Deoxynucleic Guanidines/PNA (DNG/PNA) Chimeras: Oligonucleoside Analogue Containing Cationic Guanidinium and Neutral Amide Linkages

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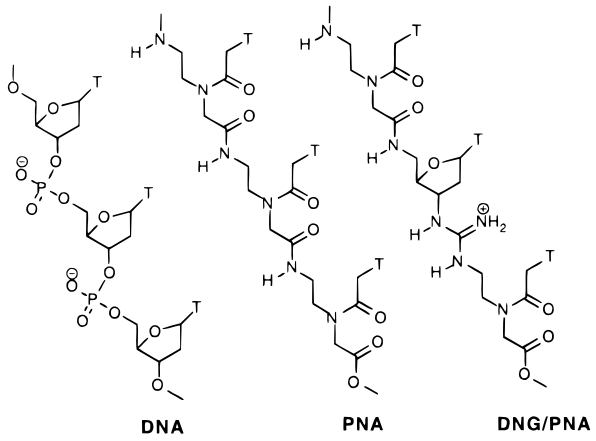
Received July 7, 1999

Considerable attention[1,2] has been paid to the modification of DNA and RNA to provide "oligonucleotides" with (i) sufficient binding at physiological temperature to ss and ds DNA and RNA, (ii) sequence specific binding, (iii) nuclease resistance, and (iv) enhanced delivery to cell targets. The hybridization processes of DNA and RNA helices are slowed due to electrostatic repulsion of the negative charges on adjacent phosphodiester linkages. This negative charge−charge repulsion can be minimized or eliminated, if the negative phosphodiester linkages are replaced by uncharged[3,4] or positively charged linkages.[5−10]

Our ongoing research in this area is focused on the development of positively charged internucleoside linkages[5−8] which provide stabilized complexes with DNA or RNA, with sequence specific binding.[11] In these studies the $-O-(PO_2^-)-O-$ linkers are replaced either by $-NH-C(=NH_2^+)-NH-$ to provide deoxynucleic guanidines (DNG)[5,6] or a $-NH-C(=S^+CH_3)-NH-$ to provide *S*-methylthiourea nucleosides (DNmt).[7] However, the electrostatic attractive forces between DNA or RNA and longer strands of DNG or DNmt could well become nonspecific if electrostatic attraction between polycationic and polyanionic structures becomes more significant than the specific hydrogen bonding interaction between heterocyclic bases. A means of lowering the electrostatic interactions would be to create mixed ODNs in which strands would consist of mixed sequences of positive and negative linkers or positive and neutral linkers. We have reported[8] the synthesis of DNG/DNA chimeras having mixed anionic $-O-(PO_2^-)-O-$ and cationic $-NH-C(=NH_2^+)-NH-$ linkers.

In peptide nucleic acid polymers (PNA), the general structure and lack of electrostatic repulsion (Scheme 1) between PNA and its DNA/RNA targets results in their high binding affinity.[12] However, the prospect of PNA's as drugs has limitations, such as poor cell membrane permeability,[13,14] small rate constants for association with DNA and RNA,[15,16] and the high thermal stability of helical PNA·DNA or PNA·RNA structures which may lead to decreased sequence specificity at physiological temperature.[17] An attempt to increase the rate of PNA association with DNA involved linking two PNA molecules with a positively charged $lysine_3$/$aminohexyl_2$ linker.[15]

Scheme 1



4 $H_3\overset{+}{N}$ - t t t t T * t t t t t - L

5 $H_3\overset{+}{N}$ - T * t t t t t t t t T * t - L

6 $H_3\overset{+}{N}$ - t t t T * t T * t t t t - L

7 $H_3\overset{+}{N}$ - T * t t t T * t t t T * t - L

8 $H_3\overset{+}{N}$ - t t t t t t t t t t - L

9 5'- A A A A A A A A A A - 3'

10 5'- A A A A G A A A A A - 3'

L = HN-$(CH_2)_6$-OH

* indicates guanidinium linkage

Upper and lower cases represent nucleobases and PNA units in sequences respectively.

