CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200700068

Adaptive Recognition of Small Molecules by Nucleic Acid Aptamers through a Label-Free Approach

Tao Li,[a, b] Bingling Li,[a, b] and Shaojun Dong*[a]

Abstract: We report a novel label-free method for the investigation of the adaptive recognition of small molecules by nucleic acid aptamers using capillary electrophoresis analysis. Cocaine and argininamide were chosen as model molecules, and the two corresponding DNA aptamers were used. These single-strand DNAs folded into their specific secondary structures, which were mainly responsible for the binding of the target molecules with high affinity and specificity. For molecular recognition, the nucleic acid structures then underwent additional conformational changes, while keeping the target molecules stabilized by intermolecular hydrogen bonds. The intrinsic chemical and physical properties of the target molecules enabled them to act as indicators for adaptive binding. Thus any labeling or modification of the aptamers or target molecules were made obsolete. This label-free method for aptamer-based molecular recognition was also successfully applied to biological fluids and therefore indicates that this approach is a promising tool for bioanalysis.

Keywords: aptamers · capillary electrophoresis · label-free · molecular recognition · nucleic acids

Introduction

Nucleic acid binders, also known as aptamers, are obtained through an in vitro selection process called systematic evolution of ligands by exponential enrichment (SELEX).[1,2] These selected binders can recognize a large variety of target molecules with high specificity and affinity.[3] Compared with antibodies, aptamers are relatively easy to obtain and more stable towards biodegradation.[4] In addition, their specificity and affinity towards binding target molecules are equal or superior to those of antibodies.[5] These unique properties make aptamers ideal recognition elements for detecting specific target molecules. To date, they have widely been used for the recognition and detection of various targets from small molecules to proteins or even whole cells,[6–24] indicating their tremendous potential in biological applications. To monitor the molecule–aptamer interactions, labeling and/or modification of the aptamers or target molecules with indicators is generally necessary. However, a label-free method for aptamer-based analysis would make the above steps obsolete, making the process simple, rapid and low in cost. The development of such a method has thus become increasingly attractive.[25–30]

In aptamer-based analysis, some intrinsic properties of the target molecules may be beneficial for the development of a new label-free method. Cocaine contains a tertiary amino group and can generate strong electrochemiluminescence (ECL) emission on a platinum electrode in the presence of tris(2,2′-bipyridyl)ruthenium(II) $[Ru(bpy)_3]^{2+}$.[31] Arginine can be electrochemically oxidized on a copper electrode to generate strong electrochemical (EC) signal;[32,33] its amide argininamide also possesses a high EC activity. However, when the targets are bound by aptamers, they show no luminescent or electrochemical activity. These chemical and physical properties of the target molecules allow us to use them as indicators for the molecule–aptamer interactions with no requirement for labeling and/or modification of aptamers or target molecules.

In general, to bind target molecules, the corresponding aptamers will undergo conformational changes to form specific secondary structures that are mainly responsible for the adaptive binding. A 30-mer anti-cocaine aptamer can form a three-dimensional structure, with a lipophilic cavity serving as the binding pocket for cocaine.[6] A 24-mer DNA aptamer recognizing the guanidinium group specifically can form a

[a] Dr. T. Li, Dr. B. Li, Prof. Dr. S. Dong
State Key Laboratory of Electroanalytical Chemistry
Changchun Institute of Applied Chemistry
Chinese Academy of Science, Changchun, Jilin 130022 (China)
Fax: (+86) 431-8568-9711
E-mail: dongsj@ciac.jl.cn

[b] Dr. T. Li, Dr. B. Li
Graduate School of the Chinese Academy of Sciences
Beijing, 100039 (China)

