JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Rapid Detection of a Cocaine-Binding Aptamer Using Biological Nanopores on a Chip

Ryuji Kawano,[†] Toshihisa Osaki,[†] Hirotaka Sasaki,[†] Masahiro Takinoue,[§] Satoko Yoshizawa,[‡] and Shoji Takeuchi*[,†,‡,§]

[†]Bio Microsystems Project, Kanagawa Academy of Science and Technology (KAST), Kawasaki City, Japan

[‡]Laboratory for Integrated Micro Mechatronic Systems (LIMMS), CNRS-IIS (UMI2820), The University of Tokyo, Tokyo, Japan

[§]Institute of Industrial Science (IIS), The University of Tokyo, Tokyo, Japan

Supporting Information

ABSTRACT: This paper describes a methodology for the rapid and highly selective detection of cocaine using a membrane protein channel combined with a DNA aptamer. The DNA aptamer recognizes the cocaine molecule with high selectivity. We successfully detected a low concentration of cocaine (300 ng/mL, the drug test cutoff limit) within 60 s using a biological nanopore embedded in a microchip.

Channel current recordings of membrane proteins reconstituted in artificial bilayer lipid membranes (BLMs) are a powerful tool for the identification of analytes.[1,2] In particular, the transmembrane toxin α-hemolysin (αHL) has been used as a sensing element for the detection of organic molecules,[3] water-soluble polymers,[4] peptides,[5,6] and DNA.[7,8] The use of nanopore detection has many advantages. For example, the protein nanopore allows label-free detection with a high signal-to-noise ratio at the single-molecule level. However, if the target molecule is much smaller than the protein pore, the detection signal cannot be observed. For small-molecule detection, Bayley and co-workers used a cyclodextrin (CD) or its derivatives as a recognition site in the αHL pore.[1] The CD adapters are proposed to occupy the protein pore, thus reducing the pore size.[9] However, the CD adapters do not have specific selectivity for small molecules, and use of the CD detection system requires mutation of the protein channel.

Here we utilized a DNA aptamer combined with the αHL channel for highly selective small-molecule detection. Aptamers are nucleic acids that have been engineered to have specific recognition properties for small molecules; the conformation of the aptamer is changed by ligand binding.[10] We propose a DNA aptamer methodology in which the difference between the target-bound and non-target-bound states of the aptamer is clearly observed (Figure 1a). The DNA aptamer is single-stranded before binding to the target molecule. The single-stranded DNA (ssDNA) can pass through the αHL pore because the constricted region of the channel (1.5 nm) is larger than the diameter of the ssDNA (~1 nm), as depicted in the left panel of Figure 1a. In contrast, the size of a ligand-bound aptamer does not allow it to pass through the pore (Figure 1a, right). In addition, the presence of the ligand-bound aptamer retained in the pore can be clearly observed because of the large difference between the channel currents for the translocated and captured states. Recognition of a target molecule is easy when the aptamer is retained in the pore.

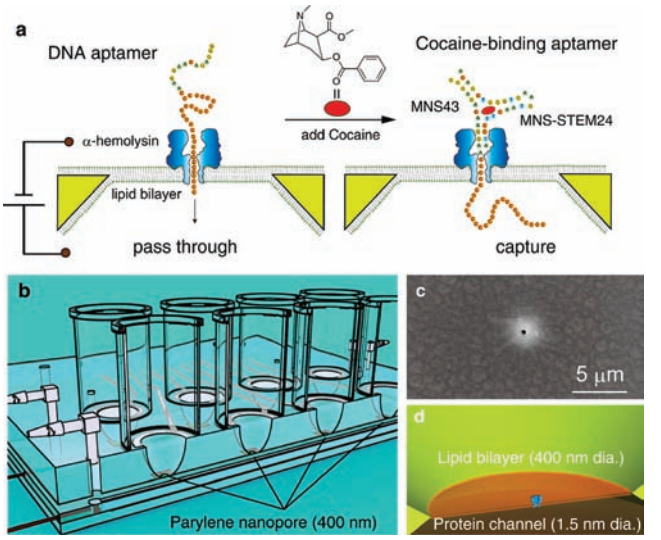

Figure 1. (a) Schematic overview of detection using the cocaine-binding aptamer (CBA). The DNA aptamer can pass through the pore in the absence of cocaine (left). However, in the presence of cocaine, the CBA cannot pass through and is captured in the pore (right). (b) Microfluidic device with parylene nanopores. (c) Scanning electron microscopy image of a parylene nanopore. (d) The bilayer lipid membrane in the parylene nanopore is stable because of the small membrane area (~400 nm).

