Assembly of Nanoscale Scaffolds from Peptide Nucleic Acids (PNA)

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ABSTRACT

Current models of G-quadruplex (multiple stacking G-tetrads) formation based on DNA are large structures containing at least four consecutive guanine bases. PNAs containing one to four guanines were synthesized using standard Fmoc peptide coupling conditions. Monitoring the assembly of short PNA quadruplexes with variable cation concentrations as well as with various cations shows an independence of PNA G-quadruplexes on cation concentration with short sequences. The strategy for synthesis allows modification of both ends of a PNA sequence. This in theory would allow one to synthesize multivalent nanoparticles containing up to eight additional substituents. A quadruplex containing four strands with four guanine residues in each strand occupies a volume of only about 8 nm^3. Consequently, depending on the modifications, this approach should allow directed-assembly of nanomaterials with volumes in the 10 to 100 nm range.

Keywords: peptide nucleic acid, quadruplexes, scaffold, self-assembly

1 DNA AS A SCAFFOLD

Deoxyribonucleic acid or DNA has been demonstrated to be a useful scaffold for self-assembling nanomaterials [1-5]. Using complementary and non-complementary sequences of single stranded DNA (ssDNA) self-assembly through hybridization yields an unlimited variety of higher order structures. The ability to direct assembly in three dimensions through the design of linear sequences is especially appealing. DNA can also be utilized as a scaffold for the direction of organic reactions through appropriate arrangement of reactants linked to DNA strands. DNA facilitated reactions include conjugate addition, reductive amination, amine acylation, oxazolidine formation, nitro aldol, nitro Michael, Wittig, 1,3-nitrone cycloaddition, Huisgen cycloaddition, and Heck coupling [3].

2 PNA AND QUADRUPLEXES

Peptide nucleic acids, or PNA, which retains the nucleic acid bases adenine, cytosine, guanine, and thymine on a pseudo-peptide backbone, can provide a unique scaffold for the construction of biologically functional nanomaterials. PNA hybridizes to natural nucleic acids by Watson-Crick base pairing and can be used as a carrier for complementary DNA or RNA. PNA has been shown to have a lower tolerance for mismatches as well as increased stability as measured by melting temperature [2, 6, 7]. The PNA backbone, comprised of 2-aminoethylglycine unit repeats, can be modified in much the same fashion as a normal peptide backbone using solid phase synthesis methods as well as standard coupling conditions for Fmoc peptide synthesis. Synthetic modification allows for the direct incorporation of peptides and molecular probes with relative ease. In addition, PNA sequences can be designed to self-assemble into nanoparticles. Compact structures based on G-tetrad formation are currently under study in our lab. Current models of G-quadruplex (multiple stacking G-tetrads) formation based on DNA are large structures containing at least four consecutive guanine bases. Because complementary PNA strands typically self-associate with higher affinity than DNA strands, we initially focused on the tetrad assembly properties of shorter

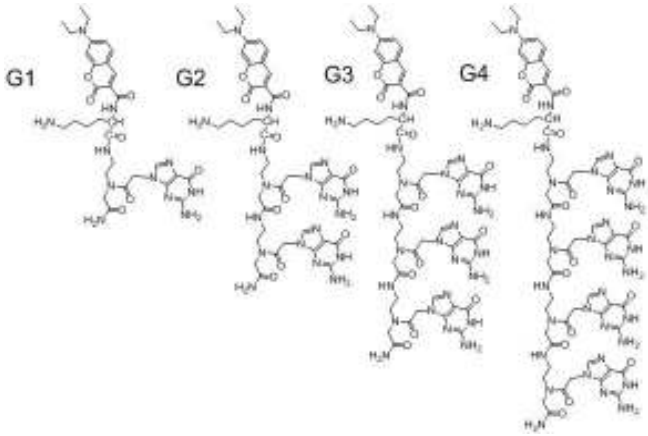

Figure 1. Structures of PNA utilized in this study. Fluorescently labeled PNA with varying lengths referred to as G1, G2, G3, and G4.

sequences. PNAs G1-G4 (Figure 1) were synthesized by the strategy mentioned above. The association and stability of the PNA G-quadruplex has several implications in the nanotechnology field. The strategy for synthesis allows modification of both ends of a PNA sequence. This in theory would allow one to synthesize multivalent nanoparticles containing up to eight additional substituents. A quadruplex containing four strands with four guanine residues in each strand occupies a volume of only about 8 nm^3. Consequently, depending on the modifications, this approach should allow directed-assembly of nanomaterials with volumes in the 10 to 100 nm range. The effects of modifications of the PNA on assembly properties are currently under study.

