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Binding properties of positively charged deoxynucleic guanidine (DNG), AgTgAgTgAgT and DNG/DNA chimeras to DNA

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ABSTRACT

The melting properties of hexameric oligonucleotide AgTgAgTgAgT, in which the phosphodiester linkages of the DNA have been replaced by guanidium linkages, have been evaluated. Using the juvenile esterase gene as a target, the binding of a 20-mer DNG/DNA chimera that includes AgTgAgTgAgT is more than $10^{5.7}$ stronger than the binding of 20-mer composed solely of DNA.

The fungus *candida albicans* infects humans and is particularly pernicious for immunopromised hosts, such as cancer and AIDS patients. Because the number of humans infected by fungi and the occurrence of resistant strains are increasing, finding new compounds to treat this infection is important.[1] In vitro results suggest that oligonucleotides are promising compounds for targeting DNA or RNA in fungal pathogens.[2,3]

The high affinity and specificity of Watson–Crick hybridization have made oligonucleotides attractive agents for diagnostic and therapeutic applications.[4] Oligonucleotides have several advantages for targeting DNA or RNA. Pairing rules between an oligonucleotide and DNA or RNA facilitate rational design. Gene expression can be reduced by RNase H cleavage of target RNA[5] or by blocking transcription and translation.[6] Oligonucleotides can also serve as suicide inhibitors[2,3] or to misfold RNA.[7] Polynucleotides are easily synthesized[8] and this allows rapid testing of oligonucleotides of different composition. Properties such as higher affinity and nuclease stability can be programmed into oligonucleotides by modifying the backbone, sugar, or base.[4] The primary goal of these modifications has been to improve biostability and cellular uptake of the oligonucleotides in order to optimize their tissue and cell distribution for a particular molecular target. The mechanism of action of antisense oligonucleotides requires specific hybridization of the oligonucleotide at its complementary site on the mRNA.

We have reported the synthesis and binding properties of deoxynucleic guanidine (DNG), wherein the negatively charged phosphodiester linkages of DNA have been entirely replaced by positively charged guanidium linkages.[9,10] DNG binds strongly to target DNA because the repulsive electrostatic interactions of duplex DNA are replaced by close attractive electrostatic interactions in DNA·DNG duplex. From computational studies,[10a,11,12] DNG is anticipated to maximize its interaction with DNA by maintaining its positive charge in proper alignment with the backbone of the negatively charged phosphodiester linkage of the opposite strand. A pentameric thymidyl oligomer of DNG, with four positively charged guanidium linkages, has been shown to bind to poly-adenine DNA with unprecedented high affinity in a 2:1 thymine–adenine complex.[10a] Since interaction with poly-guanine, cytidine, and thymine was not observed, DNG maintains fidelity of base-pair recognition. Computational molecular modeling suggests that the DNG·DNA duplex primarily retains a B-DNA conformation while the DNG·RNA duplex adopted an A-type structure.[10b] In addition to these results, the guanidium linkage was shown to have nuclease resistance[13] and positively charged backbones may give rise to cell membrane permeability through electrostatic attraction of the guanidine moiety to the negatively charged phosphate groups of the cell surface. Because of the potential antisense/antigene activity of DNG, further studies on the synthesis and properties of guanidium linked oligonucleotides are warranted.

The use of oligonucleotides in cell culture poses several challenges. For example, uptake into mammalian cells is not efficient and oligonucleotides are often degraded after entering cells.[5] Transfection agents, small molecules, and peptides have been used to circumvent these problems in mammalian cells.[14] However, little is known about oligonucleotide uptake into other organisms, such as fungi. Cell membranes and metabolism differ substantially between fungi and mammalian cells, thus uptake and metabolism of oligonucleotides may differ. Disney et al. have reported that oli-

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gonucleotides (hexamer; GCCTCT) are readily taken into *C. albicans* in an energy-dependent manner, producing intracellular concentrations as much as 100-fold higher than the concentrations in the media.[15] They also have shown that hexameric oligonucleotides are not significantly degraded in *C. albicans* after incubations of 12 h and that the intracellular concentration due to oligonucleotide uptake by mammalian cells is at least 10-fold lower than for *C. albicans*. This means that uptake may provide selectivity for targeting fungi with oligonucleotides.

The hexameric oligonucleotide ATATAT is one of most overrepresented of the yeast genomic downstream sequences.[16a] ATATAT is an essential nucleotide involved in regulation of transcription and translation processes in yeast cells. Here, we report the DNA binding properties of the hexameric DNG AgTgAgTgAgT and the synthesis of 20 base paired DNG/DNA chimeras having mixed anionic phosphodiester linkages of DNA and cationic guanidinium linkages of hexameric AgTgAgTgAgT. The structure of the DNG/DNA chimera is based on the oligonucleotide sequence targeting the juvenile hormone esterase gene.[16b] The hybridization properties of the DNG/DNA chimeras with complementary DNA have been evaluated using spectroscopic techniques.