 WILEY InterScience DISCOVER SOMETHING GREAT

© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

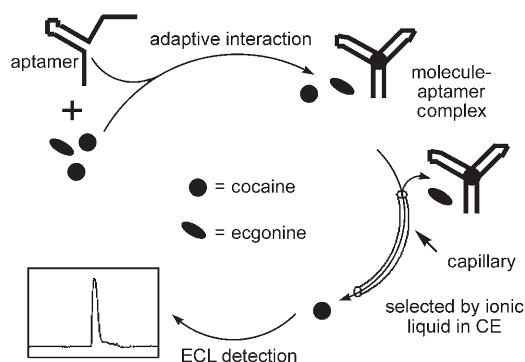
hairpin structure with a ten-residue loop,[12] encapsulating argininamide (Arm) in the folded nucleic acid structure.[13] In the presence of potassium ions, several guanine-rich DNA aptamers selected by Sen et al. can fold into a G-quartet structure which is responsible for the binding of hemin.[16] The G-quadruplex conformer can be also observed in the other guanine-rich DNA aptamers.[18] These specific nucleic acid folds play a key role in aptamer-based molecular recognition.

Recently, there is an increasing interest in the application of capillary electrophoresis (CE) for aptamer-based analysis.[34–40] Using CE, Bowser et al. introduced a new SELEX method (CE-SELEX), with which they have performed a selectivity study against human IgE.[34] Since then, a number of aptamers has been selected by this method.[35–37] In addition, the CE method has widely been applied in various fields from chiral separation to protein detection.[38–40] However, the application of this technique to aptamer-based molecular recognition has not reported so far.

Herein, we introduce a novel label-free method for the investigation of the adaptive recognition of small molecules, that is, cocaine and Arm, by DNA aptamers using the CE method.

Results and Discussion

Cocaine recognition by a three-dimensional DNA fold using CE-ECL: In the presence of $[Ru(bpy)_3]^{2+}$, cocaine generates a strong ECL emission on a platinum electrode at a potential of over 1.05 V as previously reported.[31] Under the same conditions, it was observed that its hydrolysate, ecgonine, also possesses a high ECL activity. The maximal ECL emission of both cocaine and ecgonine was observed at pH 8.5. However, when cocaine was adsorbed by the corresponding aptamer, this target molecule showed no ECL activity. These properties of cocaine allowed it to be used as an indicator for the molecule–aptamer interaction, indicating the adaptive binding by a decrease of its ECL intensity. Thus we introduced a label-free method for aptamer-based recognition of cocaine using CE analysis (Scheme 1). Herein, the ECL method was applied in aptamer-based analysis for the first time.



Scheme 1. Label-free method for aptamer-based recognition of cocaine from its hydrolysate (ecgonine) using CE-ECL analysis assisted with ionic liquid selector. Ionic liquid was used as the selector for the sample injection in the CE process, enriching cocaine and excluding the cocaine–aptamer complex and ecgonine from the capillary.

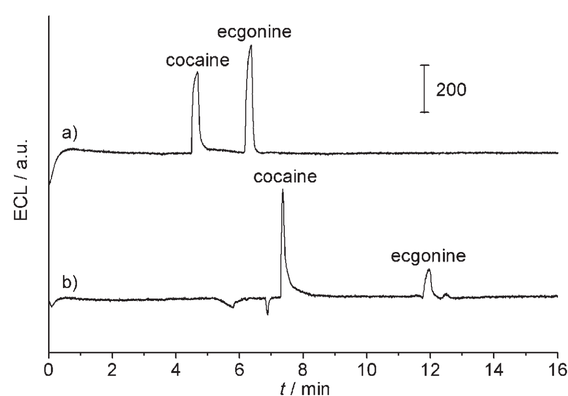


Figure 1. CE-ECL analyses for a mixture of 600 μM cocaine and 400 μM ecgonine using 20 mM phosphate (pH 7.0) in a) absence and b) presence of 1% $BMIMBF_4$ running buffer. The upright bar refers to the ECL intensity of 200 a.u.

