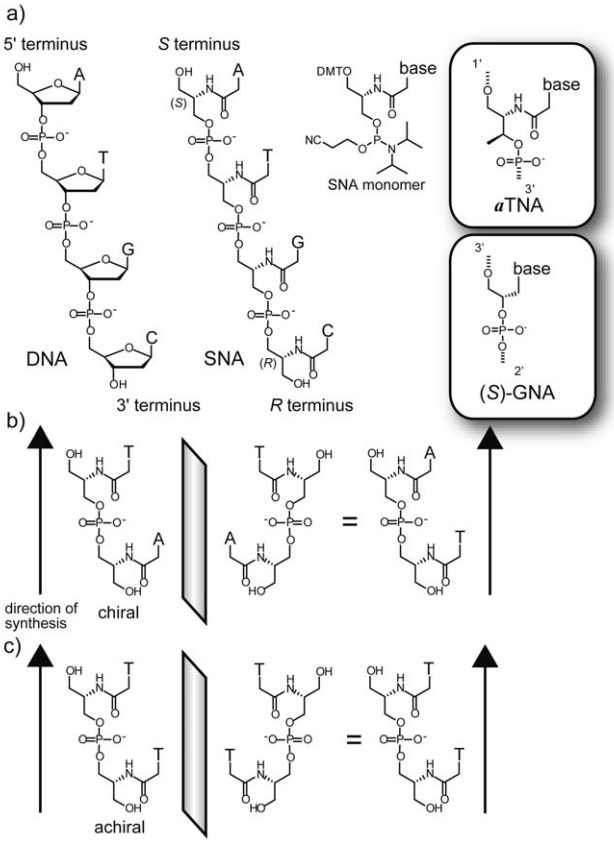

Figure 1. a) Chemical structures of DNA and SNA. *S* and *R* termini were named according to the chirality of the terminal residue. The SNA monomer for the DNA synthesizer is also shown (DMT = dimethoxytrityl). b) The mirror image of SNA with an asymmetrical sequence ((*S*)-AT-(*R*)) is identical to SNA with the reverse sequence ((*S*)-TA-(*R*)). c) SNA with a symmetrical sequence ((*S*)-TT-(*R*)) is identical to its mirror image.

The chemical structure of the SNA oligomer is shown in Figure 1a. Serinol (2-amino-1,3-propanediol), which, like DNA, has three carbon atoms in its backbone, is an achiral diol. However, modification of the two hydroxy groups with different functional groups to form an SNA monomer (Fig-

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ure 1a) results in a chiral monomer. We synthesized four chiral SNA phosphoramidite monomers by attaching four nucleobases (A, T, G, C) through the amide bond of L-serine[18–20] to avoid racemization, as described in the Supporting Information.[21–23] When these optically pure monomers were conjugated, the obtained SNA oligomers showed unique stereochemical properties, as depicted in Figure 1b,c. For example, the mirror image of a symmetrical oligomer, such as T→T, is identical to the original dimer; thus, an SNA oligomer with a symmetrical sequence is achiral (i.e., a meso compound). On the other hand, when an A→T dimer is synthesized from monomers, its mirror image does not coincide with the original enantiomer because the unsymmetrical A→T dimer is chiral. However, interestingly, its enantiomer is identical to the dimer of reversed sequence, T→A. More specifically, the chirality of the oligomer can be exactly inverted by reversing the sequence of the chiral SNA monomers: two enantiomers can be synthesized from the same chiral monomers by programming the sequences correctly, but not from their enantiomeric monomers.[24] These unique properties of SNA might provide insight into the selection of D-ribose as a scaffold for nucleic acids.

Sequences of SNA oligomers are shown in Figure 2. *R* and *S* termini are named according to the chirality of the terminal residues: the terminal residue incorporated in the first step of oligomer synthesis has the *R* configuration, whereas the other terminal residue incorporated in the last step of synthesis has the *S* configuration. **S1** is a random 8-mer SNA oligomer, and **S2** is its complementary strand in an antiparallel orientation. **S4**, which has the reverse sequence of **S1**, is the enantiomer of **S1**. Similarly, **S3** is the enantiomer of **S2**. Accordingly, the **S3**/**S4** duplex is the enantiomer of the **S1**/**S2** duplex, and the helicity of the duplex should be inverted. Furthermore, the two enantiomeric duplexes **S1**/**S3** and **S2**/**S4** are parallel duplexes. On the other hand, the duplex composed of **S5** and **S6**, which have symmetrical, complementary sequences, is achiral.

