Tunable Aptamer Capillary Electrophoresis and Its Application to Protein Analysis

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Development of protein biomarkers for disease diagnosis and treatment requires the determination of multiple proteins that are present at trace levels. The sensitivity of the current assays for multiple proteins is often inadequate for detection of low-abundance proteins. Although affinity polymerase chain reaction, affinity capillary electrophoresis, proximity ligation, and nanotechnology have improved detection for specific proteins, these assays are usually applicable to a single protein at a time.

We describe here a tunable aptamer capillary electrophoresis assay enabling the ultrasensitive analysis of multiple proteins and protein isoforms. The key concept is tuning the electrophoretic mobility of proteins with DNA aptamers to achieve efficient separation of multiple proteins or protein isoforms. We introduce aptamers of varying nucleotide length as charge modulators to modify the electrophoretic mobility of proteins, tailored for the separation of the various protein-aptamer complexes in free solution. This systematic approach extends the applications of charge modulation in affinity assays and end-labeled free-solution electrophoresis of DNA.

The principle of modulating the mobility of proteins may be expressed in the following equations, showing the dependence of electrophoretic mobility (μ) on the net charge (Z) and the mass (M) of the protein. In free-zone capillary electrophoresis, the electrophoretic mobility of a protein is proportional to its net charge, and inversely proportional to the frictional forces acting upon it in solution.

\[
\mu \approx C_p \frac{Z}{M^{\alpha}} 
\]  
(1)

where \( C_p \) is a constant for a given protein, and \( \alpha \) is a factor (0−1) describing the shape of the protein molecule. Upon the binding of an aptamer, the electrophoretic mobility of the protein is shifted to

\[
\mu_c \approx C_p \frac{Z + n\Delta Z}{(M + n\Delta M)^{\alpha}} 
\]  
(2)

where \( n \) is the number of nucleotides making up the aptamer, \( \Delta Z \) is the change in charge per nucleotide, and \( \Delta M \) is the change in size per nucleotide. Under the pH conditions (pH 7−9) typically used for capillary electrophoresis separation, \( \Delta Z \) is nearly −1 owing to the phosphate group in the nucleotide and the effect of the counterions in solution. Because most proteins carry a small net charge, and the change in mass \( \Delta M \) (~320 amu) per nucleotide is much smaller than the mass of a protein \( M \), the contribution of \( n\Delta Z \) to the shift in mobility is often much more significant than that of \( n\Delta M \). Therefore, eq 2 can be approximated to

\[
\mu_c \approx C_p \left( \frac{Z}{M^{\alpha}} + \frac{n\Delta Z}{M^{\alpha}} \right) 
\]  
(3)

Thus, the electrophoretic mobility of the proteins can be rationally controlled by modulating the length of the aptamer \( n \), taking into account the size of the protein \( M \), to achieve the desired value of the \( n\Delta Z/M^{\alpha} \) term in eq 3.

To demonstrate the proof of principle, we first showed the tuning of mobility for the human immunodeficiency virus reverse transcriptase (HIV-RT) by binding it with a 49-nt aptamer, an 80-nt aptamer, and two 81-nt aptamers (Figure 1a). The aptamers, having a similar mass-to-charge ratio, migrate through the capillary at a similar mobility (−2.81 × 10^−4 cm² V⁻¹ s⁻¹). Upon binding of HIV-RT to the aptamers of varying length, the mobilities are shifted to −0.58 × 10^−4, −0.83 × 10^−4, and −1.45 × 10^−4 cm² V⁻¹ s⁻¹, respectively. An excellent linear association \((r^2 = 0.999)\) between the mobilities of the three HIV-RT aptamer complexes (Figure 1a) and the length of aptamers supports the validity of eq 3. Similarly, the mobility of the thrombin−aptamer complexes shifted to −2.22 × 10^−4 cm² V⁻¹ s⁻¹ when using a full-length (76 nt) aptamer and further shifted to −1.68 × 10^−4 cm² V⁻¹ s⁻¹ when using a truncated (38 nt) aptamer (Figure 1b). Aptamers of different lengths can be made by maintaining the core sequence responsible for binding and either extending or truncating the aptamers at the ends. Thus, the mobilities of both large and small proteins (e.g., HIV-RT, 120 kDa; thrombin, 36 kDa) can be readily tuned using aptamers of appropriate length to achieve the desired separation.

Having achieved tunable mobility of proteins through their binding to tailored aptamers, we further applied the principle to the analysis of HIV-RT, thrombin, platelet derived growth factor (PDGF-BB), and human immunoglobulin E (IgE). Figure 2a shows a series of electropherograms from the analyses of these four proteins in mixture solutions containing varying concentrations of proteins (1−100 nM) and the corresponding aptamers for these proteins. The four protein-aptamer complexes are well resolved from one another and from the free (unbound) aptamers. Furthermore, adsorption of the basic proteins (IgE, pI ≈ 9.0; PDGF-BB, pI = 9.5−10.5) on the negatively charged fused-silica capillary surface would have been a problem, if there were no aptamers binding to these proteins. The binding of the aptamers to the proteins makes the complex negatively charged, thereby eliminating the adsorption problem and focusing the protein-aptamer complexes into narrow zones. The ability to focus proteins and to tune their electrophoretic mobility using aptamers is the key to the successful analysis of multiple proteins using free zone capillary electrophoresis.

Another important benefit of aptamer binding to the proteins is the incorporation of fluorescent aptamers as probes to enable laser induced fluorescence (LIF) detection of proteins that are otherwise not amenable to high sensitivity LIF detection. We labeled the aptamers at the 5’ end with highly fluorescent 6-carboxyfluorescein and excited it with an argon ion laser (488 nm). The binding of fluorescent aptamers to nonfluorescent proteins makes proteins amenable to highly sensitive LIF detection at 515 nm. Detection limits were 250 pM for IgE, 100 pM for HIV-RT and thrombin,
by reducing any nonspecific binding of the serum proteins to the fluorescent aptamers.

Building on the success of separation and detection of the four proteins, we also applied the tunable aptamer capillary electrophoresis technique to the analysis of PDGF isomers, (Figure 2b). The sequences of PDGF A and B chains are ~60% identical, and the molecular weights of PDGF-AB (27 kDa) and PDGF-BB (25 kDa) are similar, making their separation difficult. However, using a 33-nt aptamer that binds to the B chain but not to the A chain of PDGF, we were able to tweak the electrophoretic mobilities of the PDGF isomers for their separation. As a result, PDGF-BB bound to a single aptamer is well resolved from PDGF-BB that is bound to two aptamer molecules (Figure 2b). To our knowledge, this is the first demonstration of the differentiation and detection of PDGF-AB and PDGF-BB isomers in a mixture by using an aptamer as a fluorescent probe.

In conclusion, we have developed a tunable aptamer capillary electrophoresis technique and demonstrated its application to the simultaneous determination of pM levels of four proteins in a single analysis. The principle was also applied to the separation and detection of two PDGF isomers. The multiplex capability and high sensitivity were accomplished by introducing tunable aptamers both as charge modulators for electrophoretic separation and as fluorescent affinity probes for ultrasensitive fluorescence detection. The assay is not limited to the four proteins shown here, and the principle can be extended to the simultaneous analysis of other species to which aptamers can bind, including proteins, peptides, carbohydrates, and whole cells. The throughput of the assay can be further enhanced by using electrophoresis systems with multiple capillaries9 or microfluidic devices with multiple channels. The assays for multiple proteins are potentially useful for biomarker development, clinical testing, and medical diagnostics.

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Supporting Information Available: Detailed experimental procedures and the sequence of aptamers used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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