According to a previous report,[39] the molecule–aptamer complex might be subject to dissociation during the CE process when injected into the capillary. To avoid the potential interference, a novel CE selector, that is an ionic liquid (IL), was used. When a hydrophilic 1-alkyl-3-methylimidazolium-based IL, such as 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), was used as an additive in the running buffer of CE, a new phenomenon was observed as shown in Figure 1. By the use of 1% (v/v) IL additive, the peak intensity (peak height) of cocaine was increased to 135%, while that of ecgonine was decreased to 23%. In addition, the background signal was almost unchanged compared with no use of an IL. We assumed that in the presence of IL additive cocaine was enriched whereas ecgonine was excluded from the capillary in the sample injection process. The above phenomenon might attribute to the unique properties of ILs. It was well known that IL possessed high electrical conductivity.[41] The use of an IL additive made the conductivity of the running buffer much higher than that of the sample solution. At an electrokinetic mode, the actual voltage for sample injection was increased and a field-amplified effect was achieved, enriching the target molecules greatly. On the other hand, the intrinsic structure of ILs, formed by the cation associated to an anion, made the additive able to act as a dynamical modifier of CE. The cation or anion was adsorbed onto the capillary wall and led to a decreased electroosmotic flow (EOF),[42] resulting in a decrease in the injection capacity of the targets. For positively charged molecules, the field-amplified effect was significant and the samples were greatly enriched. For those with a negative charge, however, the field-amplified effect was not obvious and the negative effect of decreased EOF was dominant. As a result, the negative targets were excluded from the capillary. In the binding buffer (20 mM phosphate of pH 7.0), cocaine ($pK_a \approx 8.6$) was positively charged, while

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

ecgonine (pI ≈5.3) was negatively charged. Therefore, the selective sample injection in the CE process was accomplished by the use of IL additive. There would be some significant advantages in the application of IL to aptamer-based CE analysis. Above all, the negative molecule–aptamer complexes were excluded from the capillary by the use of IL selector. It intended that the potential interference from the dissociation or adsorption of the cocaine–aptamer complex in the capillary was effectively avoided. Secondly, the detection sensitivity of positive target molecules was improved. A detection limit (2 μM) of cocaine was obtained by CE-ECL in the presence of 1% IL, which was lower than that reported previously.[8]

To investigate cocaine recognition, a 30-mer DNA aptamer (A1) selected by Stojanovic et al.[6] was used. Just before use, this aptamer was hybridised at room temperature for 1 h to form a one-stem fold. In the presence of cocaine, it continued to fold into the three-dimensional structure with a lipophilic cavity (Scheme 1). This cavity served as the binding pocket, enclosing cocaine with an affinity of K_d ≈20 μM.[6] It was very important that the cocaine was bound by aptamer A1 with high specificity, which resulted in a decrease in the ECL signal. Thus the target molecule itself acted as an indicator. Its combination with aptamer A1 resulted in a decrease in the ECL signal. As shown in Figure 2, after incubation with the aptamer, the signal of cocaine gradually decreased when the aptamer concentration was increased, while the ecgonine signal remained almost unchanged throughout. We assumed that the aptamer was highly selective for cocaine compared with ecgonine. From the results, the binding affinity (K_d ≈37 μM) of aptamer A1 was easily obtained. Compared with the results previously reported,[6] the affinity of aptamer A1 decreased to about 50% in our experiments, which might attribute to the low ionic strength of the binding buffer. Because a high salt concentration would have some negative effect on CE analyses, we used a the binding buffer with low ionic strength for the cocaine recognition. As reported previously,[6] this aptamer could also recognize cocaine in the presence of two of its metabolites, that is, benzoyl ecgonine and ecgonine methyl ester. The two ester groups of cocaine should mainly be responsible for the adaptive binding. In the presence of cocaine, aptamer A1 with one binding site underwent certain conformational changes to form a three-dimensional structure, with a lipophilic cavity at the junction. In this DNA fold, cocaine was encapsulated and stabilized by the intermolecular interactions. In the whole process, the formation of the first binding site was one necessary step, obviously influencing the affinity of aptamer A1. Accordingly this aptamer should be completely hybridized just before use.