2.1 Monitoring Quadruplex PNA

The melting or disassociation of a G-quadruplex can be monitored by following the hypochromic effect that occurs with an increase in temperature at 295 nm [8, 9]. While this effect has been reported and studied using DNA, the same effect is also seen with PNA, since the alignment of the transition dipoles within the plane of the bases remains unchanged, even though the backbone is different. With fluorophore modified PNA we have observed this effect with sequences containing from one to four bases (PNA G1-G4, Figure 1). The thermal melting profile for these tetrads is shown in Figure 2. In turn, the assembly of G-quadruplexes can be monitored by a hyperchromic effect that occurs when the quadruplex is assembled. Monitoring the assembly of short PNA quadruplexes with variable cation concentrations as well as various cations shows an independence of PNA G-quadruplexes on cation concentration with short sequences. When comparing to the established relationship between DNA and cation concentration, DNA exhibits a strong dependence and PNA does not exhibit this dependence [10].

3 STABILITY OF PNA QUADRUPLEXES

For analysis of the melting of the synthesized PNA sequences, the sequences were diluted into 10 mM sodium cacodylate buffer pH 7.2 containing 100 mM sodium chloride just before starting the melting experiments. This particular buffer was chosen because it does not absorb in the UV region in question and it does not exhibit a shift in pKa with changes in temperature. The UV absorbance was recorded at a wavelength of 295 nm as the temperature was increased from 25°C to 90°C and subsequently cooled back to 25°C. After holding the sample at 25°C for 100 minutes, the sample was again heated to 90°C. All of the heating and cooling ramps were conducted at a rate of 0.2°C/min. At this wavelength, G-quadruplexes exhibit a hypochromic effect as the temperature is increased to and above the melting temperature of the structure. This unique characteristic of the spectrum can be used to identify and monitor the melting or unfolding of the quadruplex. Various concentrations of PNA strands were chosen for analysis. These solutions were made just before starting the melting experiments.

3.1 Thermal Stability

Initially all of the sequences tested showed the ability to form quadruplexes as evident from the melting curves showing the characteristic hypochromic effect at 295 nm (Figure 2). The initial slope of the melting curves indicates that the addition of guanine bases adds to the low temperature stability of the quadruplex, but the melting temperature is, interestingly enough, unaffected. This would indicate that the mechanism of dissociation is unaffected by the length of the G-tract. This finding is contrary to what has been shown with DNA G-quadruplexes [10, 11]. The properties of PNA_4 quadruplexes are not yet known. It is known that PNA_4 quadruplexes can form and be analyzed via electro-spray ionization mass spectrometry with a guanine tract containing only three guanine residues [12]. In this report, it should be noted that the PNA quadruplex studied displayed a $T_{1/2}$ of 24°C. It should also be noted that this structure is much different in that the lysine residue is placed at the carboxy-terminus as well as the presence of the free acid. The structures that have been synthesized and studied in our laboratory thus far have shown a remarkable increase in stability over the PNA that has been studied previously. DNA G-quadruplexes are stabilized by monovalent cations and destabilized by small cations, such as lithium ions. Because it has been reported that PNA does not follow this trend, at least with long sequences, this concept was then tested with the short PNA sequences.

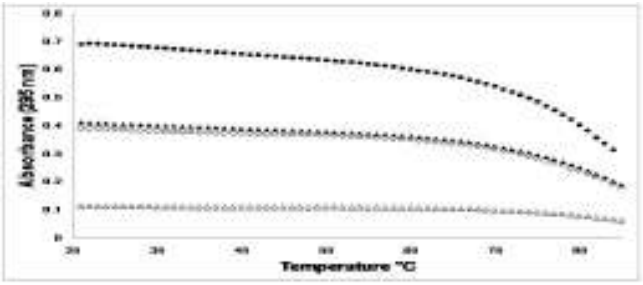

Figure 2. Melting curves for PNA of varying lengths at 100 μM. G1 (filled circles, •), G2 (filled triangles, ▲), G3 (open circles, ○), and G4 (open triangles, Δ). It is evident from the hypochromic effect seen at higher temperatures that a quadruplex is formed in all cases.