We attempted to apply this aptamer method to rapid cocaine sensing. Analytical techniques for the rapid detection of cocaine are important in border-control situations or drug investigations. Detection of the cocaine molecule with a DNA aptamer has been studied using electrochemical[11,12] and fluorescent[13,14] probes. An αHL pore has also been used to elucidate the folding/unfolding mechanism of a single DNA aptamer.[15−17] To apply the aptamer method to cocaine detection, we designed a cocaine-binding aptamer (CBA) with a long DNA tail, as depicted in the right panel of Figure 1a and in Figure S1 in the Supporting Information. In our method, the captured state of the CBA can

Received: March 23, 2011
Published: May 09, 2011

dx.doi.org/10.1021/ja2026085 | J. Am. Chem. Soc. 2011, 133, 8474–8477

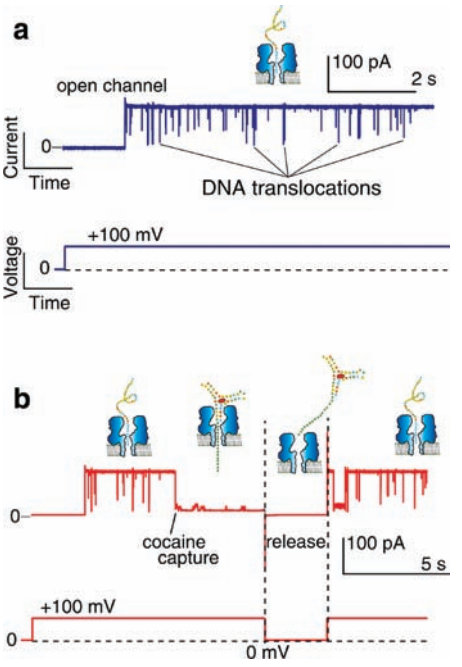

Figure 2. Typical current−time traces for DNA aptamers (a) without and (b) with cocaine in 1.0 M KCl, 10 mM PBS, and 1 mM EDTA (pH 7.4).

clearly be observed because of the addition of the long tail, and the cocaine concentration can be determined by measuring the time until capture. In this study, we verified the feasibility of this system and demonstrated rapid cocaine detection with a microfluidic device (Figure 1b−d).

The CBA used in this study comprised two oligonucleotides, MNS43 and MNS-STEM24 (Figure S1). The cocaine aptamer consisted of a two-oligonucleotide chain with the following sequences:[14] MNS43, 5′-GGGAGACAAGGAA$(C)_{30}$; MNS-STEM24, 5′-ATCCTTCAATGAAGTGGGTCGACA. The concentrations of these DNA fragments were maintained at 50 μM in the buffer. Cocaine solutions were then diluted to concentrations of 1, 10, 30, 50, and 100 μM (1 μM ≈ 300 ng/mL). The dissociation constant of this type of aptamer with cocaine is 0.4−10 μM,[14] and the detection limit should exist at 1 μM.[18] Aminobenztropine (ABT) was used in the control experiment as a cocaine analogue. The DNA and cocaine or ABT were placed in the buffer for several minutes before measurements were recorded. Following protein reconstitution, cocaine capture or control measurements were attempted at 100 mV (cis side negative). After the CBA was captured, the applied voltage was turned off to release the CBA from the pore, and then the voltage of 100 mV was applied again. We defined a CBA capture event as an 80 ± 5% current blockade for longer than 500 ms. Generally, the time of ssDNA translocation (∼100-mer) is not longer than 100 ms.[19] All of the measurements were performed at 23 ± 1 °C in a clean room with less than 60% humidity. The concentration dependence was measured using a glass micropore, and rapid cocaine detection was demonstrated using a microfluidic device.