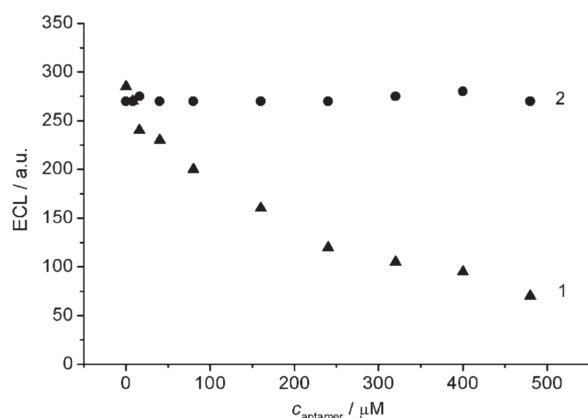
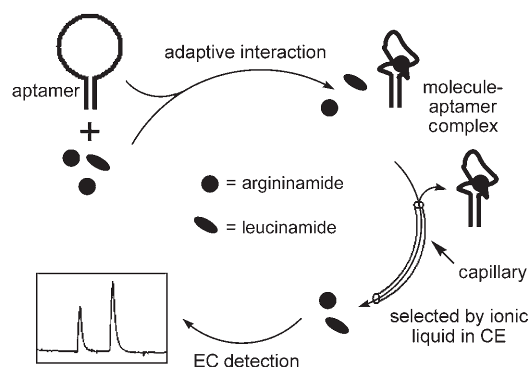


Figure 2. Dependence of ECL intensities of cocaine and its analogue on the concentrations of aptamer A1 after incubation for 1 h at room temperature in 20 mM phosphate of pH 7.0. 1) 800 μM cocaine, 2) 1.3 mM ecgonine.

Arm recognition by a DNA hairpin loop using CE-EC: As reported previously,[32,33] most amino acids could be sensitively detected on a copper electrode under strong alkaline conditions. Under the same conditions, it was observed that some amino acid amides such as Arm and leucinamide (Lem) generated strong EC signal as well. For Arm detection by CE-EC, the maximal signal was observed at 0.7 V in 60 mM NaOH, and a detection limit (5 μM) of Arm was obtained. Similar to cocaine, this target molecule showed no EC activity when it was bound by the corresponding aptamer. These properties of this target molecule allowed it to act as an indicator for the molecule–aptamer interaction. Therefore the above label-free method for molecular recognition was also applicable to Arm when EC detection was adopted (Scheme 2). The IL selector was used as well to avoid the potential interference from the dissociation or adsorption of the Arm–aptamer complex in the capillary.



Scheme 2. Label-free aptamer-based recognition of argininamide from its analogue (leucinamide) using CE-EC analysis assisted with ionic liquid selector. Ionic liquid was used as the CE selector, excluding the argininamide–aptamer complex from the capillary and enriching argininamide and leucinamide.

For Arm recognition, a 24-mer DNA aptamer (A2) selected by Frankel et al.[12] was used. After hybridization, this aptamer formed a hairpin structure with a ten-residue loop, binding Arm with a K_d of ≈100 μM.[12] In the presence of Arm, the hairpin loop folded into an adaptive conformation, allowing the target molecule to penetrated into the nucleic acid fold where intermolecular hydrogen bonds were formed exclusively with bases, as shown in Scheme 2. The

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

adaptive binding of Arm by the aptamer A2 was highly specific, not affected by the analogue Lem (Figure 3, top). The molecule–aptamer interaction resulted in a decrease of the EC signal of Arm, which was dependent on the aptamer concentration (Figure 3, bottom). From this graph, a binding affinity (K_d ≈103 μM) of aptamer A2 in 20 mM phosphate (pH 7.0) was easily obtained. This affinity was close to that reported previously.[12] We assumed that the binding of Arm by the aptamer A2 was almost not influenced by the ionic strength of the binding buffer. The specific interaction between aptamer A2 and arginine was observed as well (data not shown), but the affinity for the binding of arginine by the aptamer was much lower. The results indicated that the guanidinium groups of the two molecules were mainly responsible for the adaptive binding. This positive group was easy to penetrate into the negative hairpin loop where a conformational change would occur. The target molecules were encapsulated in the nucleic acid fold and stabilized by intermolecular hydrogen bonds. Compared with Arm, arginine could hardly enter the DNA fold due to a negative carboxyl group, which prohibits the formation of hydrogen bonds and made the arginine–aptamer complex unstable. This might account for the relatively low affinity (K_d ≈2.5 mM) for binding arginine to aptamer A2.

