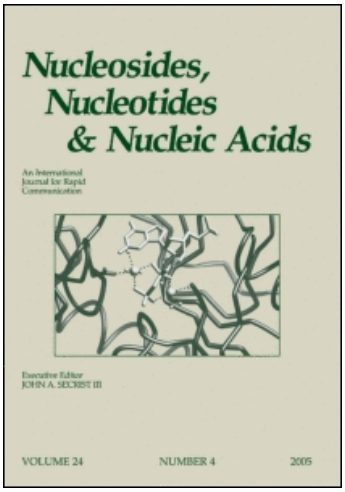

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Application of Microchip Electrophoresis in the Analysis of RNA Aptamer-Protein Interactions

Fumiko Nishikawa[a]; Hidetoshi Arakawa[b]; Satoshi Nishikawa[c]
[a] Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan [b] School of Pharmaceutical Sciences, Showa University, Shinagawa, Tokyo, Japan [c] Age Dimension Research Center, AIST, Tsukuba, Ibaraki, Japan

To cite this Article Nishikawa, Fumiko , Arakawa, Hidetoshi and Nishikawa, Satoshi(2006) 'Application of Microchip Electrophoresis in the Analysis of RNA Aptamer-Protein Interactions', Nucleosides, Nucleotides and Nucleic Acids, 25: 4, 369 — 382
To link to this Article: DOI: 10.1080/15257770600683953
URL: http://dx.doi.org/10.1080/15257770600683953

Nucleosides, Nucleotides, and Nucleic Acids, 25:369–382, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600683953

Taylor & Francis
Taylor & Francis Group

APPLICATION OF MICROCHIP ELECTROPHORESIS IN THE ANALYSIS OF RNA APTAMER-PROTEIN INTERACTIONS

Fumiko Nishikawa □ *Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan*

Hidetoshi Arakawa □ *School of Pharmaceutical Sciences, Showa University, Shinagawa, Tokyo, Japan*

Satoshi Nishikawa □ *Age Dimension Research Center, AIST, Tsukuba, Ibaraki, Japan*

□ *DNA and RNA can be separated by microchip electrophoresis (ME) and detected using an intercalating fluorescent dye. The advantages of this method are short sensing times (< 3 min), avoidance of a radioisotope labeling detection system, relatively low costs, and reduced labor intensity. In the present study, RNA aptamer-protein or -peptide interactions were analyzed using ME and the regression of free aptamers corresponding to unbound RNA was detected as the target protein or peptide increased in a dose-dependent manner. Our results demonstrate the applicability of this method to simple, rapid ligand screening in the interactions between oligonucleotides and their targets.*

Keywords *In vitro* selection; Microchip electrophoresis; RNA aptamer; SYBR gold

INTRODUCTION

Aptamers are nucleic acid ligands that recognize a wide range of target molecules with high affinity and specificity. They are generated by *in vitro* selection of a randomized oligonucleotide pool in a technique known as sys-

Received 7 December 2005; accepted 10 January 2006.

We thank Dr. K. Ohno and Mr. C. Nakata (Showa University, Japan) for help with the experimental technique. We also thanks Mr. H. Nishijima (KAINOS Laboratories, Inc., Japan) for supporting ME instrument. We gratefully acknowledge Mr. S. Sekiya and Dr. P. K. R. Kumar for providing ΔNS3 and the RE peptide.

This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

Address correspondence to Satoshi Nishikawa, Age Dimension Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan. E-mail: satoshi-nishikawa@aist.go.jp

tematic evolution of ligands by exponential enrichment (SELEX).[1–3] The development of *in vitro* selection and amplification technology has allowed the identification of specific aptamers that bind target molecules with high affinity and even discriminate between closely related targets. Their binding affinities are comparable to, or higher than, those of antibodies and antigens. In comparison to antibodies, aptamers have the advantages of (a) simple preparation by chemical or enzymatic synthesis, (b) the potential to introduce nuclease resistance by modification of 2′ OH, (c) thermal stability, (d) discrimination of structural differences, (e) ease of surface attachment for analysis, (f) ease of binding property modification through minor changes in sequence, (g) minimization of experimental animal usage, and (h) preparation for targets that are toxic or not inherently immunogenic. Aptamers are suitable as analytical, diagnostic, and therapeutic tools in applications requiring molecular recognition and are consequently gaining increased attention for their potential uses (reviewed in Tombelli et al.[4]).

Several techniques have been used in the studies of binding between aptamers and proteins: nitrocellulose filtration, affinity separation such as covalent binding to a titer plate or streptavidin (SA)-coated beads, surface plasmon resonance (SPR) technology, and mobility shift assays. With the exception of SPR, all of these methods necessitate radioisotope or fluorescent labeling of a probe (oligonucleotide or protein) for detection purposes. Electrophoresis does not require labeled probes and can be used for efficient separation of free aptamers and aptamer-target complexes by the changes in electrophoretic properties that arise from structural transitions or size changes.

The miniaturization of analytical and biochemical instruments has developed rapidly in the last decade. Since the first demonstrations in the 1990s by Manz et al.[5] and Harrison et al.,[6] many improvements have been made to the technique of microchip electrophoresis (ME). In addition to peptide, protein, and chiral compound analysis, ME can be used to size DNA and RNA and analyze DNA sequences. The principle of ME in electrophoretical separation is similar to that of capillary electrophoresis (CE), which has recently been demonstrated by Boser et al. as an alternative SELEX selection procedure (CE-SELEX).[7–10] ME offers the following features: (a) minimized sample volume, (b) quick measurement with simple procedures, and (c) digital management of analytical results. It can be used in the biotechnological and medical fields to realize cost reduction and speed up complicated analysis by microchips[11–13] (reviewed in Zhang et al.[14]).

