Direct Quantitative Analysis of Multiple microRNAs (DQAMmiR) with Peptide Nucleic Acid Hybridization Probes

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Introduction

DQAMmiR is a hybridization-based approach capable of direct, quantitative analysis of multiple miRNAs by capillary electrophoresis (CE).[1] In which, the excess of the DNA hybridization probes is separated from the miRNA-probe hybrids, and the hybrids are separated from each other in CE using two types of mobility shifters: single-strand DNA binding protein (SSB) added to the CE running buffer and peptide drag tags conjugated with the probes (Figure 1).

Here we introduced the second-generation DQAMmiR which utilized peptide nucleic acid (PNA) rather than DNA hybridization probes and requires no SSB in the CE running buffer. PNA probes are electrically neutral while PNA–miRNA hybrids are negatively charged, and this difference in charges can be a basis for separation of the hybrids from the probes. Another essential step for DQAMmiR is to separate the hybrids from each other. Theoretical estimation of the electrophoretic mobility of PNA–miRNA hybrid indicated that the PNA–miRNA hybrids with different numbers of base pairs could be separated from each other without drag tags, while the peptide drag tags on the PNA probes would still be useful mobility shifters for PNA–miRNA hybrids of the same length. The experimental results confirmed this, suggesting that DQAMmiR with PNA hybridization probes would be a promising method for practical applications.

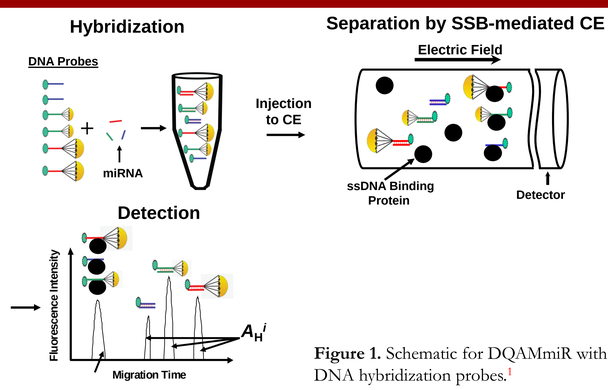

Figure 1. Schematic for DQAMmiR with DNA hybridization probes.[1]

Results

1. Separation of the excess of a PNA probe from a PNA–miRNA hybrid by CE

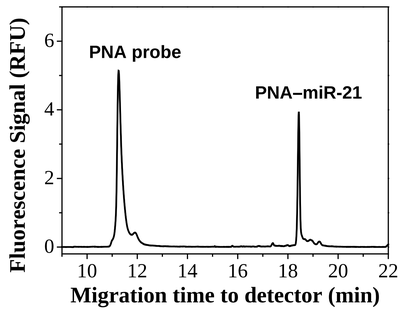

Figure 2. CE separation of a PNA probe from a PNA–miR-21 hybrid in a running buffer of 20 mM Borax, 20% acetonitrile, pH 9.0. The sample was prepared by incubating 2 nM miR-21 with 10 nM PNA probe with a sequence fully complementary to miR-21. Separation was driven by an electric field of 312.5 V/cm at 20 °C.

2. Theoretical prediction of the electrophoretic mobility of PNA–miRNA hybrid

$$\mu_{hyb} = \frac{e}{2\pi z_i \eta \lambda_B}\left[\left(\ln\frac{2N_{hyb}b}{d_{hyb}} - 0.72\right)^{-1} + \frac{3R_{H,tag}}{N_{hyb}b}\right]^{-1}$$

$$R_{H,tag} = (0.22 \pm 0.11) N_{tag}^{0.57\pm0.02}$$

μ_{hyb}: electrophoretic mobility of PNA–miRNA hybrid.
N_{hyb}: number of base pairs.
b: rise per base pair.
d_{hyb}: diameter of the helix
$R_{H,tag}$: hydrodynamic radium of the peptide drag tag.
N_{tag}: number of amino acid in the peptide drag tag.

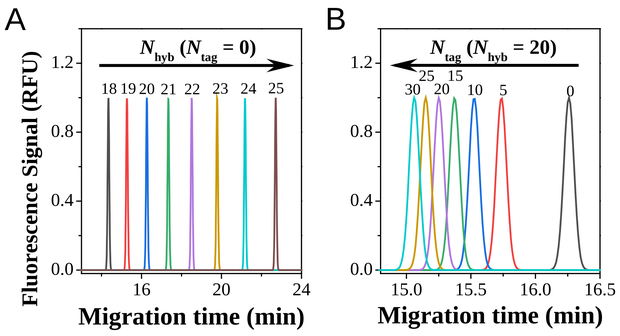

Figure 3. Simulated electropherograms of different PNA–RNA hybrids. **A**) PNA–RNA hybrids with numbers of base pairs (N_{hyb}) in a range of 18−25. **B**) 20-bp PNA–RNA hybrids with lengths (numbers of amino acids) of peptide tags (N_{tag}) in a range of 0−30. Migration times of peaks shown above were estimated by using $\mu_{EOF} = 3.14 \times 10^{-4}$ cm^2·V^{-1}·s^{-1} which was experimentally measured for our CE running buffer containing 20 mM Borax and 20% (v/v) acetonitrile at pH 9 at 20 °C.

3. Proof-of-principle experiments of DQAMmiR with PNA hybridization probes.

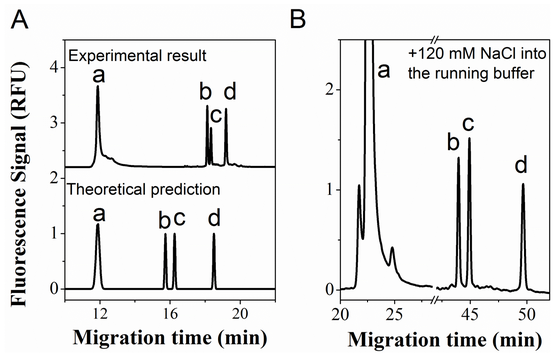

Figure 4. A) Comparison of experimental and predicted separation of three PNA–miRNA hybrids in 20 mM Borax, 20% acetonitrile, pH 9. **B**) Baseline separation of the three hybrids from each other by using a high-ionic-strength CE running buffer: 20 mM Borax, 120 mM NaCl, 20% acetonitrile, pH 9.0. Peak assignment: (a) excess PNA probes, (b) PNA–miR-147a (20-bp, 5aa), (c) PNA–miR-378g (20-bp), and (d) PNA–miR-21 (22-bp).

4. Quantitation of three miRNAs by DQAMmiR with PNA hybridization probes.

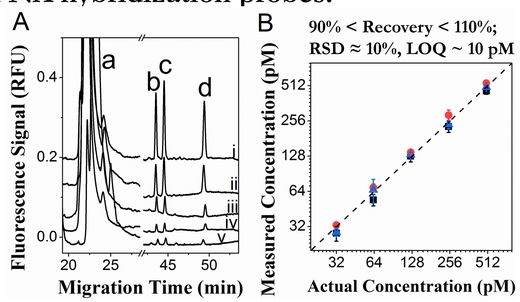

Figure 5. A) Electropherograms of PNA facilitated-DQAMmiR measurements. Peak assignment: (a) excess PNA probes, (b) PNA–miR-147a (20-bp, 5aa), (c) PNA–miR-378g (20-bp), (d) PNA–miR-21 (22-bp). Electropherograms i–vi correspond to target concentrations of 500, 250, 125, 63, and 32 pM, respectively. **B**) Quantitation of three miRNAs simultaneously by PNA-facilitated DQAMmiR. Concentrations measured with DQAMmiR are shown with respect to their actual values determined by light absorbance at 260 nm. miR-21, miR-147a, and miR-378g, are represented by black rectangles, red circles, and blue triangles, respectively. The dashed line ($y = x$) represents a line corresponding to 100% recovery. Error bars show one standard deviation from mean values obtained from three experiments.

Conclusions

DQAMmiR utilizing electrically-neutral PNA probes instead of negatively-charged DNA probes was achieved.

- The negatively-charged PNA-miRNA hybrids were separated from the neutral PNA probes by CE without any additional mobility shifter, such as SSB.
- The PNA–miRNA hybrids can be separated from each other by utilizing their differences in either (i) the number of base pairs, or (ii) the length of peptide drag tags.

Our proof-of-principle study demonstrated that PNA-facilitated DQAMmiR was able to quantify three miRNAs simultaneously with high accuracy (90% < Recovery < 110%) and precision (RSD ~ 10%) with LOQ of 14 pM by using a commercial CE instrument.

1. Wegman, D. W.; Krylov, S. N. *Angew. Chem. Int. Ed.* **2011**, *50*, 10335-10339.

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