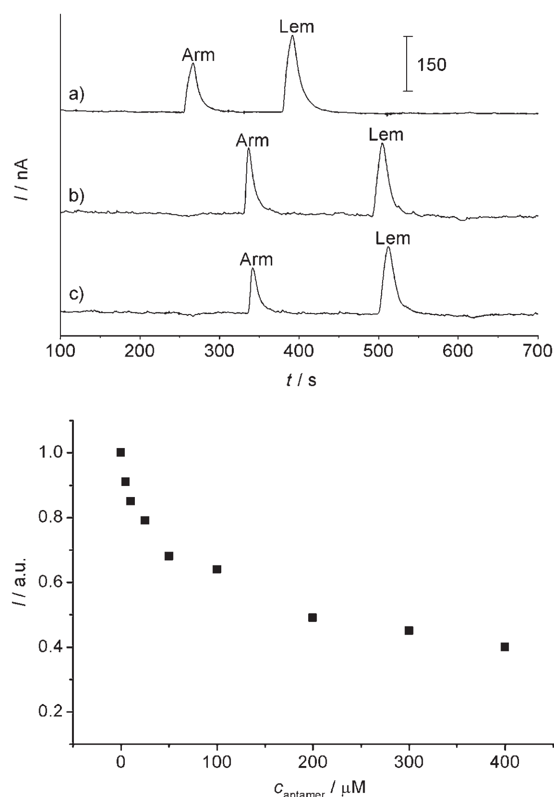


Figure 3. CE-EC analyses for the recognition of Arm by aptamer A2. Top) Electropherograms of a mixture of 500 μM Arm and 4 mM Lem before (a, b) and after (c) incubation with 250 μM aptamer A2 for 2 h at 4 °C; 20 mM phosphate of pH 7.0 in the absence (a) or presence (b, c) of 1 % IL was used as running buffer. The upright bar refers to the current of 150 nA. Bottom) The dependence of relative current intensity of Arm on the aptamer concentration. The current was normalized to the value obtained before incubation with the aptamer.

Practicality and generality of the method: To test the practicality of the above method, cocaine samples prepared from biological fluids such as FBS were investigated. It is commonly known that oligonucleotides are subject to degradation by nucleases in the serum. In addition, there might be much potential interference in aptamer-based molecular recognition performed in biological fluids. However, as shown in Figure 4, cocaine could be specifically bound by aptamer A1 not affected by other substances in FBS. Two important factors might be beneficial for the biological application of our method. Above all, the aptamers selected in a physiological buffer should also be able to bind target molecules with high specificity and affinity in biological fluids. Secondly, most of proteins in serum were excluded from the capillary by the use of a IL selector, avoiding the potential interference effectively.

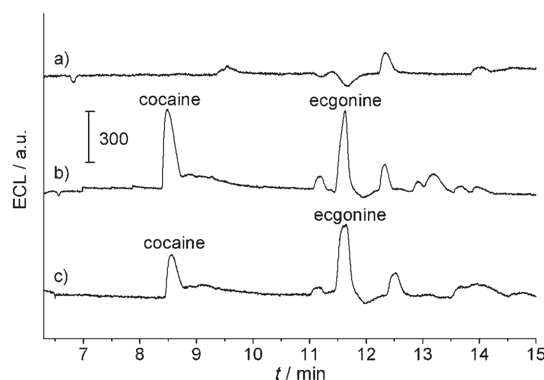


Figure 4. Application to specific recognition of cocaine in the biological fluids using CE-ECL analysis. a) 25 % fetal bovine serum (FBS); b) a mixture of 2 mM cocaine and 1.5 mM ecgonine in 25 % FBS; c) the mixture of 2 mM cocaine and 1.5 mM ecgonine in 25 % FBS after incubation with 1 mM aptamer A1 at room temperature for 1 h. The upright bar refers to the ECL intensity of 300 a.u.

