Sequence-Specific Oligonucleotide Purification Using Peptide Nucleic Acid Amphiphiles in Hydrophobic Interaction Chromatography

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We present a methodology to perform sequence-specific separations of oligonucleotides using peptide nucleic acids covalently linked to alkane chains, or "PNA amphiphiles (PNAAs)". The PNAA/DNA duplexes are discriminated from unbound DNA using hydrophobic interaction chromatography on a phenyl-substituted Sepharose column. Nearly quantitative recovery is achieved at concentrations of 50 μM after incubation of oligomers with a stoichiometric amount of PNAA for 1 min or so. The method exhibits high sequence specificity, selectivity, and resolution when applied to mixtures of various oligomers up to 60 base pairs in length.

Introduction

Recent advances in our understanding of the human genome have the potential to provide treatments and diagnoses of genetic diseases as well as identification of individuals and organisms by the genetic material they leave behind. Unlocking this potential will require the development of novel methods to extract and purify DNA with increasing resolution and speed. For example, therapeutic plasmid purification procedures typically employ commercially available kits utilizing anionic exchange media to isolate nucleic acids (*1*) as an alternative to gel electrophoresis. Other methods have been devised with higher throughputs in mind, including membrane chromatography (*2*), fast protein liquid chromatography (*3*), and hydrophobic interaction chromatography (*4*−*6*). While effective in discriminating chromosomal DNA and RNA from plasmid DNA, these high-throughput methods are not sequence-specific, leaving open the possibility that undesired genes be delivered to patients. Sequence-specific purifications would also be desirable when RNA is extracted for gene expression analysis, particularly when one wishes to study trace mRNA without interference from other RNA. Finally, many genomic sensing methods, such as quantitative PCR (*7*), would benefit from a "pre-enrichment" step to remove other nucleic acids that may encourage template bias (*8*) or polymerase inhibition.

Sequence-specific nucleic acid purification is typically achieved by hybridizing all or part of the DNA target to an immobilized probe, for example, an oligonucleotide grafted to a solid surface (*9*), a magnetic bead (*10*), or a polymer gel (*11*). For effectiveness, many samples need to undergo extensive preprocessing before the hybridization step to avoid surface fouling and attendant probe deactivation.

Surfactant-based separations have been developed for protein purification. Their responsiveness, complex phase behavior, and compatibility with aqueous systems have given them important advantages in niche applications. DNA extraction protocols have also been developed utilizing cationic surfactants that nonspecifically complex with nucleic acids from cell lysates. The surfactant/DNA complex can be recovered by treatment with organic solvents or other means.

We have been developing a series of surfactants that hybridize to particular sequence targets, "tagging" them for removal from complex mixtures. Hybridization is achieved using a peptide nucleic acid (PNA) moiety. PNA is a synthetic DNA mimic composed of a polyamide backbone and pendant nucleobases. PNA binds ssDNA and RNA in the conventional Watson−Crick motif to form antiparallel duplexes that are slightly more stable than the natural counterpart (*12*, *13*). Owing to this added stability, PNA readily forms stable, sequence-specific associations with dsDNA through a strand-invasion mechanism, allowing for hybridization to dsDNA under nondenaturing conditions (*14*−*16*). This is an important feature of PNA with regard to bioseparations, as plasmids and PCR products can be tagged without full separation of the dsDNA strands. A "PNA amphiphile (PNAA)" is a PNA peptide covalently linked to alkane chains. Since the PNA peptide and attached alkanes are both nonpolar, charged amino acids are attached to the PNA peptide headgroup of the PNAA to impart sufficient hydrophobic/hydrophilic contrast for micellization and water solubility. Our previous work (*17*, *18*) has demonstrated that properly designed PNA amphiphiles micellize at moderate concentrations, and bind complementary DNA oligomers with about the same sequence specificity and stability as unmodified PNA peptides.

