Sequence Specific Separation of Target DNA in Micellar Electrokinetic Chromatography

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Submitted to: TA008- New Developments in Bioanalytical CE and Microdevice Technology

We present the use of hydrophobically labeled peptide nucleic acid (PNA) probes for the multiplexed detection and separation of oligomeric DNA in micellar electrokinetic chromatography (MEKC). MEKC is an operational mode of capillary electrophoresis in which a surfactant is added to the running buffer at a concentration above its critical micelle concentration. In addition to differences in electrophoretic mobilities, separation of compounds can be achieved through differences in the partitioning of a molecule into the micellar core of the migrating surfactant assemblies. Due to the nearly constant charge-to-mass ratio of DNA targets, the electrophoretic mobility of DNA is largely insensitive to its length and sequence. In addition, the negatively charged backbone of DNA renders it sufficiently hydrophilic, limiting interaction with the surfactant assemblies. Interaction with the micellar subphase has been promoted through the use of a PNA probe appended to an aliphatic tail, forming a peptide nucleic acid amphiphile (PNAA).

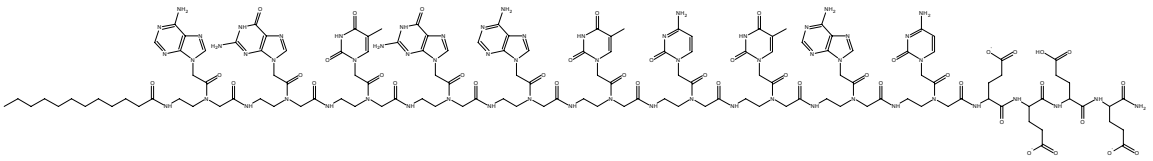

Figure 1 Structure of the C_{12} tail PNAA used in this study. The left portion of the molecule is the 12 carbon long aliphatic tail. The center portion represents the PNA sequence, agtgatctac. The right portion are four glutamic acid residue required for water solubility.

Previous work has established that PNAA will sequence specifically bind target DNA, detectable by hydrophobic interaction chromatography and capillary zone electrophoresis. In capillary zone electrophoresis, the PNAA probe is sufficient to alter the DNA's electrophoretic mobility for short DNA oligomers. Targeting longer sequences of DNA becomes problematic though as the impact of the aliphatic tail is diminished. In MEKC, the effective mobility of an analyte is a function of many factors, the most important of which are the analyte's intrinsic electrophoretic mobility, the partitioning behavior of the analyte with the pseudo-stationary phase, and the mobility of the micellar subphase. This additional separating mechanism obtainable through the use of MEKC offers improved resolving power between free and target DNA. Alterations of the pseudo-stationary phase composition and the PNAA probe architecture have been performed and demonstrate a significant impact on the partitioning behavior of the PNAA/DNA duplex.

The ability to tune the partitioning behavior of the PNAA/DNA duplex coupled with the extreme sequence specificity of the PNAA probe offers the unique ability to selectively

separate a target DNA sequence from a complex mixture of non-complementary DNA oligomers of varying length. Furthermore, by coupling a specific aliphatic tail to the desired PNA probe sequence, the migration time of a DNA target of known length can be predicted, and the introduction of a suitably dissimilar PNAA probe leads to the emergence of a second PNAA/DNA duplex population, with its own characteristic migration time. Through judicious choice of multiple aliphatic tail lengths and respective PNAA probe sequences, multiplexed detection and separation of oligomeric DNA of various lengths from 10 to ~500 is possible.

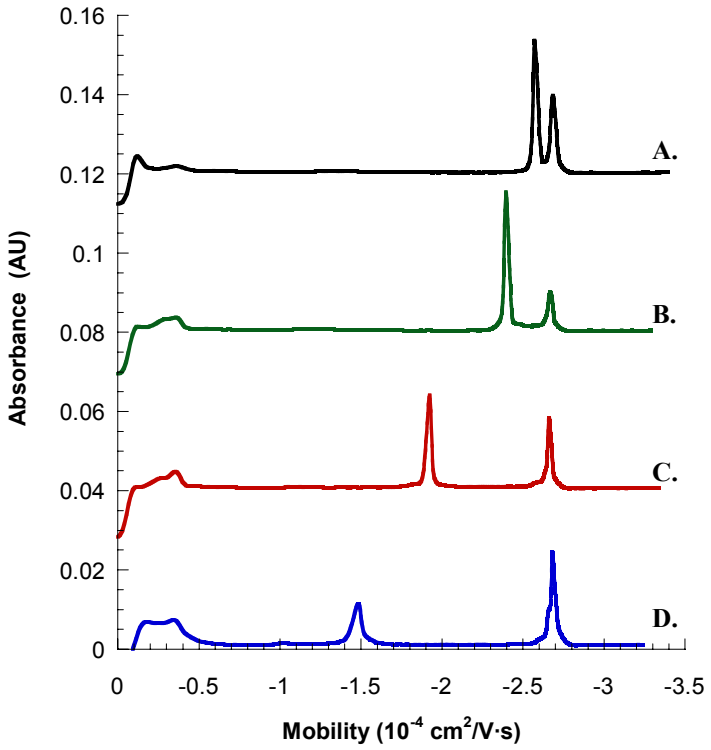

Figure 2 MEKC of C_{12}-C_{18} tailed PNAA with complementary 60 base DNA. The valley at mob=0 is the surfactant-free sample plug followed by the free PNAA peak and the peak at mob= -2.7 is unbound DNA. The peaks from mob = -1.5 to -2.5 are the PNAA/DNA duplex, **A.**) C_{12} Tail PNAA, **B.**) C_{14} Tail PNAA, **C.**) C_{16} Tail PNAA, **D.**) C_{18} Tail PNAA.