The sequences of the complementary and the binding segments were reported previously,[14] and the structure of the reported CBA is a three-way junction (Y-shaped structure). This CBA structure produced two problems for the channel recordings. First, the Y-shaped aptamer without the long tail easily and

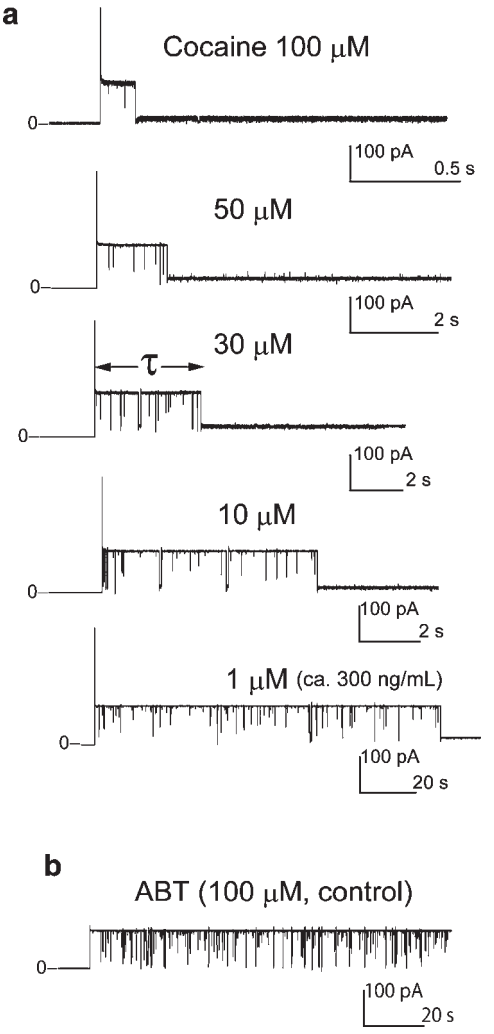

Figure 3. (a) Typical current−time traces for DNA aptamers (50 μM each) with cocaine at concentrations from 1 μM (∼300 ng/mL) to 100 μM using a glass micropore in 1.0 M KCl PBS buffer (pH 7.4) at 100 mV. (b) Channel recording for DNA aptamers with ABT as a control under the same conditions in as the cocaine experiment.

spontaneously escaped from the channel because it lingered in the channel vestibule.[20] Second, the blocking-current amplitude of the vestibule block was ∼50% of the full blocking amplitude.[21] Thus, we modified one of the oligonucleotides by adding a long (30-mer) cytosine overhang, as mentioned above. As a result, long blocking currents for CBA were observed (i.e., the capture state of the CBA became stable). Additionally, this 30-mer overhang occupied the β-barrel region of the channel; a minimum of a 25-mer nucleotide is required to enter the β-barrel region from the entrance of the αHL.[22] When the β-barrel was occupied with the cytosine overhang, a distinct blocking current appeared at 85% of the full blocking amplitude.[19,23] Homopolymers of DNA and RNA give distinguishable blocking-current amplitudes.[24] This distinctive blocked state ensured that the CBA could be readily detected because the other blocking currents (e.g., from impurities or protein gating) were usually observed at other amplitude levels. These results indicate that the overhang plays an important role when the CBA is captured in the pore.