First, we measured the melting temperatures (T_m) of these duplexes (Figure 3). The **S1**/**S2** duplex showed a typical sigmoidal curve, whereas single-stranded **S1** and **S2** did not show any transition above 20 °C. A Job plot of absorbance at 260 nm revealed that **S1** and **S2** formed a 1:1 complex (Figure 3a, inset). Furthermore, the CD spectrum of **S1**/**S2** was different from the sum of the spectra of single-stranded **S1** and **S2** (Figure 3b).[25] These results clearly demonstrate that **S1** and **S2** form a duplex. The T_m value of the duplex was determined to be 51.1 °C (Table 1), which is significantly higher than that of the corresponding DNA (**D1**/**D2**: 29.0 °C) and RNA duplexes (**R1**/**R2**: 38.9 °C). Thus, the SNA duplex was much more stable than the DNA and RNA duplexes.[26] Because the **S3**/**S4** duplex is the enantiomer of **S1**/**S2**, the T_m value of **S3**/**S4** (51.2 °C) was the same as that of **S1**/**S2** (51.1 °C; Table 1 and Figure 3c). We also found that the parallel combination of **S1**/**S3** showed a typical sigmoidal curve, and we determined its T_m value to be 15.9 °C. This T_m value is about 35 °C lower than that of the antiparallel **S1**/**S2** duplex. Thus, the SNA oligomer strongly recognized antiparallel complementary strands in the same way that DNA and RNA do. We further examined the sequence specificity of the SNA oligomer. The incorporation of one base mismatch (T–T mismatch) into the SNA duplex (**S1**/**S7**) lowered its T_m value to 38.7 °C,

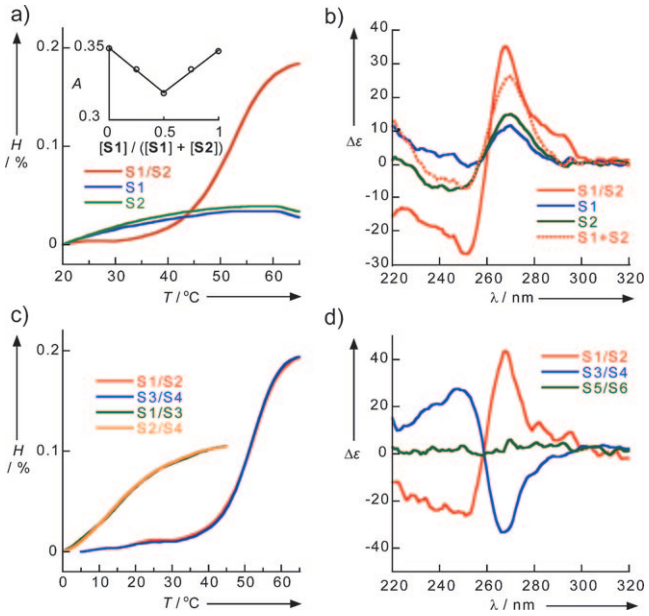

Figure 3. a) Melting profiles of **S1**/**S2** and single-stranded **S1** and **S2**. Inset: Job plot of the hybridization between **S1** and **S2**. b) CD spectra at 20 °C of **S1**/**S2**, single-stranded **S1**, and single-stranded **S2**, and the sum of the spectra of **S1** and **S2**. c) Melting profiles of antiparallel duplexes (**S1**/**S2**, **S3**/**S4**) and parallel duplexes (**S1**/**S3**, **S2**/**S4**). d) CD spectra of duplexes **S1**/**S2**, **S3**/**S4**, and **S5**/**S6** at 20 °C. *H* is hyperchromicity; $\Delta\varepsilon$ is the molar circular dichroism per duplex ($L\,mol^{-1}\,cm^{-1}$). Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), [oligonucleotide] = 2.0 μM (for melting profiles) or 4.0 μM (for CD measurements).