Hexameric oligonucleotide **1**[17] (Fig. 1) was synthesized from commercially available 2′-deoxyadenosine and 2′-deoxythymidine

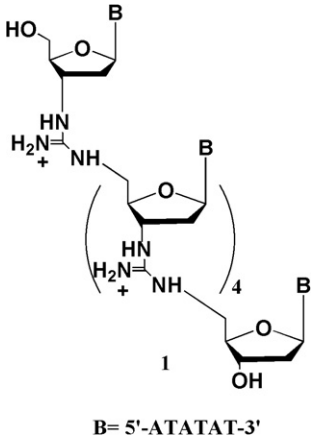


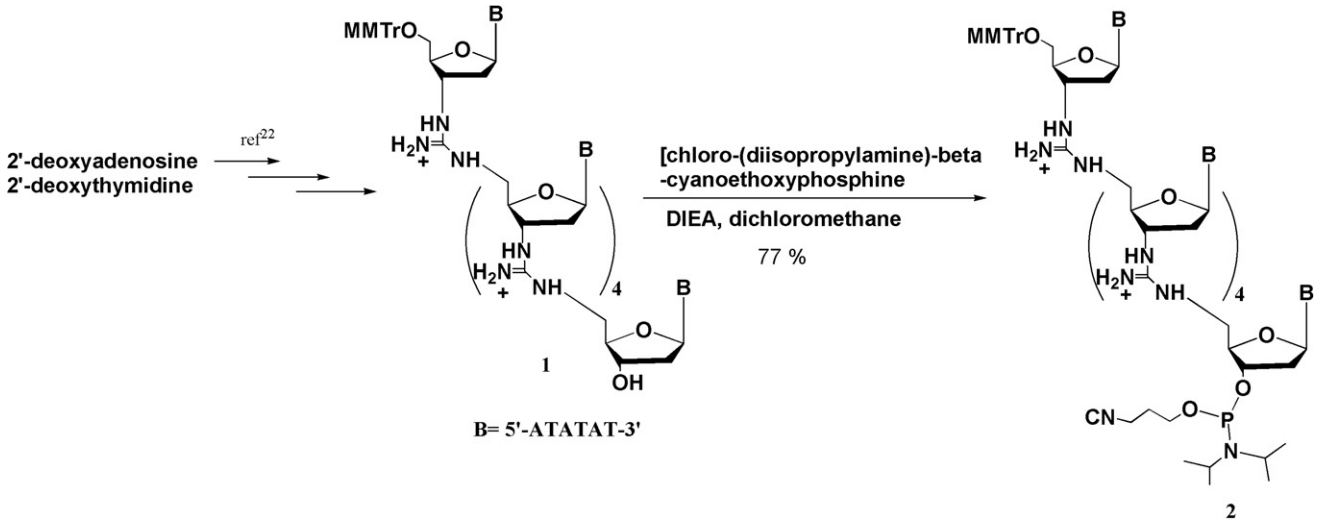
Figure 1. Structure of hexameric DNG, AgTgAgTgAgT ('g' indicated guanidinium linkage).

by a previously reported method through several steps.[16] Phosphoramidite **3**[19] was synthesized (Scheme 1) to facilitate the solid-phase synthesis of oligonucleotide chimeras containing both the standard phosphodiester and guanidium linkages.[20] Compound **2**[18] was activated for use in solid-phase synthesis by phosphitylation using [chloro-(diisopropylamino)-β-cyanoethoxyphosphine] to provide the desired phosphoramidate **3**. Phosphoramidite **3** was used as a building block to introduce guanidium linkages at desired positions in the chimeric oligonucleotides. The chimeras were synthesized using an automated solid-phase synthesizer with 5′-trityl groups which allows HPLC purification.[21] The final detritylated and HPLC purified oligonucleotides were analyzed by mass spectrometry (ESI) and found to be the desired chimeric products. As shown in Table 1, 3′-end (**6**), 5′-end (**5**), and 3′, 5′-end (**7**) mismatched chimeras were synthesized to examine sequence specificity.

The binding stoichiometry[22] of AgTgAgTgAgT and DNA was determined by the method of continuous variation[23] to generate mixing curves of the absorbance versus mole fraction of AgTgAgTgAgT and DNA (Fig. 2). This method is based on the assumption that a decrease in absorbance is proportional to the number of base pairs hydrogen bonded between the interacting species. Increasing mole fraction of AgTgAgTgAgT to the DNA (pH 7.0 and $\mu = 0.12$ with KCl at 20 °C) lowered the UV absorbance at 260 nm. An inflection point at 0.5 mol fraction indicated the formation of AgTgAgTgAgT·DNA duplex with the expected 1:1 stoichiometry.

The stability of the duplexes formed by AgTgAgTgAgT and DNA was studied by thermal denaturation experiments (Fig. 3).[24,25] To confirm the effect of the guanidium linkage of the AgTgAgTgAgT on the thermal stability (T_m) of the duplexes, the T_m values for an unmodified DNA·DNA duplex was also determined. As expected, AgTgAgTgAgT binds to DNA with much higher affinity ($T_m = 56$ °C) than complementary DNA (extrapolated T_ms to 5 °C, data not shown). Although the AgTgAgTgAgT·DNA duplex (Fig. 3) exhibited broad melting transition states, this anomaly is similar to DNA·RNG melting curves.[25,26] This can be explained by the electrostatic interaction between the positive charge of DNG and the negative charge of DNA, which provides an additional bonding, in spite of breaking down the Watson–Crick base pairing.

To study the sequence specificity of the binding of DNG with complementary DNA, AgTgAgTgAgT was allowed to form duplexes with complementary DNA (AgTgAgTgAgT·AATATA) and single-mismatch (5′- or 3′-end) DNAs (AgTgAgTgAgT·TATATT). These



Scheme 1. Synthesis of AgTgAgTgAgT phosphoramidate.