In the current study, we examined the use of ME to detect RNA aptamer-target interactions without any modification of the aptamer. The system showed an RNA concentration limit for detection, with a comparable sensitivity to the K_d value.

MATERIALS AND METHODS

Preparation of RNA

dsDNA was generated by PCR amplification of plasmids using previously described primers[15–19] and EX taq (TAKARA). It was used as a template for *in vitro* transcription by T7 RNA polymerase (T7 Ampliscribe Kit, Epicentre Technologies) as previously described.

Proteins and Peptides

HCV NS3 and HCV NS3 protease domain proteins were expressed and purified as previously described.[15,16] Human activated protein C was kindly provided by Dr. T. Nakagaki (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). GST was expressed using the GST expression vector pGEX-6P-1 and purified according to the manufacturer's instructions (Amersham Biosciences). Protein concentrations were measured using a Bio-Rad protein assay (BioRad, Hercules, CA). RE peptides that spanned the arginine-rich region of the Tat protein of human immunodeficiency virus type-1 (HIV-1) were synthesized chemically and purified by HPLC: RE (amino acids 49-86, RKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDPTGPKE, 38 mer). Compositions were confirmed by reverse-phase HPLC (Peptide Institute, Inc.) after hydrolysis.

Electrophoresis

To analyze RNA-protein interactions, free RNAs were analyzed with an ME instrument (model SV1100; Hitachi Electronics Engineering Co. Ltd., Tokyo, Japan). The wells were connected with a 0.1-mm-wide, 0.03-mm-deep cross-channel with a 10-mm-long injection channel and a 45-mm-long separation channel in each lane (Figure 1). The channel was filled with 0.6% hydroxypropyl methylcellulose (HPMC) (Sigma) in 1 × TBE buffer containing 0.5 μg/ml ethidium bromide (Sigma), a 1:10,000 dilution of SYBR Green I (TAKARA) or 1:10,000 dilution of SYBR Gold (Molecular Probes, Inc.), and the reaction solution containing the internal standard was loaded into the sample well of the microchip. Fluorescein sodium salt (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) was used as an internal standard at a final concentration of 1 μM.

The program was run at 300 V for 60 s (injection time), at 750 V for 100 s (separation time), and at a return voltage of 130 V. Detection was accomplished using a blue light-emitting diode (LED; excitation wavelength 470 nm; emission wavelength 580 nm). The observed peak intensity of RNA was normalized by dividing by the peak intensity of internal standards. Prior to sample analysis, the chip was pre-run by injection of binding buffer alone. Any remaining sample solution was removed from the sample well and the

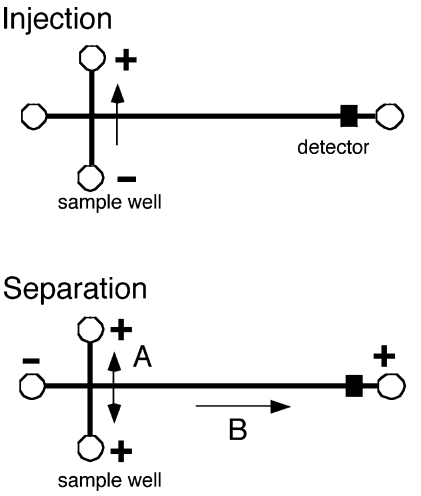

FIGURE 1 Schematic drawing of the microchip electrophoresis (ME) apparatus. The arrow shows the direction of electrophoresis. White circles represent the wells connected with the channels. In the injection mode, the program was run at 300 V for 60 s. In the separation mode, the program was run for 100 sec with a return voltage (A) of 130 V and a separation voltage (B) of 750 V. The samples in the cross-junction then migrated into the separation channel. The detector uses an LED light source.

well rinsed with deionized water before reusing the lane for loading of the next sample solution. Experiments were performed at least twice.

Binding Reaction

We followed the protocol previously described for each RNA aptamer.[15–19] Before mixing, RNAs were denatured in their individual binding buffer at 90°C for 2 min and allowed to cool at room temperature for 10 min, following which the internal standard was added. The reaction mixtures (10 μl) were incubated at room temperature for 30 min and analyzed by ME. The binding buffers used are as follows: aptamer #5, G925-s50 [25 mM Mops-NaOH (pH 7.0), 3 mM $MgCl_2$]; aptamer G9-II [50 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 5 mM $CaCl_2$]; aptamer APC-99 [10 mM sodium citrate (pH 7.6), 150 mM KCl]; aptamer Tat T34 [10 mM Tris-HCl (pH 7.8), 70 mM NaCl].

RESULTS AND DISCUSSION

The development of ME has enabled products to be rapidly sized by electrophoresis.[13,14] This technology can detect small amounts of DNA or RNA within three minutes using a simple instrument without the need for slab gels or staining steps. Accordingly, the method is cost-effective and requires minimal manual labor. Therefore, we used ME to analyze RNA aptamer-target interactions based on the principle illustrated in Figure 1.

The ME instrument is composed of an electrophoretic separator and a fluorescence detector. The experimental procedure is as follows: (a) HPMC gels are filled in the electrophoresis channel before use, (b) the reaction solution is loaded onto the sample well, (c) the injection mode and separation mode are applied, and (d) size-fractionated reaction products are detected. The injection voltage causes the RNA aptamer to move to the intersection in the injection channel and then migrate to the separation channel under the separation voltage. Fluorescent dye is intercalated by RNAs as they migrate through the gel, while an external blue LED excites emission and an integrated photodiode beneath the channel captures the fluorescent signal. The output from the detector indicates the electropherogram with the migration time and intensity of the sample RNA. The aptamers used in this study are listed in Figure 2.