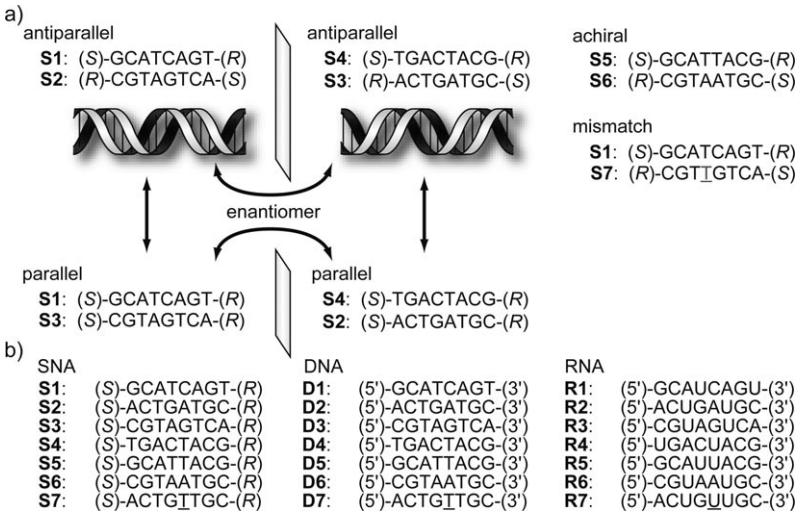

Figure 2. a) Schematic illustration of the relationship between SNA duplexes. b) Sequences of SNA, DNA, and RNA.

Table 1: Melting temperatures of SNA, DNA, and RNA duplexes.

Sequence	Direction of duplex	T_m [°C][a] SNA	DNA	RNA	SNA/DNA	SNA/RNA
1/2	antiparallel	51.1 (**S1/S2**)	29.0 (**D1/D2**)	38.9 (**R1/R2**)	23.5 (**S1/D2**)	35.0 (**S1/R2**)
					21.2 (**D1/S2**)	32.2 (**R1/S2**)
3/4	antiparallel	51.2 (**S3/S4**)	23.1 (**D3/D4**)	37.9 (**R3/R4**)	21.4 (**D3/S4**)	33.3 (**R3/S4**)
					20.0 (**S3/D4**)	36.2 (**S3/R4**)
1/3	parallel	15.9 (**S1/S3**)	<10 (**D1/D3**)	<10 (**R1/R3**)	<10 (**S1/D3**)	<10 (**S1/R3**)
					<10 (**D1/S3**)	<10 (**R1/S3**)
2/4	parallel	15.9 (**S2/S4**)	<10 (**D2/D4**)	<10 (**R2/R4**)	<10 (**D2/S4**)	<10 (**R2/S4**)
					<10 (**S2/D4**)	<10 (**S2/R4**)
5/6	–	47.0 (**S5/S6**)	28.1 (**D5/D6**)	34.3 (**R5/R6**)	19.1 (**S5/D6**)	30.3 (**S5/R6**)
					18.5 (**D5/S6**)	26.3 (**R5/S6**)
1/7	antiparallel	38.7 (**S1/S7**)	<10 (**D1/D7**)	23.1 (**R1/R7**)	<10 (**D1/S7**)	14.5 (**R1/S7**)

[a] Conditions: [oligonucleotide] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

which is 12 °C lower than that of fully matched **S1**/**S2**. The decrease in T_m was comparable to that observed for the DNA and RNA duplexes, which indicates that the bases in the SNA oligomer formed Watson–Crick base pairs and recognized complementary nucleobases, as in DNA and RNA.