It occurred to us that positive charges could be placed directly on the backbone of PNA by injecting DNG and/or DNmt monomers into the PNA sequence. The characteristics of DNG, DNG/DNA chimera, DNmt, and PNA helical structures suggested to us that the synthesis and investigation of DNG/PNA chimeric oligos (Scheme 1) is warranted. It was anticipated that placement of spaced DNG positive charges in the PNA structure would increase the rate constant for association with DNA and RNA[15] and enhance cell membrane permeability.[18]

In this paper, we report fully automated solid-phase synthesis of DNG/PNA chimeras (Scheme 1) and their binding properties with complementary DNA. For the incorporation of guanidinium linkage into PNA to provide DNG/PNA chimeras, we synthesized the DNG/PNA dimer **1** (Scheme 2). The guanidinium linkage of dimer **1** was protected with a trichloroethoxy carbamate[19] and remains protected throughout the synthesis of DNG/PNA chimeras. The guanidinium linkage was incorporated into DNG/PNA chimeras **4**−**7** by using Pharmacia GA Plus synthesizer which

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10.1021/ja9923540 CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/21/1999

Scheme 2

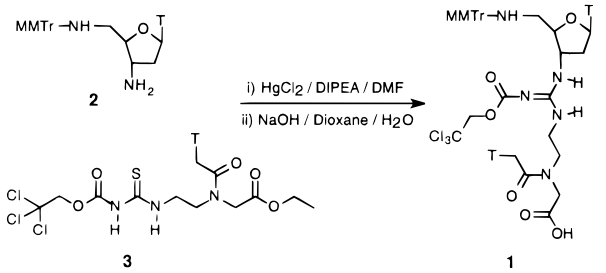

Table 1. Results of Melting Temperature Experiments[a]

exp. no.	complex	T_m (°C)	hypochromicity (%)
1	**(8)**$_2$**·9**[23]	54.5	17.8
2	**(4)**$_2$**·9**	29.5	27.0
3	**(5)**$_2$**·9**	52.5	25.4
4	**(6)**$_2$**·9**	25.5	15.8
5	**(7)**$_2$**·9**	25.5	13.7
6	**(8)**$_2$**·10**	38.5	
7	**(5)**$_2$**·10**	39.5	

[a] The melting temperature experiments were carried out on a Cary 100 Bio. UV/Vis spectrophotometer, the heating and cooling rates were 0.2 °C/min. 3 μM of DNA **9** or **10** were hybridized with 1 equiv of the DNG/PNA Chimera **4**−**7**. The buffer used was 100 mM NaCl, 10 mM phosphate, pH 7.1. The T_m values were determined using the first derivative of the plot of A_{260} vs temperature

was programmed for peptide coupling reaction with 45 min of coupling time. The coupling reagent used was PyBop in DMF/NEM.[20] Each coupling was followed by a capping reaction of truncated oligomers. After completion of synthesis, oligomers were cleaved from solid support by NH_4OH treatment such that the resulting DNG/PNA chimeras **4**−**7** possess hydroxyhexylamides at the C-terminus (Scheme 1). The DNG/PNA chimeras were then treated with Cd/AcOH which deprotects guanidinium groups[21] and the terminal MMTr. These DNG/PNA chimeras **4**−**7** were then purified by RP-HPLC. FAB MS analysis of purified DNG/PNA chimera (tetramer) $H_2N-t\,T*t\,t-L$ ($C_{50}H_{73}N_{18}O_{16}$ 1182.24) indicated the expected mass.

Binding of the DNG/PNA Chimeras 4−7 with Complementary DNA. The chimeras **4**−**7** have one guanidinium linkage at center, guanidinium linkages at both termini, two guanidinium linkages at center, guanidinium linkages at both termini, and one guanidinium linkage in the center, respectively (Scheme 1). The stoichiometry of binding of the DNG/PNA chimeras **4**−**7** to DNA **9** was determined by the continuous variation method[22] and found to be 2:1 indicating formation of [(DNG/PNA)$_2$·DNA] triplexes. Relative stabilities of [(PNA)$_2$·DNA] and [(DNG/PNA)$_2$·DNA] triplexes were determined by comparing their T_m values which were obtained from UV-melting curves. Examination of Table 1 shows that the [(DNG/PNA)$_2$·DNA] triplexes composed of DNG/PNA chimeras **4, 6,** and **7** with DNA **9** have lower melting temperatures than the [(PNA)$_2$·DNA] triplex [**(8)**$_2$**·9**].[23] The T_m values for [**(5)**$_2$**·9**] and **[(8)**$_2$**·9**] are quite similar. In DNG/PNA chimera **5** the guanidinium linkages have the greatest separation being located at terminal positions. However, all DNG/PNA·DNA triplexes [**(4**−**7)**$_2$**·9**] have greater stability than the corresponding DNA·DNA triplex [$(T_{10})_2$·dA_{10}] ($T_m \sim 10$ °C). These are sought after properties. The percentage hypochromicity accompanying