4 ION EFFECTS

The stability of the resulting PNA quadruplexes has been tested under various conditions. It has been reported that for PNA quadruplexes containing at least three guanine bases, cation concentration does not add to the stability of the complex [12]. Structures of assembly with less than three guanines have not previously been studied. Also it

should be noted that the thermodynamic properties of PNA quadruplexes of such short sequences has not been determined, as is the same with the kinetic parameters of PNA quadruplex assembly.

4.1 Ion Concentration And Assembly

Kinetic data for assembly of quadruplexes at 22°C was recorded. PNA samples that contained no quadruplex component were used for this analysis. The PNA of varying lengths (G2 and G3) was diluted into 10 mM cacodylate buffer pH 7.2. Chloride salts containing either sodium or potassium were added to the sample so that the final concentration was one of the following: 50, 100, 200, 300, 400, or 500 mM. Upon addition of the sample to the salt buffer, the absorbance at 295 nm was recorded. Absorbance readings were taken for 16 hours, and following the conclusion of the time recordings, plots were constructed of the absorbance versus time. These resulting plots showed that no sharp increases in absorbance were recorded. This indicates that the cation concentration did not accelerate the assembly of the quadruplexes at 22°C on this time scale. A slight increase in absorbance was noted in the PNA G3 sample, but this increase was not significant enough to indicate dependence on cation concentration.

4.2 Temperature And Assembly

Because lower temperatures have been shown to increase the rate of quadruplex assembly in DNA [9, 10], the kinetic experiments were repeated at 3.5°C. This decrease in temperature should aid in the assembly of the PNA quadruplexes if the same effect with DNA is carried over to the PNA constructs. Again the PNA samples were added to 10 mM sodium cacodylate buffer containing 100, 300, or 500 mM sodium chloride. The absorbance readings were recorded every five minutes for 16 hours. Once all of the readings were recorded, it was noted that again there were no increases in absorbance for any of the concentrations used with any of the PNA samples (Figure 3), which indicates that the decrease in temperature again did not accelerate the assembly of the PNA quadruplexes on this time scale.

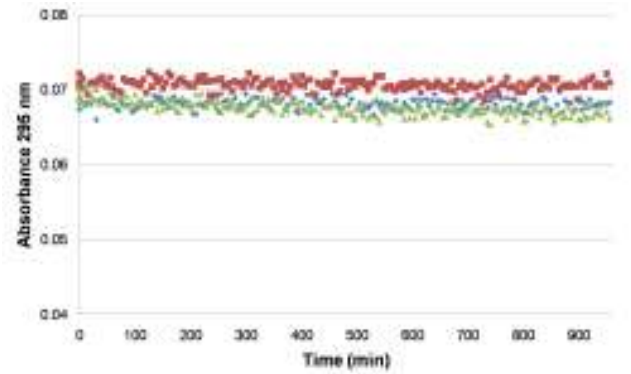

Figure 3. Representative plot (G2 shown) of the assembly of PNA quadruplexes from a 100 µM solution with various sodium ion concentrations (100 mM NaCl Blue Diamond, 300 mM NaCl Red Square, and 500 mM NaCl Green Triangle). It is apparent that there is no assembly occurring at 3.5°C on this time scale.

4.3 Ion Size And Assembly

Because ion size is important for stabilizing quadruplex DNA, the size of the cations present in solution was probed. Again PNA samples were diluted into 10 mM sodium cacodylate buffer pH 7.2 containing 100 mM lithium chloride (destabilizes quadruplex DNA), 100 mM sodium chloride (stabilizes quadruplex DNA), or 100 mM potassium chloride (highly stabilizes quadruplex DNA). The absorbance was again recorded every five minutes for 16 hours at 295 nm. The resulting plots that were obtained indicated that PNAs G1 and G2 did not assemble at 3.5 **°C** regardless of the cation size. These plots showed no increase or decrease in absorbance over the course of the experiment; however, PNA G3, and to a much larger degree PNA G4, showed a decrease in absorbance over the time of the experiment. The slight decrease was most evident in PNA G4 samples that contained potassium (Figure 4). Interpretation of this result will require additional studies.