It was suggested that a general label-free method for the molecular recognition could be developed using CE analyses, based on the intrinsic properties of target molecules. This method would be applied to aptamer-based recognition of other small molecules such as hemin and adenosine when chemiluminescence, fluorescence and/or ultraviolet detection were adopted, further extending the application of the method presented herein.

Conclusion

In summary, we have developed a novel label-free method for the investigaton of the adaptive recognition of small molecules by nucleic acid aptamers using CE analysis. Cocaine and Arm were chosen as model molecules. The intrinsic properties of target molecules allowed their use as indi-

© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

cators for the aptamer–molecule interactions, without any need for labeling and/or modification of aptamers or target molecules. Through this label-free approach, the adaptive binding of target molecules by nucleic acid aptamers was investigated. It was shown that the developed method is simple, easy, and relatively low in cost. In addition, it was the first time that the ECL method was applied to aptamer-based analysis so far, paving the way for further application of ECL in this field. This label-free method for aptamer-based molecular recognition has been proved applicable in biological fluids as well, indicating that it was promising for aptamer-based bioanalysis. Based on the other characters such as catalytic, fluorescent, and ultraviolet properties of small molecules, the applications of the developed method can further be extended.

Experimental Section

Materials: Two DNA aptamers (A1, 5′ GAC AAG GAA AAT CCT TCA ATG AAG TGG GTC 3′; A2, 5′ GAT CGA AAC GTA GCG CCT TCG ATC 3′) were synthesized (Sangon Biotechnology Co. Ltd. Shanghai, China) for molecular recognition of cocaine and Arm. Hydrophilic 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$) IL was obtained from Fluka Chemie (Buchs, Neu-Ulm, Switzerland). Cocaine was purchased from the State Narcotic Laboratory (Beijing, China). Arm, leucinamide (Lem) and tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate $[Ru(bpy)_3]^{2+}$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Tianjin Haoyang Biological Manufacture Co., Ltd (TBD, Tianjin, China). Cocaine was dissolved in 20 mM phosphate of pH 7.0 immediately before use to avoid hydrolysis. The stock solution of ecgonine was prepared by hydrolyzing cocaine in diluted NaOH and then adjusted to pH 7.0 with HCl. The 10 mM solution of $[Ru(bpy)_3]^{2+}$ was freshly prepared by diluting the 50 mM stock solution with 0.1 M phosphate (pH 8.5) just before use. 18 MΩ water purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Instrumentation: A CE setup described in our previous work[43] was used. Briefly, an uncoated fused-silica capillary (Yongnian Optical Fiber Co. Ltd., Hebei, China) with 25 μm inner diameter was cut to 60 cm in length and used for CE separation. A high-voltage power supply (Shanghai Nucleus Institute, Shanghai, China) was used to supply high voltage for CE. Samples were injected at 20 kV for 10 s at the electrokinetic mode. Electrophoresis was carried out at ambient temperature with a voltage of 20 kV using 20 mM phosphate (pH 7.0) in the presence or absence of 1 % (v/v) $BMIMBF_4$ as running buffer. For ECL detection of cocaine, a platinum disk electrode of 500 μm diameter was used. The ECL emission was detected at 1.2 V (vs Ag/AgCl) with a Model MCDR-A Chemiluminescence Analyzer Systems (Xi′An Remax Science & Technology Co. Ltd., Xi′An, China). The distance between the working electrode and the end of capillary was 125 μm. The voltage of photomultiplier tube was set at 850 V. For EC detection of Arm, a copper disk electrode of 350 μm diameter was used. The amperometric signal was detected in 60 mM NaOH at 0.7 V with a Model CHI800 Voltammetric Analyzer (CH Instruments, Austin, TX). The distance between the working electrode and the end of capillary was 200 μm.