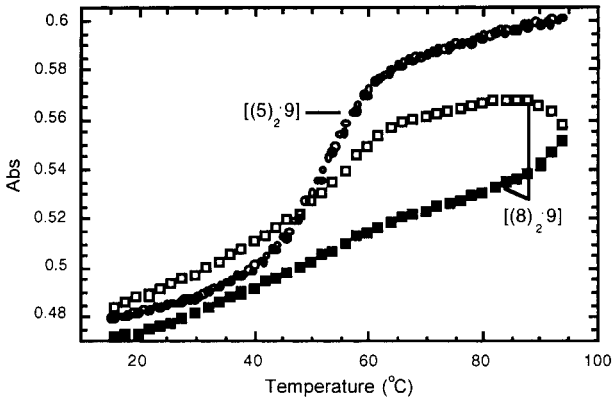

Figure 1. Absorbance vs temperature profiles for DNG/PNA chimera **5** and PNA **8** hybridized to DNA **9**. Dissociation (heating) curves for [**(5)**$_2$**·9**] and [**(8)**$_2$**·9**] triplexes are designated by (●) and (■), similarly (○) and (□) represent association (cooling) curves, respectively.

formation of [(DNG/PNA)$_2$·DNA] triplexes [**(4)**$_2$**·9**] and [**(5)**$_2$**·9**] exceeds that for the [(PNA)$_2$·DNA] triplex [**(8)**$_2$**·9**] indicating better base stacking in [**(4)**$_2$**·9**] and [**(5)**$_2$**·9**]. The triplexes containing one mismatch [**(8)**$_2$**·10**] and [**(5)**$_2$**·10**] exhibited a 16 and 13 °C decrease in T_m, respectively, in comparison to fully complementary [**(8)**$_2$**·9**] and [**(5)**$_2$**·9**] triplexes. This demonstrates that binding of DNG/PNA chimera **5** with complementary DNA **9** is sequence specific.

The association process of DNA hybridization can be monitored by a decrease in absorbance with lowering temperature.[24] Hysteresis is observed between heating and cooling curves because the rates of association of PNA with DNA to form either duplex or triplex is, at usual concentrations, slower than the rates of dissociation.[16] As an example, the heating and cooling curves of the [(PNA)$_2$·DNA] triplex [**(8)**$_2$**·9**] show significant hysteresis (Figure 1). By substituting deoxynucleic guanidines for terminal PNA of **8** in the structure [**(8)**$_2$**·9**] the heating and cooling curves of the resultant [**(5)**$_2$**·9**] were found to be almost overlapping (Figure 1). Clearly the rate of association of **5** with **9** is greater than observed in the formation of [**(8)**$_2$**·9**] and the off and on rates for **[(5)**$_2$**·9**] are comparable.

In conclusion, we have successfully developed fully automated solid phase synthesis chemistry for the insertion of positively charged guanidinium linkage into PNA to provide DNG/PNA chimeras. The results from binding studies show that [(DNG/PNA)$_2$·DNA] triplexes are more stable than DNA·DNA triplex [$(T_{10})_2$·dA_{10}]. The insertion of guanidinium linkage at internal sites of PNA results in destabilization due to possible structural changes in the backbone. Placement of guanidinium linkages at both the termini of PNA as in **5** results in the [(DNG/PNA)$_2$·DNA] triplex [**(5)**$_2$**·9**], which has stability comparable to the [(PNA)$_2$·DNA] triplex [**(8)**$_2$**·9**]. The binding of DNG/PNA chimera with complementary DNA is sequence specific with the formation of triplexes. The most important finding in this investigation is that the association process of DNG/PNA **5** with DNA **9** is faster than the association of PNA **8** with DNA **9**. This increase in the rate by substitution of a DNG/PNA into a PNA extends the potential utility of PNA in diagnostics, biomolecular probes, and antisense/antigene therapeutics.

Acknowledgment. We gratefully acknowledge continuing support by the National Institute of Health (3 R37 DK09171-3451).

Supporting Information Available: Mass spectrum of tetramer DNG/PNA, HPLC chromatograms of DNG/PNA chimeras **4**−**7**, and Job's plot for [**(8)**$_2$**·9**] and [**(5)**$_2$**·9**] triplexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9923540

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