Typical current−time traces for aptamers with and without cocaine are shown in Figure 2. DNA aptamers that formed

dx.doi.org/10.1021/ja2026085 |J. Am. Chem. Soc. 2011, 133, 8474–8477

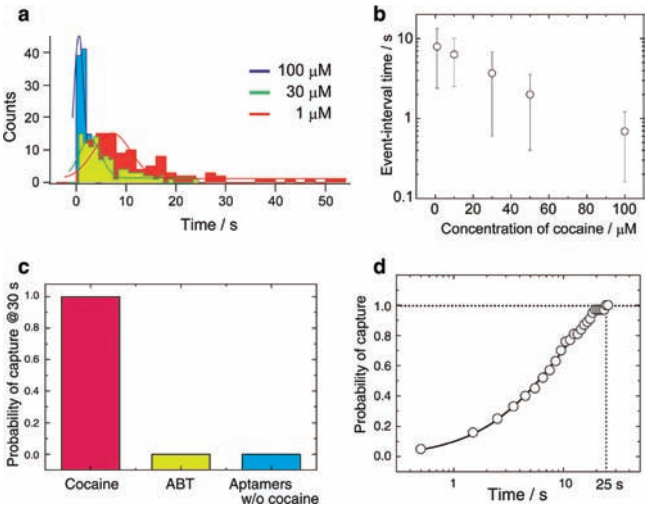

Figure 4. (a) Typical histograms of the event-interval time (τ) for CBA capture. Solid lines are fits of the histograms to Gaussian curves, which were used to obtain peak values. (b) Cocaine concentration dependence of τ. The cocaine concentration ranged from 1 to 100 μM. (c) Selectivity of this method in the microfluidic device. The probability of CBA capture with cocaine was compared with the probability of CBA capture without cocaine at 30 s after the channel was opened. ABT and DNA aptamers were used as controls under the same experimental conditions as in Figure 3. (d) Probability of CBA capture as a function of time in the microfluidic device. The probability of cocaine detection became 1 in just 25 s for a concentration of 3 μg/mL in the device.

single-stranded chains passed through the pore in the absence of cocaine. Spikelike current blockades were observed during ssDNA translocation (Figure 2a). In contrast, in the presence of cocaine molecules, aptamers bound cocaine molecules and formed the complexes. Protein pores captured the CBAs, and the long current blockade was observed. The CBA was released from the pore by turning off the bias and then captured again by applying a voltage of 100 mV (Figure 2b). The obstructed pore occasionally opened spontaneously after a long blockade (Figure S3). This behavior may have been caused by unzipping[25] or by escape from the pore of the CBA.

We analyzed the event-interval time, τ, which is the time required for the aptamer to go from the opened to the captured state (Figure 3a) to examine the concentration dependence of the response. This time was observed to increase as the cocaine concentration decreased (Figure 3a). Histograms of τ at different cocaine concentrations are shown in Figure 4a. The histograms were fitted to Gaussian curves, and the mean time values were plotted against the concentration (Figure 4b). The lowest concentration was set equal to the federal workplace cutoff value for cocaine.[26] The mean values of τ ranged from ~1 to 10 s in this method. The captured state was not observed when the cocaine analogue ABT was added as a control instead of cocaine (Figure 3b). Rapid detection using a biological nanopore is important for use as a real-time drug test.

Rapid sensing of cocaine was demonstrated using a microfluidic device. This device had eight chambers, and each chamber had a nanosized platform for durable channel recordings, as previously described.[27] The probability of cocaine detection was measured in this experiment. The response using DNA aptamers with cocaine was compared with that using DNA aptamers with ABT and that using DNA aptamers alone. Because of the inherent selectivity of the aptamer for its target, the differences between cocaine and the controls were clearly shown with the device (Figure 4c). Next, rapid detection of cocaine using DNA aptamers was tested in our microfluidic device. The results indicated that cocaine at a concentration of 3 μg/mL could be sensed in just 25 s with high selectivity using our microchip system (Figure 4d). The detection times ranged from 60 s at 300 ng/mL to 5 s at 30 μg/mL (Figure S4).

In conclusion, biological nanopores conjugated with DNA aptamers in a microchip allow the rapid detection of cocaine. In this system, cocaine at a concentration equivalent to that of the drug test cutoff limit was recognized within less than 1 min with high selectivity. Furthermore, this system could be applied not only to cocaine but also to a wide variety of targets, as several hundred aptamers have already been developed. This method is expected to aid in the development of real-time drug testing technology and the rapid sensing of small molecules using biosensors on a chip.