Adaptive interactions: For adaptive binding action, the two aptamers were dissolved in the binding buffer (20 mM phosphate of pH 7.0). Just before use, they were heated at 88 °C for 10 min to dissociate any intermolecular interaction, then cooled and allowed to hybridize. Aptamer A1 was hybridized at room temperature for 1 h to form one binding site of the three-dimensional structure, while aptamer A2 was hybridized at 4 °C for 30 min to form a hairpin structure with a ten-residue loop. Then the aptamer solutions were mixed with the targets dissolved in the binding buffer. The interaction between aptamer A1 and cocaine was kept for 1 h at room temperature, while aptamer A2 was incubated with Arm at 4 °C for 2 h.

Molecular recognition: As precolumn preparation, the target molecules and the analogues were incubated with aptamers to form corresponding molecule–aptamer complexes, accompanied by a decrease in the concentration of free target molecules. Then the mixtures were used as samples and measured using CE with ECL or EC detection. Target molecules generated ECL or EC signal on the electrode surface and were used as indicators for the molecule–aptamer interactions, reflecting the adaptive binding by the decrease of their signal intensities. As control experiments, target molecules without incubation with aptamers were subsequently measured by CE analysis.

Biological application: Cocaine samples in biological fluids were prepared by addition of 100 μL of 20 mM cocaine and 150 μL of 10 mM ecgonine to 250 μL of FBS. Then 10 μL of biological sample was mixed with 10 μL of 2 mM aptamer A1. After incubated at room temperature for 1 h, the mixture was analyzed by CE-ECL. A mixture of 2 mM cocaine and 1.5 mM ecgonine dissolved in 25 % FBS was used as control.

Acknowledgements

This work is supported by the National Natural Science Foundation of China with the Grants 20335040, 20427003 and 20675078 and Chinese Academy of Sciences KJCX2.YW.HO9.