We measured the CD spectra of the duplexes to evaluate their helicity (Figure 3d). **S1**/**S2** showed symmetrical positive and negative Cotton effects, which indicated that this duplex formed a right-handed helix. As expected, **S3**/**S4** (the enantiomer of **S1**/**S2**) with its reversed sequence showed exactly inverse CD signals: negative and positive Cotton effects appeared at around 260 nm. On the other hand, **S5**/**S6**, which has symmetrical sequences, showed no induced CD, because the **S5**/**S6** duplex is achiral. Thus, the helicity of the SNA duplex can be modulated by the sequence. Since the symmetrical SNA duplex **S5**/**S6**, which does not exhibit helicity, showed a similar T_m value (47.0 °C) to that of chiral SNA (**S1**/**S2**: 51.1 °C), the helical preference (i.e., helical preorganization) of the corresponding single strands did not strongly affect the stability of the SNA duplexes. Although an SNA monomer has a simple and symmetrical structure, SNA has the prerequisites for a genetic carrier: SNA oligomers can form a highly stable duplex (considerably more stable than DNA or RNA duplexes) with an antiparallel orientation and also specifically recognize the complementary SNA sequence. However, the conformation of the SNA duplex is significantly sequence-dependent, which might be a severe disadvantage for recognition by proteins and other biomolecules. We believe that one the reasons for the selection of ribose as the scaffold for DNA and RNA is that the conformation of the resulting duplexes is not sequence-dependent.

Next, we investigated the cross-hybridization ability of SNA with DNA or RNA. As mentioned above, both GNA and *a*TNA could not hybridize with DNA or RNA.[14] Surprisingly, however, SNA can form a heteroduplex with DNA and RNA: the **S1**/**D2** duplex showed a sigmoidal melting curve (Figure 4a), unlike single-stranded **S1** or **D2**. Furthermore, the CD spectrum of **S1**/**D2** was completely different from the sum of the spectra of single-stranded **S1** and **D2** (Figure 4b). These results demonstrate that the SNA oligomer can hybridize with DNA. The T_m value of **S1**/**D2** was determined to be 23.5 °C, which is comparable to that of the corresponding native duplex **D1**/**D2** (29.0 °C). Similarly, the melting profile and CD spectrum of **S1**/**R2** revealed that the SNA oligomer could also form a duplex with RNA: the T_m value of **S1**/**R2** was 35.0 °C, which is also comparable to that of **R1**/**R2** (38.9 °C). The T_m value of **S1**/**R2** was even higher than that of **D1**/**R2** (27.3 °C), which indicates the potential of SNA as an antisense agent. On the other hand, **S4**, the enantiomer of **S1**, hybridized with neither **D2** nor **R2** (see Table 1). On the basis of these results, we regarded the *S* and *R* termini of the SNA oligomer as equivalent to the 5′ and 3′ termini of natural oligonucleotides with a ribose scaffold, respectively. Interestingly, both **S3** and **S4** formed a stable duplex with DNA and RNA in an "antiparallel" fashion,

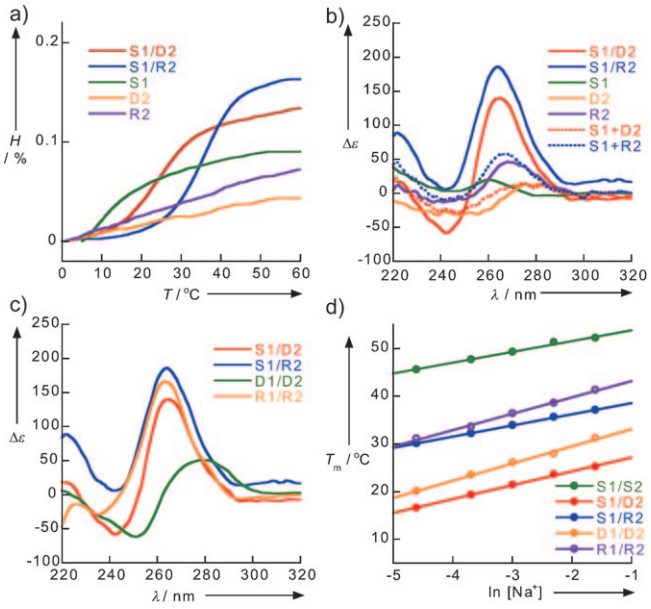

Figure 4. a) Melting profiles of **S1**/**D2**, **S1**/**R2**, and single-stranded **S1**, **D2**, and **R2**. b) Comparison of the CD spectra at 0 °C of **S1**/**D2** and **S1**/**R2** with those of single-stranded **S1**, **D2**, and **R2**, and the sum of the spectra of **S1** and **D2**, and **S1** and **R2**. c) Comparison of the CD spectra at 0 °C of SNA–DNA (**S1**/**D2**), SNA–RNA (**S1**/**R2**), DNA (**D1**/**D2**), and RNA (**R1**/**R2**) duplexes. d) Dependence of the T_m values of **S1**/**S2**, **S1**/**D2**, **S1**/**R2**, **D1**/**D2**, and **R1**/**R2** on salt concentration. Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), [oligonucleotide] = 2.0 μM (for melting profiles) or 1.0 or 4.0 μM (for CD measurements).

although the **S3**/**S4** duplex formed a left-handed helix (see **3**/**4** in Table 1). The achiral SNA oligomers **S5** and **S6** also formed duplexes both with DNA and RNA (see **5**/**6** in Table 1). Accordingly, the helical preference of the SNA oligomer in the single-stranded state did not much affect its ability to recognize DNA or RNA. CD spectra of the **S1**/**D2** and **S1**/**R2** duplexes are shown in Figure 4 b,c. The exciton couplet shows that these heteroduplexes form a right-handed helix. Since these CD spectra were similar to that of **R1**/**R2**, rather than that of **D1**/**D2**, we think that SNA–DNA and SNA–RNA duplexes both form an A-form-like structure.

Finally, we determined thermodynamic parameters for **S1**/**S2** as well as for cross-hybridized **S1**/**D2** and **S1**/**R2** (see Table S2 in the Supporting Information). **S1**/**S2** was 5.6 and 3.8 kcal mol^{-1} more stable than **D1**/**D2** and **R1**/**R2**, respectively, in terms of the $\Delta G°_{37}$ values of these duplexes. The ΔH value of **S1**/**S2** was more negative than those of **D1**/**D2** and **R1**/**R2**, and the ΔS value was also more negative. These results indicate that the SNA duplex is stabilized by a larger enthalpy change. Since the dependence of the T_m value of **S1**/**S2** on the salt concentration was smaller than that of **D1**/**D2** and **R1**/**R2** (Figure 4 d: the slope of the plot was greater for **D1**/**D2** and **R1**/**R2** (at 3.6 and 3.4, respectively) than for **S1**/**S2** (2.3)), the high stability of the SNA duplex is partly attributable to the decrease in electrostatic repulsion between phosphate anions.[27,28] The SNA monomer has three atoms between the backbone and the base, whereas DNA and RNA have only two: the one-atom-longer interior linker should expand the helix radius and lessen the electrostatic repulsion between phosphate groups. The dependence of the T_m values of **S1**/**D2** and **S1**/**R2** on salt concentration was also weaker than that observed for the DNA and RNA duplexes. This observation indicates that decreased electrostatic repulsion as well as the flexibility of the serinol scaffold also contribute to the stabilities of the SNA–DNA and SNA–RNA duplexes. In previous studies, the incorporation of SNA monomers into DNA destabilized the duplex.[18–20] This destabilization might reflect the structural difference between SNA and natural nucleic acids. On the other hand, the oligomer containing only SNA monomers could hybridize with DNA and RNA, in contrast to GNA and *a*TNA. As we had intended with our design, the flexible structure of the serinol scaffold facilitated cross-hybridization with natural oligonucleotides by relaxing the structural difference.

In conclusion, we synthesized a unique artificial oligonucleotide, the SNA oligomer. The helicity of the SNA duplex could be controlled by appropriate design of the SNA-oligomer sequence. The SNA duplex showed remarkably high stability in comparison with DNA and RNA, partly as a result of the decrease in electrostatic repulsion. Furthermore, the SNA oligomer sequence-specifically recognized its complementary sequence in an antiparallel fashion with sufficient thermal stability. SNA is the first example of an artificial nucleic acid composed of a fully acyclic scaffold with a phosphodiester linkage that can cross-hybridize with DNA and RNA irrespective of the sequence. SNA oligomers may be useful as biological tools, such as antigene/antisense agents, and may find application in the development of nanomaterials.

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