Affinity Assay of Anti-NS3 Aptamer for HCV NS3 and GST

We determined whether the interaction between the anti HCV NS3 helicase RNA aptamer, #5 (Figure 2A)[15] and the HCV NS3 protein ($K_d = 20 \pm 7$ nM) was detectable by ME, after having confirmed that the internal standard fluorescein did not interfere in the interaction (data not shown). The series of electropherograms observed from the reaction of 1 μM aptamer #5 with 0–5 μM NS3 are shown in Figure 3A and reveal a dose-dependent decrease in aptamer #5 peak intensity as the amount of NS3 increased. This indicates the regression of unbound aptamer #5 to NS3 as the complex increased in the reaction mixture. No peak corresponding to the RNA-protein complex was observed during the 30-min separation time (data not shown).

When aptamer #5 and GST were mixed under the same conditions, no difference in aptamer peak intensity compared to the internal standard was observed (Figure 3A right), suggesting an absence of interaction up to 10 μM GST. The relative peak intensities of aptamer #5 in association with NS3 and GST are compared in Figure 3B and clearly indicate that ME is suitable for analyzing aptamer-protein interactions.

Amplification of RNA Peak Intensity

In the experiment described above, the quantity of aptamer RNA required for detection on an EtBr gel is at least 1 μM. Enhanced fluorescence sensitivity is essential for the detection of reduced RNA quantities; we therefore compared alternative intercalating fluorescent dyes such as SYBR Green I, II, and SYBR Gold. These sensitive reagents stain DNA and RNA with a lower level of background fluorescence than EtBr,[20] while SYBR Gold has been shown to provide the highest level of sensitivity for dsDNA, ssDNA, and RNA detection in many gel types, exhibiting more than a 1000-fold enhancement

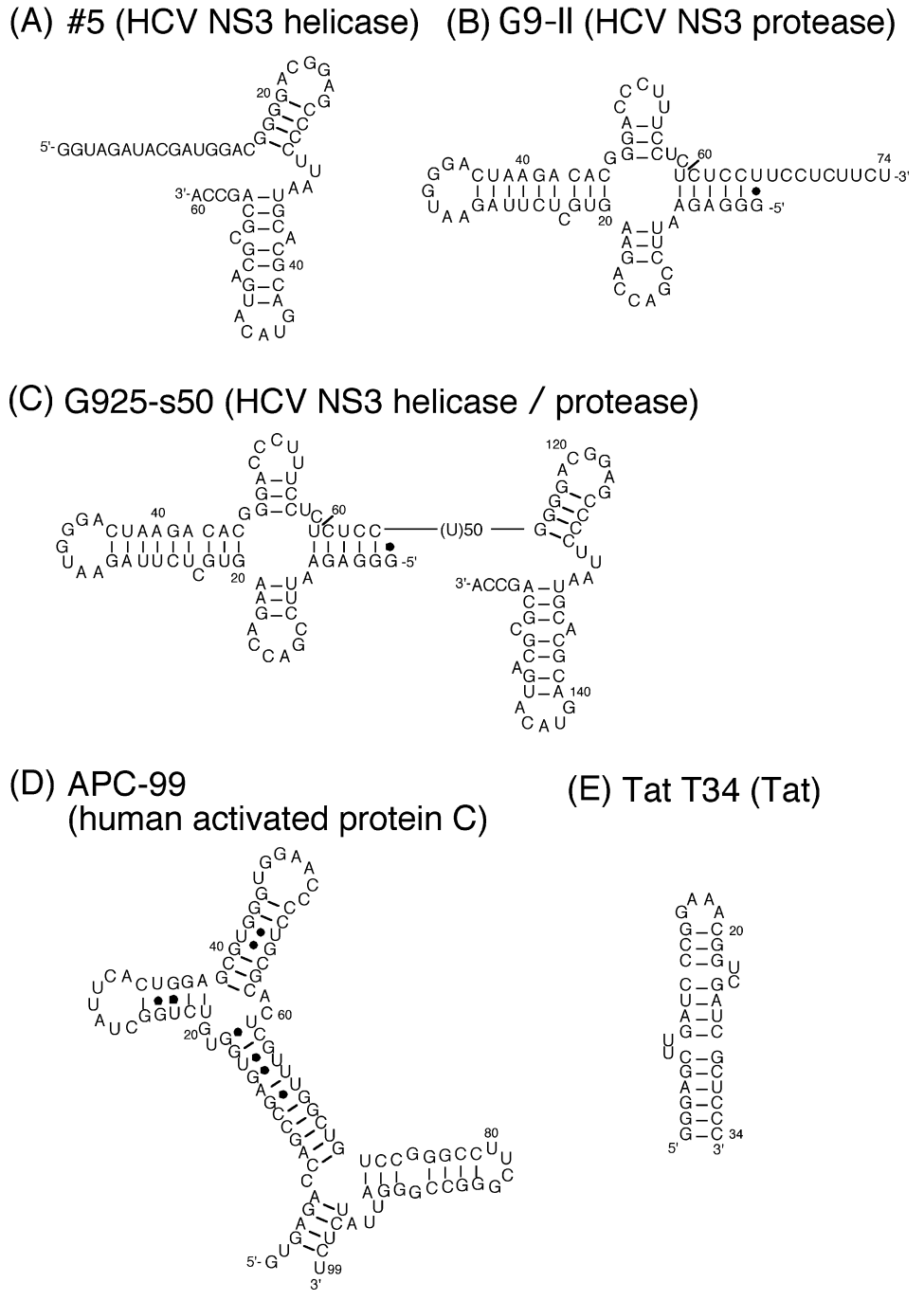

FIGURE 2 Secondary structures of RNA aptamers analyzed by ME. (A) Anti-HCV NS3 helicase RNA aptamer #5. (B) Anti-HCV NS3 protease RNA aptamer G9-II. (C) Anti-HCV NS3 RNA aptamer G925-s50. (D) Anti-human activated protein C RNA aptamer APC-99. (E) Anti-Tat RNA aptamer Tat T34.