Others have utilized PNA peptide hybridization as a means to isolate specific DNA and RNA targets, with mixed results. A "pregel" hybridization method successfully identified complementary DNA by a reduction in mobility following PNA peptide hybridization in gel electrophoresis (*19*). Another approach had biotinylated PNA probes hybridized to DNA and recovered using streptavidin-coated magnetic beads (*20*) or nickel−histidine binding (*21*). Direct linkage of PNA probes to the beads generally resulted in a loss of activity compared to hybridization in solution. Since PNA amphiphiles micellize in a concentration-dependent fashion, it should

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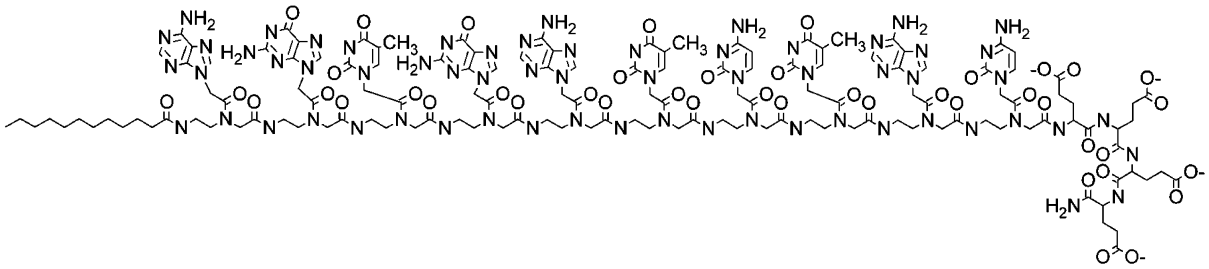

Figure 1. Structure of the PNA amphiphile used in this study, C_{12}-agtgatctac-$(Glu)_4$. The glutamic acid residues at the C terminus impart water solubility to the molecule.

be possible to bind PNA as an isolated molecule before microstructures appropriate for separations are formed, removing the surface-binding step. We also point out that difficulties in incorporating gels and microbeads into "lab-on-a-chip" devices can be overcome by using these small molecules, changing the sequence target when necessary by simply introducing a new PNA amphiphile into a separation chamber.

Here, we demonstrate that PNA amphiphiles can be used as a method to selectively recover DNA oligomers from mixtures of oligomers in hydrophobic interaction chromatography (HIC). HIC was selected as a platform for these initial studies on the basis of its mild elution conditions and ability to discriminate materials on the basis of polarity. This work sets the stage for applications of PNA amphiphiles to other separation modalities including micellar electrokinetic separations (*22*, *23*), aqueous two-phase extraction (*24*−*26*), and selective precipitation (*27*).

Materials and Methods

Synthesis of PNA Amphiphiles. PNA reagents were purchased through PerSeptive Biosystems (Framingham, MA), and solvents (high-purity) were purchased through Fisher Scientific. DNA oligomers (Integrated DNA Technologies, Coralville, IA) were dissolved in 20 mM phosphate buffer at pH 7.00 and used without further purification.

PNAAs were synthesized using a solid-phase peptide synthesis technique with Fmoc/Bhoc-protected monomers (*28*, *29*) on an Fmoc-protected PAL-PEG-PS resin with a 0.25 mmol/g loading capacity (Peptides International, Louisville, KY). Coupling of the PNA bases as well as the amino acids and lipid tail was done by activating the monomers with HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and a base solution consisting of 2,6-lutidine and *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide (DMF) for 2 min before resin introduction. Acetic anhydride was used to cap unreacted amine groups after each coupling step, and the ninhydrin test was used to verify the complete coupling. To cleave the PNAA from the resin and to remove all Bhoc side protecting groups, the resin was soaked in 2000 μL of 4:1 trifluoroacetic acid (TFA)/*m*-cresol for 2 h. Once cleaved from the support, the PNAA was precipitated by addition of at least 10-fold excess dry ether. The precipitated PNAA was allowed to cool for 5 min in a −80 °C freezer to ensure complete precipitation of the product. The solid was separated from the ether by centrifugation at 1000*g* for approximately 5 min. The top phase was carefully decanted off and the pellet resuspended with another addition of dry ether. The freezing and centrifugation process was repeated three times. Upon completion the PNAA pellet was dried under nitrogen and dissolved in deionized water for HPLC purification. Following purification, the structure of the PNAA was verified by MALDI-TOF mass spectroscopy using a sinapinic acid matrix (positive polarity). For duplex characterization, DNA was analyzed using a picolinic acid matrix (negative polarity).

The naming convention we use for the PNAA lists the sequence from left to right in the N to C sense. PNA base sequences are written in lowercase form, while alkyl chains are in uppercase text, and amino acids are written with their three-letter abbreviation. Using this convention, the PNA amphiphile used in this study is designated "C_{12}-agtgatctac-$(Glu)_4$", where C_{12} refers to the 12-carbon lipid tail and $(Glu)_4$ indicates the glutamic acid tetrapeptide appended to the C-terminus of the PNA peptide. Figure 1 shows the structure of this molecule.

HIC Purification of DNA by PNAA Hybridization. Hydrophobic interaction chromatography was used to purify single-stranded DNA targets. A 1 mL HiTrap phenyl-substituted column (Amersham Biosciences, Piscataway, NJ) and a 6% cross-linked agarose gel with a ligand density of 20 μmol/mL were used for all experiments. Separations were performed on an AKTA explorer fast protein liquid chromatography (FPLC) system (Amersham Biosciences) at room temperature. C_{12}-agtgatctac-$(Glu)_4$ was mixed with complementary target DNA in 1.5 mL microcentrifuge tubes for 1−10 min in 20 mM phosphate buffer (pH 7.0) prior to injection, unless otherwise stated. Injections were performed manually using a 50 μL sample loop which was overfilled by 50%. Elution of the PNAA/DNA duplex was performed at a flow rate of 0.3 mL/min using a linear salt gradient of 100% solvent A to 100% solvent B in 30 min [(solvent A) 1.5 M $(NH_4)_2SO_4$/10 mM Tris−HCl, pH 8.00; (solvent B) 0.1 M sodium phosphate, pH 8.00). After the injections and elution the column was washed with 0.01 M NaOH for at least 5 column volumes for storage. For quantitation, the HIC column detector was calibrated by injecting a known amount of DNA and plotting the resulting peak area versus the molar concentration of DNA.

Results

While PNAAs form stable associations with complementary DNA oligomers, a concern is preventing the dissociation of the complex during processing. For example, duplexes of PNAA and DNA oligomers will melt under typical reversed-phase HPLC conditions (water/acetonitrile gradient elution), yielding separate peaks for the PNAA and the DNA and loss of separation resolution. Better results were obtained using gentler HIC on a phenyl-substituted Sepharose support. Equimolar amounts of PNAA and DNA (50 μM each) in 20 mM phosphate buffer (pH 7.0) were mixed and allowed to stand for 1 min prior to injection. Figure 2 is a chromatogram for the elution of the resulting PNAA/DNA complex (A) along with the isolated DNA (B) and PNAA (C) at the same concentration as the complex. The complex elutes as a single peak in this case, with a much higher retention

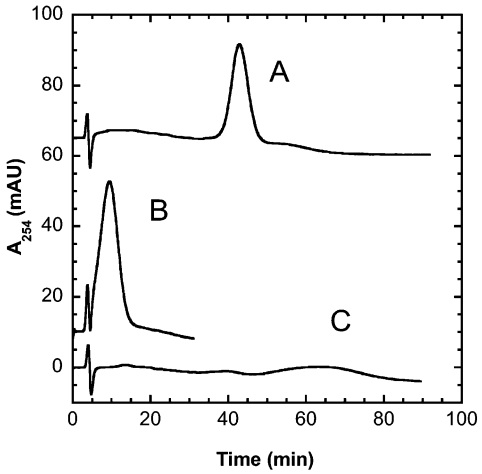

Figure 2. HIC chromatograms for elution of PNAA C_{12}-agtgatctac-$(Glu)_4$ with and without complementary DNA: A, PNAA with DNA; B, DNA; C, PNAA. The DNA sequence, 5′-GTAGATCACT-3′, is complementary to that of PNAA. The incubation time was 2.5 h. Chromatograms A and B were shifted on the ordinate for clarity.

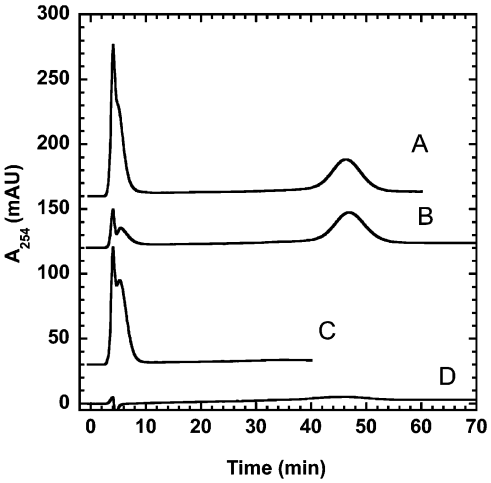

Figure 3. HIC DNA retention with C_{12}-agtgatctac-$(Glu)_4$: A, PNAA with complementary and noncomplementary DNA; B, PNAA with complementary DNA; C, complementary DNA; D, PNAA. The complementary DNA sequence is 5′-GTAGATCACT-3′, and the noncomplementary DNA sequence is 5′-TGTAC-GACTC-3′. Chromatograms A−C were shifted for clarity.

time than the isolated DNA. The pure PNAA sample has a much smaller peak, and can only be fully removed from the column by washing with 0.01 M NaOH.

Replacing the DNA oligomer with a fully noncomplementary DNA strand (5′-TGTACGACTC-3′), but using the same incubation and processing conditions, yields elution of separate PNAA and DNA peaks. When combined with an equimolar amount of complementary DNA, noncomplementary DNA does not inhibit PNAA binding to complementary DNA (Figure 3A), and the PNAA/DNA complex elutes at 47 min as in Figure 3B.

To probe the specificity of the HIC purification process, we repeated the procedure using DNA with a single-base mismatch (5′-GTAGA*G*CACT-3′, mismatch in italics) for the same PNAA. Figure 4 is the HIC chromatogram for the equimolar PNAA/complementary DNA mixture (A), along with the corresponding profile for mismatch DNA (B) and the isolated PNAA (C). The PNAA/mismatch DNA chromatogram shows the mismatch DNA peak eluting at approximately 9 min and the free PNAA peak eluting at 38 min. Comparing with Figure 4C, the small peak observed in Figure 4B at higher elution times is likely isolated PNAA. These results show that the hybridization of PNAA is highly sequence-specific, and even single mismatches can completely arrest binding and recovery. Differences in the duplex melting transition temperatures (T_m) for the various duplexes (20 mM phosphate buffer) corroborate this result: the PNAA/complementary DNA duplex has a T_m of 42 °C, while the PNAA/noncomplementary DNA duplex has $T_m = 22$ °C. This strong dependence of duplex stability on base mismatches is a hallmark of PNAs (*30*) that is retained in the PNAA system.

To confirm that the putative duplex peak actually contains the PNAA and complementary DNA, the peak in Figure 4A was fractionated, desalted, and subjected to UV−thermal analysis and MALDI-TOF mass spectroscopy. The expected hypochromicity was obtained for the duplex ($T_m = 42$ °C), and MALDI-TOF mass spectroscopy revealed the molecular weights for both the PNAA and complementary DNA. The following $[M - H]^-$ values for DNA and $[M + H]^+$ values for PNAA were obtained: 5′-GTAGATCACT-3′, 3026.1, 3048.1 (theoretical 3027 Da); C_{12}-agtgatctac-$(Glu)_4$, 3426.9, 3448.9 (theoretical 3425.7).

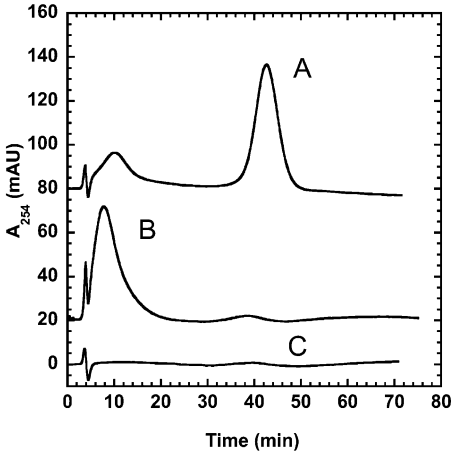

Figure 4. HIC DNA retention comparison with DNA containing a single-base mismatch, 5′-GTAGA*G*CACT-3′ (mismatched base in italics): A, PNAA with complementary DNA; B, PNAA with mismatched DNA; C, PNAA. The complementary DNA sequence is 5′-GTAGATCACT-3′, and the noncomplementary DNA sequence is 5′-TGTACGACTC-3′. Chromatograms A and B were shifted for clarity.

In an effort to challenge PNAA binding to its complement, a relatively large nucleic acid impurity, sonicated calf thymus DNA (0.1−3 kb), was introduced into the incubation mixture in an amount equimolar to that of the complementary DNA (on a nucleobase basis). Figure 5 shows the resulting HIC chromatogram following the incubation of various DNA samples with PNAA. The isolated calf thymus DNA (D) elutes at 21 min. When incubated with the PNAA (C), the calf thymus peak is unaffected. Curve B plots the elution profile for an equimolar mixture of complementary, noncomplementary, and sheared calf thymus DNA, and curve A plots elution for that same mixture with PNAA. The specific binding of PNAA with complementary DNA is marked by the 2-fold reduction in the oligomer peak, while the calf thymus peak is unaffected. Accordingly, the presence of PNAA and DNA in the putative duplex peak was verified by MALDI-TOF mass spectroscopy and UV−thermal analysis.

To pinpoint the effect of the alkane tail of the PNAA on DNA retention, a PNA peptide with the same se-

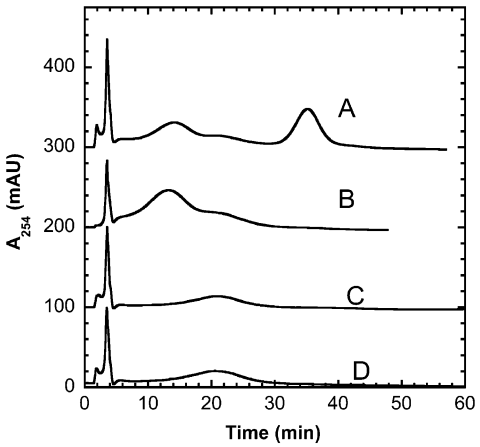

Figure 5. HIC separation of DNA using C_{12}-agtgatctac-$(Glu)_4$. The complementary DNA sequence is 5′-GTAGATCACT-3′, and the noncomplementary DNA sequence is 5′-AAAAAAAAAAAA-AAA-3′. Sheared calf thymus DNA is added to demonstrate specificity of PNAA. Key: A, PNAA with complementary DNA, sheared calf thymus DNA, and noncomplementary DNA; B, complementary, noncomplementary, and sheared calf thymus DNA; C, PNAA with sheared calf thymus DNA; D, sheared calf thymus DNA. Chromatograms A−C were shifted for clarity.

quence as the PNAA was synthesized (OH-agtgatctac-$(Glu)_4$). HIC separation was performed under conditions identical to those of HIC separation of PNAA. As shown in Figure 6, the PNA/DNA duplex has a greater retention time than the PNA and DNA controls, but the holdup of the PNA/DNA duplex is not as great as that of the PNAA/DNA duplex. The significant increase in retention time observed when the PNAA rather than PNA is used shows that the attachment of the alkane tail greatly improves the resolution between tagged and untagged DNA, while not affecting the binding thermodynamics or the sequence specificity.

We also investigated increasing the molar excess of PNAA as well as the incubation time and sample heating temperature to maximize the amount of DNA recovered in the HIC process. The amount of DNA recovered was quantified using an external standard calibration curve for the pure DNA (peak are basis). The percent DNA retained was then calculated from the size of the DNA peak remaining from a PNAA/DNA mixture. As PNAA:DNA molar ratios are increased from 1:1 to 1.5:1 (incubation time 1 min), the percentage of DNA bound to PNAA increases from 72% to 91% (Figure 7). The use of large excesses of PNAA did not increase the percent bound above 91%. A 2.5 h incubation is sufficient to retain greater than 95% of the DNA sample (at a 1:1 PNAA:DNA ratio).

We also attempted to improve the recovery yield by including an "annealing" step, where the PNAA/DNA mixture is heated to 90 °C and cooled to 10 °C at various rates prior to injection. We found that this step did not improve recovery; in fact for higher cooling rates, the recovery was actually diminished (Figure 8). Taken together, these results indicate that the amount of PNAA used can best be minimized by increasing the incubation time prior to injection. We also point out that, for many applications (such as sensing), it may not be necessary to recover all DNA, and in many cases large excesses of PNAA can be expected if recovery of an unknown, trace amount of DNA is desired.

Overhanging bases at various positions on the DNA target strand were used to probe the influence on the lipid−column interaction and PNAA/DNA retention. DNA oligomers with flanking cytosine (C) residues were

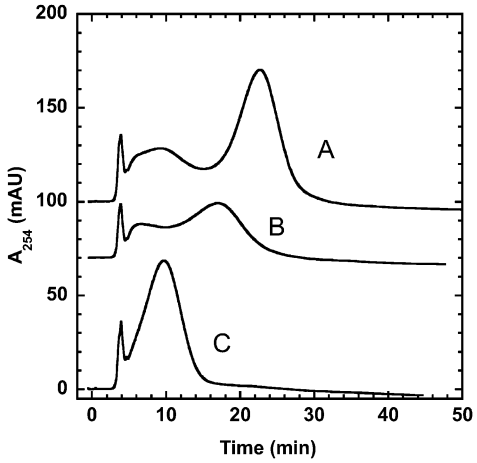

Figure 6. HIC DNA retention with PNA peptide (OH-agtgatctac-$(Glu)_4$) compared to PNAA: A, PNA peptide with complementary DNA; B, PNA peptide alone; C, complementary DNA. The complementary DNA sequence is 5′-GTAGATCACT-3′. Chromatograms A and B were shifted for clarity.

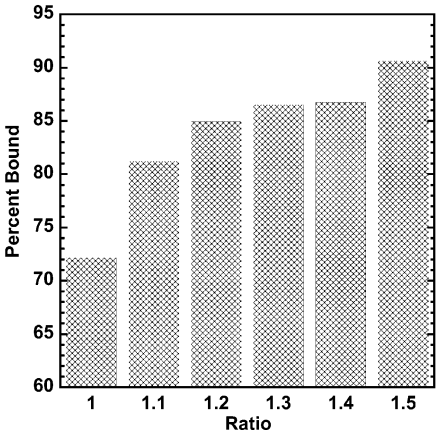

Figure 7. Percent of complementary DNA bound to PNAA as the PNAA:DNA ratio is increased. The complementary DNA sequence is 5′-GTAGATCACT-3′. Data were calculated on the basis of column calibration of target DNA.

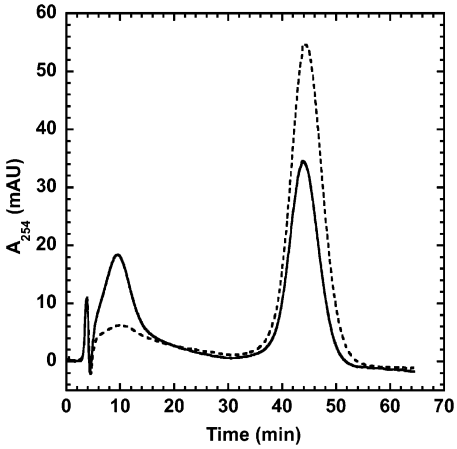

Figure 8. Retention of complementary DNA using PNAA with annealing (solid line) and without annealing (dashed line). Annealing was performed by heating to 90 °C and then cooling from 90 to 10 °C at 5 °C/min. The complementary DNA sequence is 5′-GTAGATCACT-3′.

analyzed for their effect on HIC retention. Figure 9 shows that the placement of overhangs decreases the retention time of the duplex peak. PNAA bound to a DNA target with no overhangs exhibits a retention factor of 12.2, while bases on the 5′ end of the DNA molecule decrease

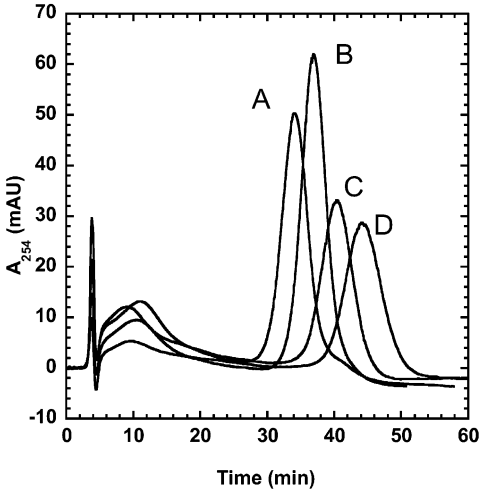

Figure 9. HIC chromatograms for PNAA binding to DNA oligomers with overhanging C bases at various positions near the target sequence: A, 5′ and 3′ overhang (5′-CCCCGTAGATCACTCCCC-3′); B, 3′ overhang (5′-GTAGATCACTCCCC-3′); C, 5′ overhang (5′-CCCCGTAGATCACT-3′); D, no overhang (5′-GTAGATCACT-3′).