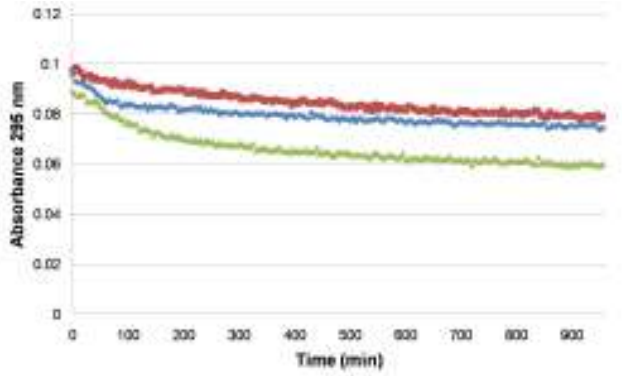

Figure 4. Representative plot (G4 shown) of the assembly of PNA quadruplexes from a 100 µM solution with various cation concentrations (100 mM LiCl Blue Diamond, 100 mM NaCl Red Square, and 100 mM KCl Green Triangle). It is apparent that there is no assembly occurring at 3.5°C on this time scale.

4.4 Ion Concentration And Stability

Because the concentration of the cation did not accelerate the assembly of the PNA quadruplexes at temperatures down to 3.5 **°C** and time periods of the order of 24 hours, additional experiments were designed to test the effect of salt concentration on the melting curve of the PNA quadruplexes. For this set of experiments, the PNA samples were stored for one week at -20°C in the Tm buffer (10 mM sodium cacodylate buffer pH 7.2) containing 100, 300, or 500 mM sodium chloride to ensure the formation of

the quadruplexes took place in the cation concentrations desired. The samples were then thawed at room temperature for 10 minutes before the start of the experiment. The UV absorbance was recorded at a wavelength of 295 nm as the temperature was increased from 25°C to 90°C and subsequently cooled back to 25°C. After holding the sample at 25°C for 100 minutes, the sample was again heated to 90°C. All of the heating and cooling ramps were conducted at a rate of 0.2°C/min. Upon conclusion of this set of experiments, the absorbance readings were plotted as a function of temperature. It was evident from these plots that the cation concentration had no effect on the temperature at which the apparent melting of the PNA quadruplexes occurred, and had no effect on PNA G1 or PNA G2 at all. However, in the temperature range 25 to 40 °C PNA G3 showed an increase in the absorbance at 295 nm suggesting a cation dependent alteration in the quadruplex PNA structure (Figure 5). The increased absorbance remained constant at this higher level until the apparent melting temperature was reached.

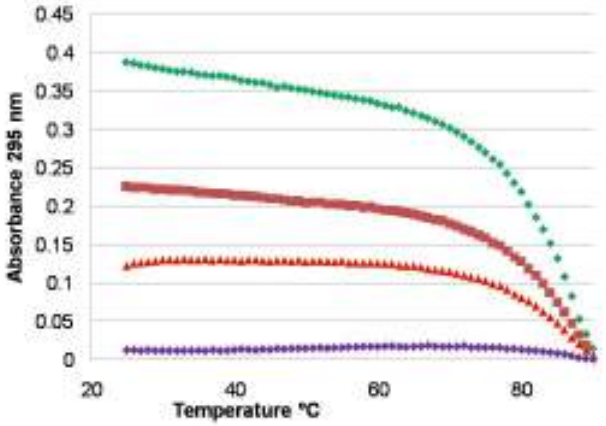

Figure 5. Representative plot of the dissociation or melting of PNA quadruplexes from a 100 µM solution formed in 300 mM sodium ion solutions (PNA G1 Green Diamond, PNA G2 Red Square, PNA G3 Red Triangle, and PNA G4 Purple Diamond).

5 CONCLUSIONS

This is the first report of short sequences of PNA, less than three guanine residues, forming quadruplex structures. The structures that are formed are four-stranded, and they are extremely stable once formed. The stability is demonstrated by the apparent melting temperatures which are greater than 80°C. The assembly of the quadruplexes on a reasonable time scale requires that the samples be frozen. Modification of both ends of the PNA will allow attachment of up to eight individual ligands, which will allow exploitation of the multivalence effect on binding target proteins. It may be possible to decrease the size of the scaffold necessary to produce this arrangement to a single guanine residue given the right conditions, resulting in the minimum possible scaffolding.

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