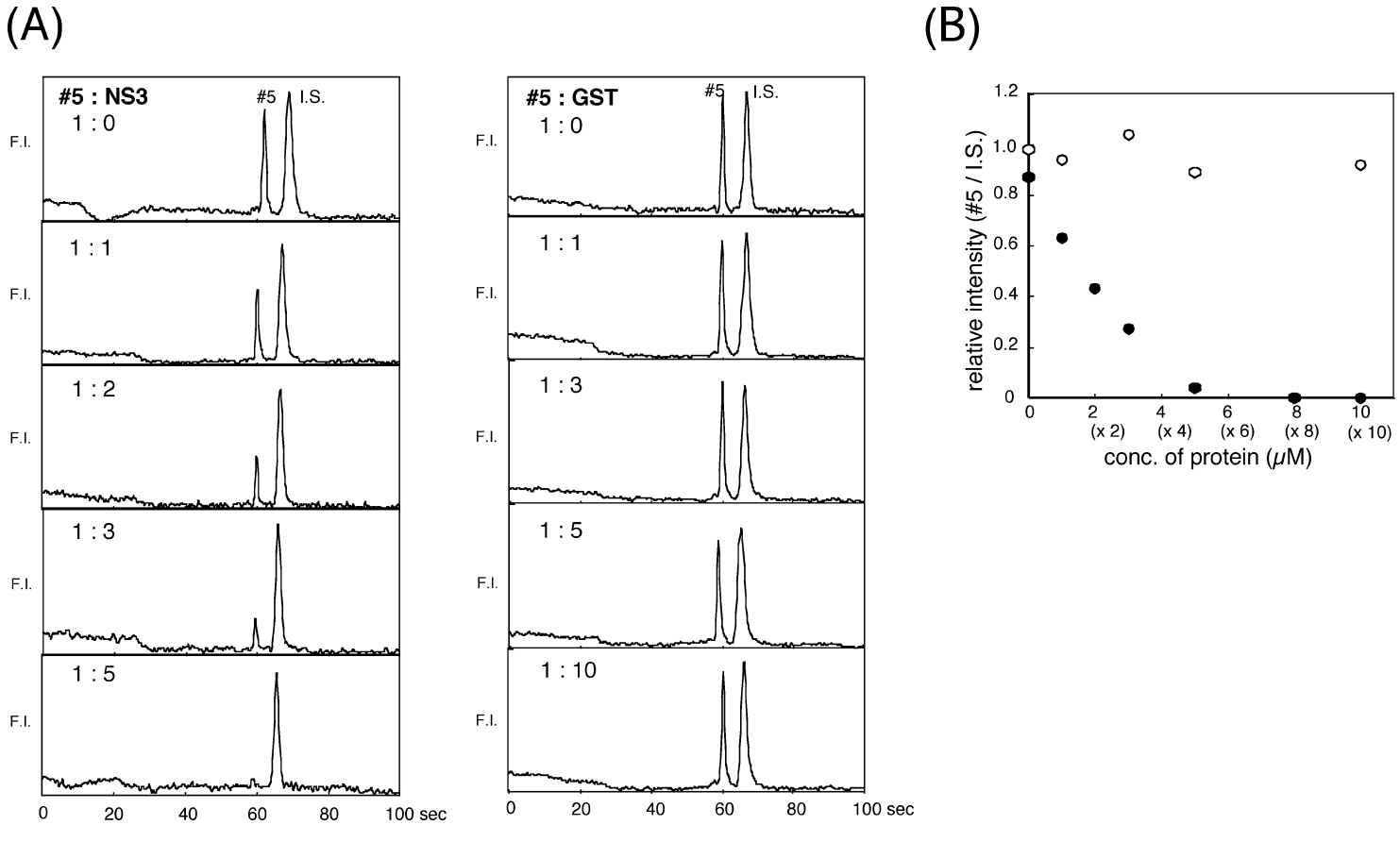

FIGURE 3 Detection of interaction between aptamer #5 and HCV NS3. (A) ME electropherograms from a 0.6% HPMC-EtBr gel obtained for interactions of 1 μM aptamer #5 with 0–10 μM HCV NS3 and GST. I.S. indicates internal standard. F.I. indicates fluorescein intensity. (B) Comparison of observed results with HCV NS3 and GST. Relative peak intensity (#5/I.S.) for HCV NS 3 is shown by closed circles and for GST by open circles. The numbers in parentheses represents the number of folds of protein for 1 μM aptamer #5.

in fluorescence by binding to nucleic acids.[21] SYBR Green I has been used in an earlier study involving a nanoliter device that mixed and amplified DNA samples and separated the products in a channel for on-line detection.[12] In order to determine the optimal dilution ratios for SYBR Green I, II, and SYBR Gold in ME, we initially tested the manufacturer's recommended dilution of 1:10,000 (data not shown). Varying quantities of aptamer #5 were electrophoresed by ME and SYBR Gold staining was shown to be 20-fold more sensitive than EtBr, being able to detect as little as 50 nM RNA, compared to detection limits of 1 μM with EtBr (Figure 3A) and 200 nM with SYBR Green I or II (data not shown). This finding was confirmed by running alternative RNAs on ME.