[1] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505–511.
[2] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818–822.
[3] T. Hermann, D. J. Patel, *Science* **2000**, *287*, 820–825.
[4] J. Liu, Y. Lu, *Angew. Chem.* **2006**, *118*, 96–100; *Angew. Chem. Int. Ed.* **2006**, *45*, 90–94.
[5] H. So, K. Won, Y. H. Kim, B. Kim, B. H. Ryu, P. S. Na, H. Kim, J. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 11 906–11 907.
[6] M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* **2001**, *123*, 4928–4931.
[7] M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* **2000**, *122*, 11 547–11 548.
[8] B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger, K. W. Plaxco, *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.
[9] P. L. Sazani, R. Larralde, J. W. Szostak, *J. Am. Chem. Soc.* **2004**, *126*, 8370–8371.
[10] J. Wang, Y. Jiang, C. Zhou, X. Fang, *Anal. Chem.* **2005**, *77*, 3542–3546.
[11] M. Koizumi, R. R. Breaker, *Biochemistry* **2000**, *39*, 8983–8992.
[12] K. Harada, A. D. Frankel, *EMBO J.* **1995**, *14*, 5798–5811.
[13] C. H. Lin, D. J. Patel, *Nat. Struct. Biol.* **1996**, *3*, 1046–1050.
[14] E. J. Merino, K. M. Weeks, *J. Am. Chem. Soc.* **2005**, *127*, 12 766–12 767.
[15] E. Vianini, M. Palumbo, B. Gatto, *Bioorg. Med. Chem.* **2001**, *9*, 2543–2548.
[16] Y. Li, C. R. Geyer, D. Sen, *Biochemistry* **1996**, *35*, 6911–6922.
[17] M. Michaud, E. Jourdan, C. Ravelet, A. Villet, A. Ravel, C. Grosset, E. Peyrin, *Anal. Chem.* **2004**, *76*, 1015–1020.
[18] Y. Xiao, B. D. Piorek, K. W. Plaxco, A. J. Heeger, *J. Am. Chem. Soc.* **2005**, *127*, 17 990–17 991.
[19] V. Pavlov, Y. Xiao, B. Shlyahovsky, I. Willner, *J. Am. Chem. Soc.* **2004**, *126*, 11 768–11 769.
[20] H. Zhang, Z. Wang, X. Li, X. C. Le, *Angew. Chem.* **2006**, *118*, 1606–1610; *Angew. Chem. Int. Ed.* **2006**, *45*, 1576–1580.
[21] V. Pavski, X. C. Le, *Anal. Chem.* **2001**, *73*, 6070–6076.
[22] C. J. Yang, S. Jockusch, M. N. J. Vicens, Turro, W. Tan, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17 278–17 283.
[23] C. Huang, Y. Huang, Z. Cao, W. Tan, H. Chang, *Anal. Chem.* **2005**, *77*, 5735–5741.
[24] J. K. Herr, J. E. Smith, C. D. Medley, D. Shangguan, W. Tan, *Anal. Chem.* **2006**, *78*, 2918–2924.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[25] M. Zayats, Y. Huang, R. Gill, C. Ma, I. Willner, *J. Am. Chem. Soc.* **2006**, *128*, 13666–13667.
[26] J. H. Choi, K. H. Chen, M. S. Strano, *J. Am. Chem. Soc.* **2006**, *128*, 15584–15585.
[27] F. L. Floch, H. A. Ho, M. Leclerc, *Anal. Chem.* **2006**, *78*, 4727–4731.
[28] D. Xu, D. Xu, X. Yu, Z. Liu, W. He, Z. Ma, *Anal. Chem.* **2005**, *77*, 5107–5113.
[29] M. C. Rodriguez, A. N. Kawde, J. Wang, *Chem. Commun.* **2005**, 4267–4269.
[30] A. N. Kawde, M. C. Rodriguez, T. M. H. Lee, J. Wang, *Electrochem. Commun.* **2005**, *7*, 537–540.
[31] Y. Xu, Y. Gao, H. Wei, Y. Du, E. Wang, *J. Chromatogr. A* **2006**, *1115*, 260–266.
[32] P. Luo, F. Zhang, R. P. Baldwin, *Anal. Chem.* **1991**, *63*, 1702–1707.
[33] J. Ye, R. P. Baldwin, *Anal. Chem.* **1994**, *66*, 2669–2674.
[34] S. D. Mendonsa, M. T. Bowser, *J. Am. Chem. Soc.* **2004**, *126*, 20–21.
[35] S. D. Mendonsa, M. T. Bowser, *J. Am. Chem. Soc.* **2005**, *127*, 9382–9383.
[36] A. Drabovich, M. Berezovski, S. N. Krylov, *J. Am. Chem. Soc.* **2005**, *127*, 11224–11225.
[37] M. Berezovski, A. Drabovich, S. M. Krylova, M. Musheev, V. Okhonin, S. N. Krylov, *J. Am. Chem. Soc.* **2005**, *127*, 3165–3171.
[38] J. Ruta, C. Ravelet, C. Grosset, J. Fize, A. Ravel, A. Villet, E. Peyrin, *Anal. Chem.* **2006**, *78*, 3032–3039.
[39] A. J. Haes, R. C. Giordano, G. E. Collins, *Anal. Chem.* **2006**, *78*, 3758–3764.
[40] I. German, D. D. Buchanan, R. T. Kennedy, *Anal. Chem.* **1998**, *70*, 4540–4545.
[41] A. A. Fannin, D. A. Floreani, L. A. King, J. S. Landers, B. J. Piersma, D. J. Stech, R. L. Vaughn, J. D. Wilkes, J. L. Williams, *J. Phys. Chem.* **1984**, *88*, 2614–2621.
[42] S. M. Mwongela, A. Numan, N. L. Gill, R. A. Agbaria, I. M. Warner, *Anal. Chem.* **2003**, *75*, 6089–6096.
[43] T. Li, J. Yuan, J. Yin, Z. Zhang, E. Wang, *J. Chromatogr. A* **2006**, *1134*, 311–316.

Received: January 16, 2007
Revised: February 23, 2007
Published online: May 15, 2007

© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim