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# Unexpectedly Stable Artificial Duplex from Flexible Acyclic Threoninol

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***Abstract:*** A new foldamer, *a*cyclic *t*hreoninol *n*ucleic *a*cid (*a*TNA), has been synthesized by tethering each of the genetic nucleobases A, G, C, and T to D-threoninol molecules, which were then incorporated as building blocks into a scaffold bearing phosphodiester linkages. We found that with its fully complementary strand in an antiparallel fashion, the *a*TNA oligomer forms an exceptionally stable duplex that is far more stable than corresponding DNA or RNA duplexes, even though single-stranded *a*TNA is rather flexible and thus does not take a preorganized structure.

A key feature of natural DNA is its spontaneous hybridization of two strands that are complementary with each other in an antiparallel fashion. Although nature uses ribose or deoxyribose as a scaffold for nucleic acids to carry genetic codes, investigation of DNA hybridization has revealed that deoxyribose is not a prerequisite for this supramolecular property.[1] Recently, surprising results were reported by Meggers et al.,[2] who demonstrated that the glycol nucleic acid (GNA: see Figure 1a) bearing phosphodiester linkages consisting of artificial oligonucleotides synthesized from an acyclic propylene glycol forms a more stable duplex in an antiparallel fashion than does natural DNA. This result proved that simple acyclic diols can form excellent scaffolds for new "foldamers" that can spontaneously be folded into a double-helical structure in a programmed manner.[3]

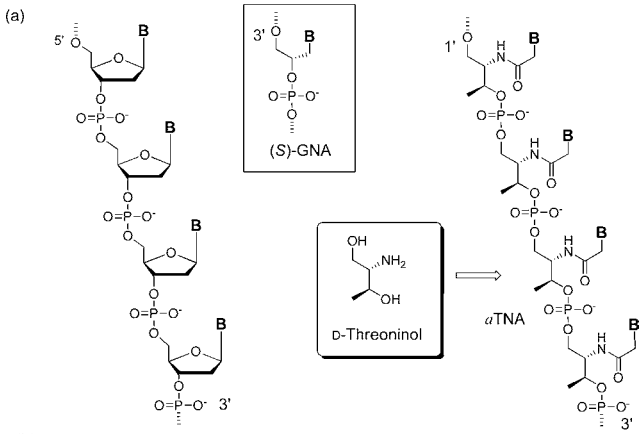


***Figure 1.*** (a) Structures of natural DNA and *a*TNA synthesized from D-threoninol. (b) Sequences of oligonucleotides synthesized in this study.

Over the past decade, we have developed unique base surrogates based on an acyclic compound with three carbons in its main chain (2-amino-1,3-butanediol, known as threoninol) to covalently incorporate various functional dyes into natural DNA.[4] These base surrogates derived from D-threoninol can be incorporated into natural DNA without destabilizing the duplex.[5] The high compatibility of D-threoninol with natural DNA as well as the stable GNA duplex prompted us to design a new foldamer with this more flexible acyclic scaffold, threoninol.

Herein we propose a new foldamer, *a*cyclic *t*hreoninol *n*ucleic *a*cid (*a*TNA),[6] prepared using D-threoninol as building block tethered to one of the genetic nucleobases A, G, C, and T. Although there have been several reports of modified DNA involving several "thymidine" *a*TNAs,[7] a fully changed *a*TNA oligomer has not been synthesized to date.[8] We found that the *a*TNA oligomer forms an exceptionally stable duplex in an antiparallel fashion with its fully complementary strand.

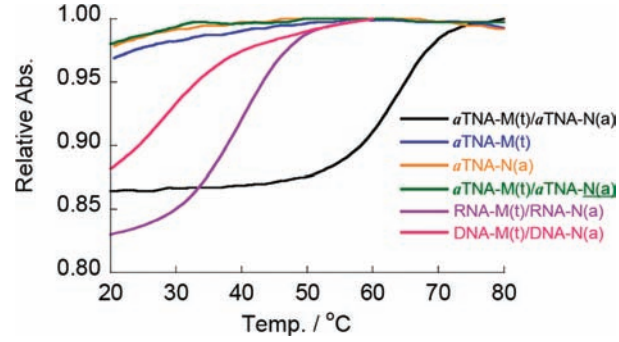


***Figure 2.*** Melting curves of antiparallel ***a*TNA-M(t)/*a*TNA-N(a)** (black), single-stranded ***a*TNA-M(t)** (blue) and ***a*TNA-N(a)** (yellow), parallel ***a*TNA-M(t)/*a*TNA-N(a)** (green), native **DNA-M(t)/DNA-N(a)** (magenta), and native **RNA-M(t)/RNA-N(a)** (purple). Conditions: [oligonucleotide] = 2 $\mu$M, [NaCl] = 100 mM, 10 mM phosphate buffer (pH 7.0).

All of the *a*TNA oligomers listed in Figure 1b were synthesized from the corresponding phosphoramidite monomers (see Scheme S1 in the Supporting Information). The melting profiles of *a*TNAs were first examined by analyzing the change of absorbance at 260 nm with temperature. As shown by the solid black line in Figure 2, the 1:1 mixture of 8-mer ***a*TNA-M(t)** and ***a*TNA-N(a)**, which are complementary to each other in an antiparallel fashion, exhibited a typical sigmoidal curve. The melting temperature ($T_m$) was determined to be as high as 62.7 °C, which is remarkably higher than that measured for the corresponding natural DNA (29.0 °C; magenta line in Figure 2) and RNA duplex (38.9 °C; purple line). In contrast, neither ***a*TNA-M(t)** nor ***a*TNA-N(a)** in a single-stranded state showed such a sigmoidal curve (blue and orange lines) above 20 °C. Furthermore, a parallel combination of complementary ***a*TNA-M(t)** and ***a*TNA-N(a)** also did not display sigmoidal melting (green line). These results demonstrate that two complementary *a*TNAs could form a remarkably stable duplex in an antiparallel fashion. Similarly, ***a*TNA-N(a)** displayed a sigmoidal curve only with an antiparallel counterstrand of ***a*TNA-M(t)** and not with



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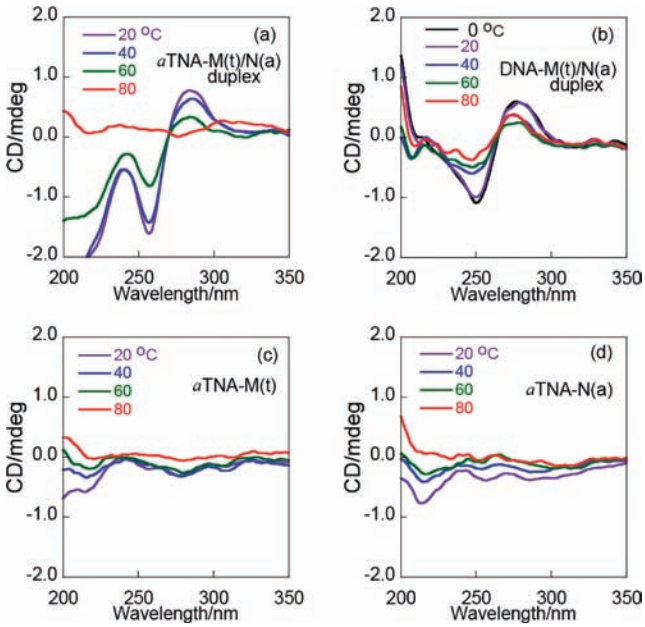


***Figure 3.*** Temperature dependence of the CD spectra of (a) ***α*TNA-M(t)/*α*TNA-N(a)** and (b) native **DNA-M(t)/DNA-N(a)** duplexes and single-stranded (c) ***α*TNA-M(t)** and (d) ***α*TNA-N(a)**. Conditions: [oligonucleotide] = 5 $\mu$M, [NaCl] = 100 mM, 10 mM phosphate buffer (pH 7.0).

parallel ***α*TNA-N(a)** (see Figure S1 in the Supporting Information). In order to substantiate that canonical Watson−Crick base-pairing dominates the stable association of *α*TNAs, the effect of mismatches on $T_m$ was examined with ***α*TNA-M(t)**. As listed in Table S1 in the Supporting Information, we found that introduction of a T−C mismatch [***α*TNA-M(t)/*α*TNA-N(c)**] significantly lowered the $T_m$ from 62.7 to 49.2 °C. Similarly, T−T and T−G mismatches destabilized the duplex by 8 and 3.7 °C, respectively.

Next, the *α*TNA duplex was analyzed by circular dichroism (CD) spectroscopy. As shown in Figure 3a, hybridization of ***α*TNA-M(t)** and ***α*TNA-N(a)** below $T_m$ for the duplex induced a symmetrical positive−negative Cotton effect similar to that seen for the typical B-type **DNA-M(t)/DNA-N(a)** duplex (compare panel b with panel a in Figure 3), demonstrating that hybridization of *α*TNA allowed the formation of a right-handed helix. However, the induced CD decreased with increasing temperature and became very weak at 80 °C, where the duplex was completely dissociated. CD analysis of single-stranded ***α*TNA-M(t)** and ***α*TNA-N(a)** revealed that the shapes of their CD spectra in the 200−300 nm region were entirely different from that of the duplex (compare panels c and d with panel a in Figure 3). Although the individual strands are chiral oligomers, their CD signals were very small at all temperatures examined here, indicating that single-stranded *α*TNA did not take a particularly preorganized structure but existed as a random coil. Nevertheless, hybridization of these strands induced formation of a right-handed double-helical structure.

Comparison of the thermal stability of *α*TNA with those of other natural duplexes revealed that the *α*TNA duplex is exceptionally stable. As described above, the $T_m$ of the ***α*TNA-M(t)/*α*TNA-N(a)** duplex was 62.7 °C. However, the corresponding DNA and RNA duplexes (**DNA-M(t)/DNA-N(a)** and **RNA-M(t)/RNA-N(a)**) yielded $T_m$ values of only 29.0 and 38.9 °C, respectively, which are far lower than that of the corresponding *α*TNA duplex. We also examined other sequences and found that the *α*TNA duplex was remarkably stable irrespective of the sequence. For example, a 12-mer *α*TNA duplex composed of only A and T gave a $T_m$ as high as 64.3 °C in the presence of 100 mM NaCl (see Table S2), which was ~40 °C higher than that of the corresponding DNA duplex (25.1 °C). The $T_m$ of a GNA duplex involving 18 A−T base pairs was reported to be 63 °C, which also exceeded the stability of the corresponding DNA duplex by 22.5 °C in the presence of 200 mM NaCl.[2a] Although we could not compare the stability of *α*TNA with GNA precisely, the *α*TNA duplex appears to be much more stable than the GNA duplex.[9]

According to Meggers,[2a] even single-stranded GNA showed a significant Cotton effect, indicating that helical preorganization of the GNA backbone had already formed in the single strand, allowing formation of a duplex that was highly stable in comparison with native ones. However, single-stranded *α*TNA did not form such a helical preorganized structure, as shown in Figure 3c,d. It should be noted that although *α*TNA is more flexible than DNA or GNA, it still formed a remarkably stable duplex in an antiparallel manner.[10]

In conclusion, we have developed from threoninol a new artificial oligonucleotide, *α*TNA, that forms an unexpectedly stable duplex in an antiparallel fashion. Even nucleobases tethered on a flexible scaffold that did not take a preorganized structure in the single-stranded state could form a duplex that was remarkably more stable than DNA or RNA duplexes by inducing formation of a right-handed double-helical structure. Our findings might allow for the new design of artificial duplexes that do not rely on rigid preorganization. Furthermore, by combining the "threoninol nucleotides"[4,5] that tether functional molecules with the present *α*TNA, functional foldamers can be prepared.

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**Supporting Information Available:** Experimental procedures for the syntheses of *α*TNAs and spectroscopic measurements, melting profiles and temperatures of duplexes, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) We also examined the cross-pairing of *α*TNA with DNA or RNA. However, unlike GNA, it hybridized with neither DNA nor RNA under the conditions employed here (see Figure S2).
(10) Flexible peptide nucleic acid (PNA) oligomers that do not have negative charges on their backbones also form remarkably stable duplexes with each other (see ref 8a).

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