We used SYBR Green I and SYBR Gold as staining agents in our study of the interaction between the anti HCV NS3 protease aptamer, G9-II (Figure 2B)[16] and the HCV NS3 protease domain (ΔNS3) during ME. For detection by SYBR Green I, 500 nM G9-II aptamer was used with 0–2000 nM ΔNS3, while 50 nM G9-II was used with 0–350 nM ΔNS3 for detection by SYBR Gold. As shown in Figure 4A, the SYBR Green I fluorescence intensity of unbound aptamer G9-II decreased linearly with increasing concentrations of ΔNS3. Although the peaks of free aptamer G9-II overlapped with those of the internal standard in detection by SYBR Gold (Figure 4B), they decreased in a dose-dependent manner as levels of ΔNS3 rose to 100 nM, and reached a plateau at more than 150 nM of ΔNS3. To overcome the inaccuracy in such a case, the use of tag-attached aptamer may solve this problem for the separation of aptamer from the internal standard. The sensitivity of SYBR Gold for detection of the RNA aptamer was shown to be superior to SYBR Green I. Although some variability was detected in the signal intensity between repeat scans, no significant decrease of signal was observed after 5–8 successive runs.

Anti-HCV NS3 Aptamer and Its Selectivity for HCV NS3 in ME

We next analyzed the dual-function aptamer, G925-s50 (Figure 2C)[17] which demonstrates high affinity ($K_d = 12 \pm 4$ nM) and selectivity for NS3. We used 50 nM G925-s50 in all analyses (Figure 5A and 5B). An interaction between G925-s50 and NS3 was detected by a decrease of unbound RNA at NS3 levels ranging from 0 to 400 nM (Figure 5A). When G925-s50 was incubated with NS3 in the presence of an equal concentration of aptamer #5 (with a weaker affinity for NS3 ($K_d = 20 \pm 7$ nM)), we were clearly able to detect the selectivity of G925-s50, shown by a dose-dependent decrease compared with aptamer #5 in the 0–400 nM range of NS3 (Figure 5B). This result indicates that ME is a useful tool for distinguishing between aptamers that have different affinities.

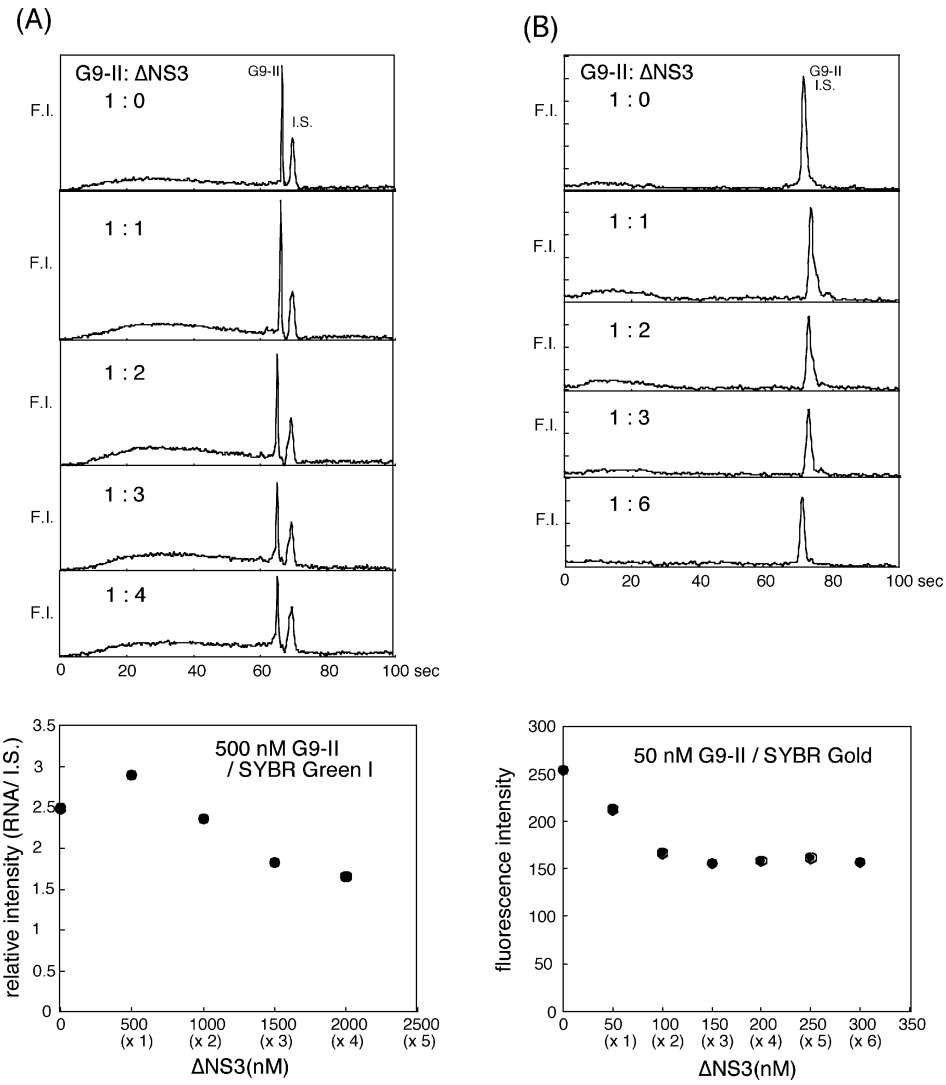

FIGURE 4 Amplification of RNA peak intensity on detection by SYBR Green I or SYBR Gold. (A) ME electropherograms from a 0.6% HPMC-SYBR Green I gel obtained for interactions of 500 nM aptamer G9-II with 0-2000 nM HCV NS3 protease domain (ΔNS3). The normalized intensity of G9-II was plotted against a range of ΔNS3 concentrations. (B) ME electropherograms from a 0.6% HPMC-SYBR Gold gel obtained for interactions of 50 nM aptamer G9-II with 0–300 nM ΔNS3. The fluorescence intensity of the overlapping peak was plotted against a range of ΔNS3 concentrations.

Relationship between the Regression of Unbound RNA and Its K_d Value

To determine the detection limits of the RNA-protein interaction with the regression of free RNA peak intensity, we used the anti-human activated protein C RNA aptamer, APC-99 (Figure 2D)[18] as it has a lower affinity for

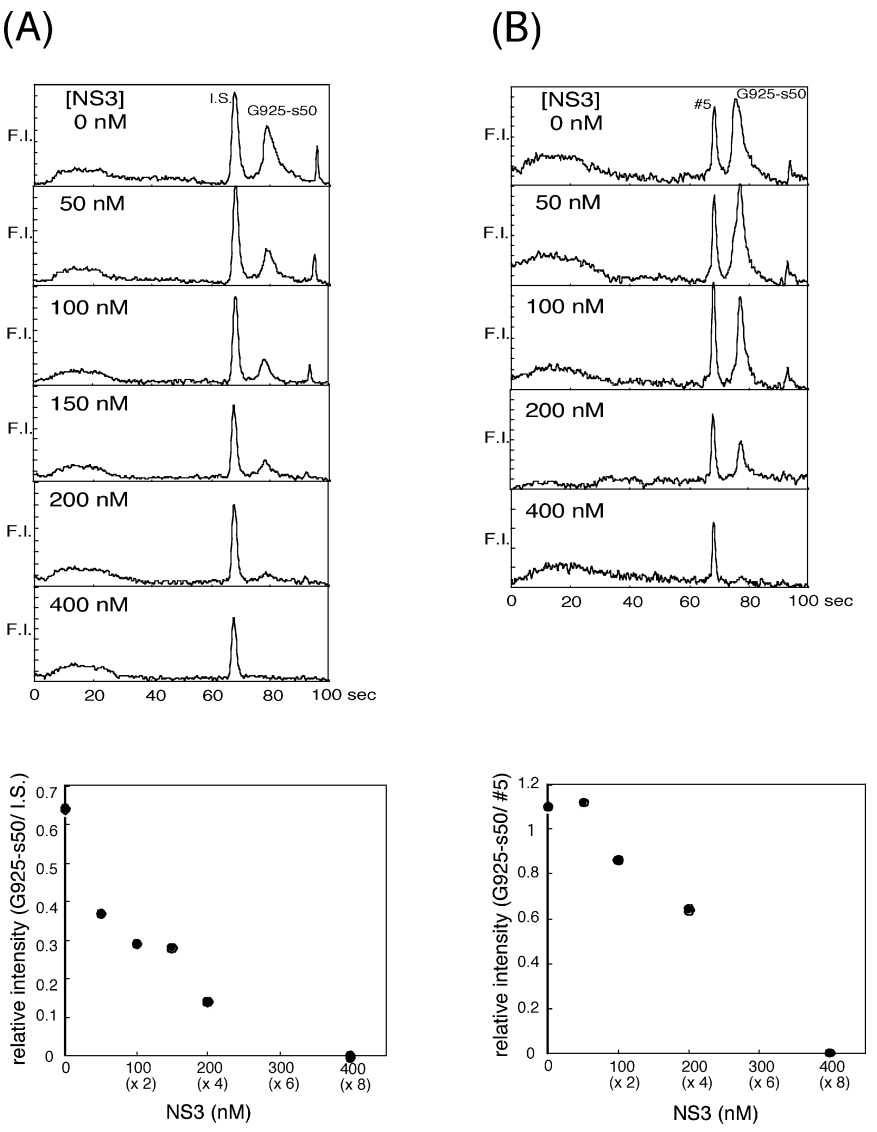

FIGURE 5 Detection of interaction between aptamer G925-s50 and HCV NS3. (A) ME electropherograms from a 0.6% HPMC-SYBR Gold gel obtained for interactions of 50 nM aptamer G925-s50 with 0–400 nM HCV NS3. The normalized intensity of G925-s50 was plotted against a range of NS3 concentrations. (B) ME electropherograms from a 0.6% HPMC-SYBR Gold gel obtained for interactions of 50 nM aptamer G925-s50 and 50 nM aptamer #5 with 0–400 nM HCV NS3. The relative intensity of G925-s50 for #5 was plotted against a range of HCV NS3 concentrations. Internal standards were not included in these conditions.

its target ($K_d = 120$ nM) than the aptamers described earlier. Using SYBR Gold dye, we detected no difference in normalized peak intensity following incubation of 20 nM and 100 nM APC-99 with a 0–2000 nM range of human activated protein C (data not shown).