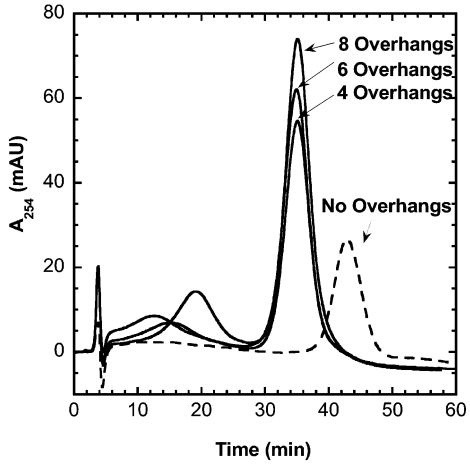

Figure 10. HIC chromatograms with overhanging C bases of various lengths (x) flanking the target DNA sequence (5′-$(C)_x$GTAGATCACT$(C)_x$-3′).

this value to 11.0, and the 3′ overhangs yield a retention factor of 10.1. Overhanging bases on both the 5′ and 3′ ends of the DNA target yield a retention factor of 9.2. Varying the overhang length does not alter duplex retention as demonstrated in Figure 10. We show that as the number of overhanging bases (on both 3′ and 5′ ends) on the free DNA target increases the retention time increases. This suggests that an upper limit for the length of the DNA target exists, at which point the retention times of the unbound DNA and PNAA/DNA duplex will be identical. Using a 60 bp DNA target with 25 overhanging thymine (T) bases on both the 5′ and 3′ ends of a complementary sequence (Figure 11), we find the isolated DNA target (dashed line) elutes slightly before the PNAA/DNA complex (solid line). The slight overlap of the peaks indicates that, under these conditions, ssDNA targets longer than 60 bp require attachment of additional surfactants for good resolution from untagged ssDNA of the same length.

Attempts were also made to optimize the HIC gradient from our standard 30 min linear gradient, giving a PNAA/DNA elution at approximately 45 min. A flatter gradient of 60 min delays PNAA/DNA elution until approximately 70 min, while a steeper gradient of 15 min

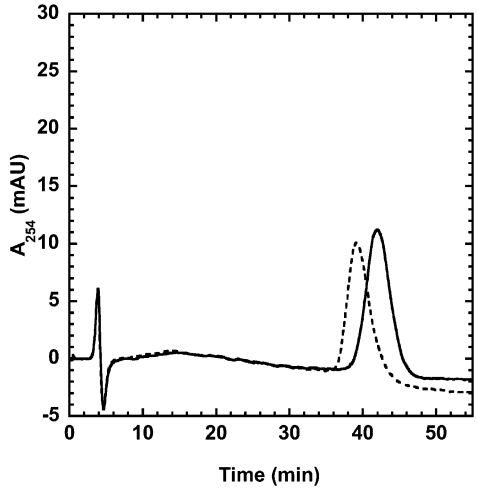

Figure 11. HIC chromatogram for PNAA binding to a 60 bp DNA target strand (5′-$(A)_{25}$GTAGATCACT$(A)_{25}$-3′): solid line, PNAA with a 60 bp DNA target; dashed line, 60 bp DNA target alone.

causes the PNAA/DNA duplex to elute at ~30 min (data not shown). The resolution and sharpness of the PNAA peak were not affected significantly by these modifications.

Discussion

The use of PNAAs as affinity tags for DNA oligomers in HIC requires that the interaction of the PNAA/DNA with the nonpolar HIC medium be significantly more favorable than that of the unmodified DNA. The unmodified PNAA is retained on the medium throughout the gradient elution, and can only be removed by a wash with 0.01 M NaOH. The unbound DNA oligomers adsorb to the medium initially, but elute as the $(NH_4)_2SO_4$ concentration is decreased. This indicates that the chemical composition of the overhanging bases plays a role in the elution time observed for the PNAA/DNA duplex. Diogo et al. (*31*) studied the interaction of various homooligonucleotides in HIC, and found that homothymine had a stronger interaction with the HIC medium compared with homoadenine. This was ascribed to the formation of secondary structures in homoadenine, decreasing the exposed hydrophobic area of the strand. Since both overhangs used in our study are homopyrimidines, we expect a stronger interaction with the HIC medium than might be observed using homopurines. Therefore, the results of Figure 11 are likely a worst-case scenario, and even better resolution could be obtained for strands with homopurine or mixed-base overhangs.