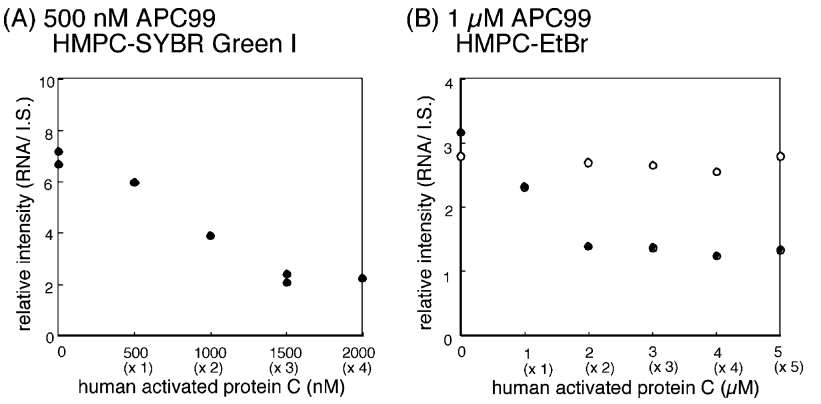

FIGURE 6 Detection of interaction between aptamer APC-99 and human activated protein C (A) Analyzed results from ME electropherograms of 0.6% HPMC-SYBR Green I gels obtained for interactions of 500 nM aptamer APC-99 with 0–2000 nM human activated protein C. (B) Analyzed results from ME electropherograms of 0.6% HPMC-EtBr gels obtained for interactions of 1 μM aptamer APC-99 with 0–5 μM human activated protein C (closed circles) and GST (open circles).

A regression of unbound aptamer was detected when 500 nM APC-99 was incubated with increasing amounts of human activated protein C (0-2000 nM, Figure 6A). Further detection by EtBr revealed a similar regression response for 1 μM APC-99 incubated with human activated protein C, up to a level of 2 μM. The binding reaction reached a plateau at $\geq$2 μM protein (Figure 6B, closed circles). Under the same conditions, discrimination for GST was detected by the absence of effect in RNA peak intensity (Figure 6B, open circles). A decrease of free RNA peak intensity can be observed under saturated conditions of more than 4-fold the K_d value of the RNA or target concentration, indicating that most of the RNA aptamer was in a target-bound state at high concentrations of target.

Detection of RNA-Peptide Interaction in ME

We examined the binding of 50 nM anti-Tat RNA aptamer Tat T34 (Figure 2E) and varying amounts of the Tat-derived peptide, RE (38 amino acids, $K_d = 120$ pM)[19] on an SYBR Gold gel. As the target size was small, the migration distance between unbound RNA and the RNA-peptide complex would be expected to be shorter than with an RNA-protein complex, but we did not detect any peak under our experimental conditions (data not shown). However, we were able to detect changes in the relative intensity of Tat T34 in a range of concentrations of the RE peptide and GST (0–250 nM). The analysis showed a decrease of free RNA with increasing levels of RE peptide and no change in the RNA peak for the GST negative control (Figure 7), suggesting that ME could even be used to detect aptamer selectivity for small targets such as peptides.

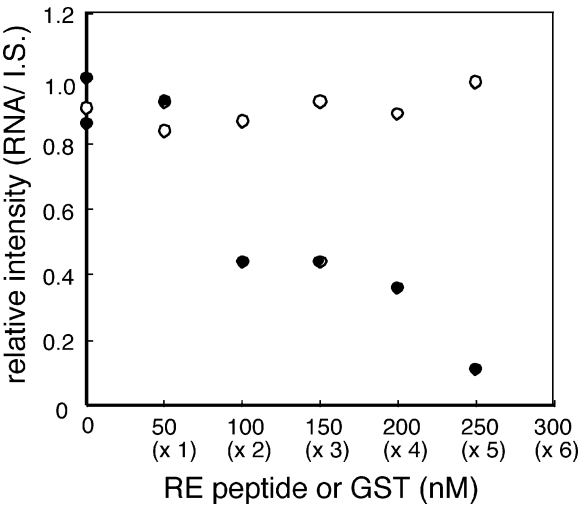

FIGURE 7 Detection of interaction between aptamer Tat T34 and RE peptide. Analyzed results from ME electropherograms obtained for interactions of 50 nM aptamer Tat T34 with 0–250 nM RE peptide (closed circles) and GST (open circles).

CONCLUSION

As reported for CE, nucleic acids bound to a target migrate at a different mobility to unbound sequences.[22] Using this principle, we have developed a simple method of ME for detecting the interaction between an RNA aptamer and its target without the need for RNA labeling. This method will overcome the difficultly involved in designing adequate labeling strategies due to the uncertainty regarding target binding sites and precise conformational changes. Moreover, the labeling of RNA aptamers is rendered extremely difficult by the instability of RNA.

In this study, RNAs were successfully stained by the highly sensitive intercalating dyes SYBR Green I and SYBR Gold. SYBR Gold enables the detection of RNA at low concentrations of $\geq$0.6 ng/μl (50 nM Tat T34). It is difficult to precisely determine the K_d value from this method as RNA-protein complexes are not always detected and may dissociate slightly during electrophoresis. However, at higher RNA concentrations ($\geq$4-fold the K_d value), a decrease in the amount of unbound RNA was detectable in the analysis. Consequently it might be possible to predict the concentration limit under the saturated conditions in which most of the RNA forms a complex with its target, with the exception of inactive structured RNA.

The advantages of using an ME instrument include speed and the elimination of slab gels, staining steps, species-specific probes, and DNA sequencing. Furthermore, minimal manual labor is required, thus making the fragment detection system more accurate and cost-effective. The current trend

of requiring vast numbers of aptamers for suitable targets means that the binding assay using ME demonstrated here will provide a valuable and convenient technique for the rapid screening and characterization of aptamers during *in vitro* selection.

REFERENCES

1. Ellington, A.D.; Szostak, J.W. *In vitro* selection of RNA molecules that bind specific ligands. Nature **1990**, 346, 818–822.
2. Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science **1990**, 249, 505–510.
3. Joyce, G.F. Amplification, mutation and selection of catalytic RNA. Gene **1989**, 82, 83–87.
4. Tombelli, S.; Minunni, M.; Mascini, M. Analytical applications of aptamers. Biosensors & Bioelectronics **2005**, 20, 2424–2434.
5. Manz, A.; Graber, N.; Widmer, H.M. Miniaturized Total chemical analysis systems: a novel concept for chemical sensing. Sensors and Actuators **1990**, B1, 244–248.
6. Harrison, D.J.; Manz, A.; Fan, Z.; Lusi, H.; Widmer, H.M. Capillary electrophoreses and sample injection systems integrated on a planar glass chip. Analytical Chemistry **1992**, 64, 1926–1932.
7. Mendonsa, S.D.; Bowser, M.T. *In vitro* evolution of functional DNA using capillary electrophoresis. Journal of the American Chemical Society **2004**, 126, 20–21.
8. Mendonsa, S.D.; Bowser, M.T. *In vitro* selection of high-affinity DNA ligands for human IgE using capillary electrophoresis. Analytical Chemistry **2004**, 76, 5387–5392.
9. Mendonsa, S.D.; Bowser, M.T. *In vitro* selection of aptamers with affinity for neuropeptide Y using capillary electrophoresis. Journal of the American Chemical Society **2005**, 127, 9382–9383.
10. Mosing, R.K.; Mendonsa, S.D.; Bowser, M.T. Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. Analytical Chemistry **2005**, 77, 6107–6112.
11. Cheng, J.; Waters, L.C.; Fortina, P.; Hvichia, G.; Jacobson, S.C.; Ramsey, J.M.; Kricka, L.; Wilding, P. Degenerate oligonucleotide primed-polymerase chain reaction and capillary electrophoretic analysis of human DNA on microchip-based devices. Analytical Biochemistry **1998**, 257, 101–106.
12. Burns, M.A.; Johnson, B.N.; Brahmasandra, S.N.; Handique, K.; Webster, J.R.; Krishnan, M.; Sammarco, T.S.; Man, P.M.; Jones, D.; Heldsinger, D.; Mastrangelo, C.H.; Burke, D.T. An integrated nanoliter DNA analysis device. Science **1998**, 282, 484–487.
13. Shimayama, T.; Arakawa, H.; Watanabe, K.; McCormic, R.M.; Watanabe, H.; Maeda, M. Ultrarapid and highly sensitive specific detection of *E.coli* O-157: H7 by allele specific PCR coupled with microchannel plastic chip capillary electrophoresis. ITE Lett, On Batteries, New Technologys and Medicine **2003**, 4, 661–671.
14. Zhang, L.; Dang, F.; Baba, Y. Microchip electrophoresis-based separation of DNA. Journal of Pharmaceutical and Biomedical Analysis **2003**, 30, 1645–1654.
15. Nishikawa, F.; Funaji, K.; Fukuda, K.; Nishikawa, S. *In vitro* selection of RNA aptamers against the HCV NS3 helicase domain. Oligonucleotides **2004**, 14, 114–129.
16. Fukuda, K.; Vishnuvardhan, D.; Sekiya, S.; Hwang, J.; Kakiuchi, N.; Taira, K.; Shimotohno, K.; Kumar, P. K. R.; Nishikawa, S. Isolation and characterization of RNA aptamers specific for the hepatitis C virus nonstructural protein 3 protease. European Journal of Biochemistry **2000**, 267, 3685–3694.
17. Umehara, T.; Fukuda, K.; Nishikawa, F.; Kohara, M.; Hasegawa, T.; Nishikawa, S. Rational design of dual-functional aptamers that inhibit the protease and helicase activities of HCV NS3. Journal of Biochemistry **2005**, 137, 339–347.
18. Gal, S.W.; Amontov, S.; Urvil, P.T.; Vishnuvardhan, D.; Nishikawa, F.; Kumar, P.K.R.; Nishikawa, S. Selection of a RNA aptamer that binds to human activated protein C and inhibits its protease function. European Journal of Biochemistry **1998**, 252, 553–562.
19. Yamamoto, R.; Katahira, M.; Nishikawa, S.; Baba, T.; Taira, K.; Kumar, P.K.R. A novel RNA motif that binds efficiently and specifically to the Tat protein of HIV and inhibits the trans-activation by Tat of transcription *in vitro* and *in vivo*. Genes to Cells **2000**, 5, 371–388.

20. Singer, V.L.; Haugland, R.P. 1999. Fluorescent imaging of nucleic acids and proteins in gels, in fluorescent and luminescent probes for biological activity, 2nd ed.; ed. W. T. Mason, pp. 51–62, Academic Press, San Diego, CA.
21. Tuma, R.S.; Beaudet, M.P.; Jin, X.; Jones, L.J.; Cheung, C.-Y.; Yue, S.; Singer, V.L. Characterization of SYBR gold nucleic acid gel stain: a dye optimized for use with 300-nm ultraviolet transilluminators. Analytical Biochemistry **1999**, 268, 278–288.
22. German, I.; Buchanan, D.D.; Kennedy, R.T. Aptamers as ligands in affinity probe capillary electrophoresis. Analytical Chemistry **1998**, 70, 4540–4545.