Figure 9 compares homocytosine overhangs on either end of the PNAA binding sequence of the DNA. DNA with overhanging bases on the 3′ end only (same side as the PNAA alkane tail in the duplex) gives a much greater decrease in elution time than that with a 5′ overhang only. This indicates that overhanging DNA bases can reduce the hydrophobic exposure of the alkane tail, decreasing the affinity of the PNAA/DNA duplex for the HIC medium. In choosing a sequence for targeting, it appears that the best results will be obtained by targeting the 3′ terminus of DNA, so that no overhanging bases will sequester the alkane tail and reduce its affinity for the medium. Figure 9 also shows that the presence of 4−8 C residues on either side of the DNA target sequence *decreases* the elution time of the PNAA/DNA duplex compared to the duplex without overhangs. Conversely,

the presence of 25 overhanging T residues yields about the same elution time for the PNAA/DNA duplex compared to the duplex without overhangs. While the reason for this apparent discrepancy is not entirely clear, it is likely that the longer, unbound DNA overhangs increase the affinity of the PNAA/DNA duplex for the column, reversing the decrease in affinity observed when 3′ overhangs interfere with the PNAA alkane tail.

The association dynamics of PNAA with DNA and with the HIC medium throughout the separation process show that the binding of the PNAA with the target DNA strand is not influenced by the gradient conditions of HIC processing. Interestingly, the PNAA/mismatch DNA duplex (Figure 4) actually has a higher T_m than the PNAA/complementary DNA duplex when measured in the HIC buffer. In the 1.5 M $(NH_4)_2SO_4$ with 10 mM Tris−HCl buffer, the mismatched DNA duplex has a T_m of 52 °C, compared to 40 °C for the complementary DNA. Hence, the only way PNAA would discriminate DNA oligomers would be via a preferential binding during the incubation step prior to HIC. After injection, any unbound DNA is quickly sequestered from the PNAA so that binding is impossible, even if the T_m is increased by the processing conditions.

As reported previously (*17*), the cmc for the PNAA in these buffers is about 20 μM. Since we performed our incubations at 50 μM, just above the cmc, PNAA/DNA hybridization involves binding of PNAA *micelles* or other aggregates to DNA. After injection, the system is diluted by HIC buffer and the PNAA concentration falls below the cmc. At this point, the aggregates likely remodel into isolated PNAA/DNA complexes that interact with the HIC column. One explanation for the need to either add an excess of PNAA or wait more than 1 h to achieve full recovery of DNA is that the activity of PNAA is compromised when it is packed into micelles. The decrease in recovery after heating to 90 °C may indicate a partial precipitation of PNAA on heating that decreases the availability of PNAA for binding. Several nonionic surfactants are known to exhibit clouding on heating; see, for example, ref *32*. These observations suggest that the use of cosurfactants to form mixed micelles of PNAA may act to improve DNA recovery. Additionally, we might expect bound micelles to have a much higher retention time in HIC and other separation methods. Such mixed micelles may also be appropriate for micellar electrokinetic separations or aqueous two-phase extractions.

Recognition of DNA by PNAA in solution offers, potentially, a kinetic advantage over other surface-based approaches to separate DNA by probe hybridization. The rate constant for PNA/DNA hybridization where one strand is immobilized on a surface was measured to be 12 ± 1.8 mM^{-1} s^{-1} (*33*), while the association rate constant for a similar PNA/DNA system in solution was found to be 8600 ± 1700 mM^{-1} s^{-1} (*34*). Since in our system PNAA/DNA binding is done in solution prior to chromatographic injection, we take advantage of the faster binding kinetics. Additionally, solution hybridization is not subject to the deactivation of surface-bound probes that may occur when nontarget DNA or other contaminants nonspecifically adsorb. We believe this approach to nucleic acid purification will be of interest to researchers in the fields of molecular biology, DNA diagnostics, and basic research.

Conclusions

We have shown that the peptide nucleic acid amphiphile C_{12}-agtgatctac-$(Glu)_4$ binds to a complementary DNA sequence with high specificity and selectivity while retaining its stability throughout the HIC chromatographic process, allowing for efficient sequence-specific nucleic acid separations. Nearly quantitative recovery of DNA can be achieved at incubation times on the order of minutes, without the need to heat or wash the sample to remove partially bound DNA. PNAA/DNA duplex peaks are well resolved from DNA impurities for short sequence lengths, but for longer sequences triplex formation or multiple binding sites on the target DNA may be necessary to retain similar capacity factors. Ongoing efforts involve targeting double-stranded DNA samples including plasmids and PCR products by taking advantage of the strand-invasion properties of PNA. We are also investigating the phase behavior and microstructure of PNAA micelles to separate DNA using two-phase aqueous micellar systems and micellar electrokinetic capillary chromatography.

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