■ ASSOCIATED CONTENT

Supporting Information. Chemicals, materials, fabrication procedures, instrumentation, and complete ref 8. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
takeuchi@iis.u-tokyo.ac.jp

■ ACKNOWLEDGMENT

We thank Maiko Onuki, Utae Nose, Yoshimi Komaki, and Wakako Mukai for technical assistance in the preparation of the microfluidic device. This work was partially supported by the Strategic International Cooperative Program of the Japan Science and Technology Agency (JST).

■ REFERENCES

(1) Gu, L. Q.; Braha, O.; Conlan, S.; Cheley, S.; Bayley, H. *Nature* **1999**, *398*, 686–690.
(2) Bayley, H.; Cremer, P. S. *Nature* **2001**, *413*, 226–230.
(3) Bayley, H.; Braha, O.; Gu, L. Q. *Adv. Mater.* **2000**, *12*, 139–142.
(4) Robertson, J. W. F.; Rodrigues, C. G.; Stanford, V. M.; Rubinson, K. A.; Krasilnikov, O. V.; Kasianowicz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8207–8211.
(5) Movileanu, L.; Schmittschmitt, J. P.; Scholtz, J. M.; Bayley, H. *Biophys. J.* **2005**, *89*, 1030–1045.
(6) Schibel, A. E. P.; An, N.; Jin, Q. A.; Fleming, A. M.; Burrows, C. J.; White, H. S. *J. Am. Chem. Soc.* **2010**, *132*, 17992–17995.
(7) Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13770–13773.
(8) Branton, D.; et al. *Nat. Biotechnol.* **2008**, *26*, 1146–1153.
(9) Gu, L. Q.; Cheley, S.; Bayley, H. *Science* **2001**, *291*, 636–640.
(10) Hermann, T.; Patel, D. J. *Science* **2000**, *287*, 820–825.
(11) Swensen, J. S.; Xiao, Y.; Ferguson, B. S.; Lubin, A. A.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Soh, H. T. *J. Am. Chem. Soc.* **2009**, *131*, 4262–4266.
(12) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.
(13) Stojanovic, M. N.; de Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* **2000**, *122*, 11547–11548.
(14) Stojanovic, M. N.; de Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* **2001**, *123*, 4928–4931.

(15) Shim, J. W.; Gu, L. Q. *J. Phys. Chem. B* **2008**, *112*, 8354–8360.
(16) Ying, Y. L.; Wang, H. Y.; Sutherland, T. C.; Long, Y. T. *Small* **2011**, *7*, 87–94.
(17) Gu, L. Q.; Shim, J. W. *Analyst* **2010**, *135*, 441–451.
(18) Freeman, R.; Li, Y.; Tel-Vered, R.; Sharon, E.; Elbaz, J.; Willner, I. *Analyst* **2009**, *134*, 653–656.
(19) Meller, A.; Nivon, L.; Brandin, E.; Golovchenko, J.; Branton, D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1079–1084.
(20) Vercoutere, W. A.; Winters-Hilt, S.; DeGuzman, V. S.; Deamer, D.; Ridino, S. E.; Rodgers, J. T.; Olsen, H. E.; Marziali, A.; Akeson, M. *Nucleic Acids Res.* **2003**, *31*, 1311–1318.
(21) Howorka, S.; Cheley, S.; Bayley, H. *Nat. Biotechnol.* **2001**, *19*, 636–639.
(22) Henrickson, S. E.; DiMarzio, E. A.; Wang, Q.; Stanford, V. M.; Kasianowicz, J. J. *J. Chem. Phys.* **2010**, *132*, No. 135101.
(23) Kawano, R.; Schibel, A.; Cauley, C.; White, H. *Langmuir* **2009**, *25*, 1233–1237.
(24) Ashkenasy, N.; Sanchez-Quesada, J.; Bayley, H.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 1401–1404.
(25) Mathe, J.; Visram, H.; Viasnoff, V.; Rabin, Y.; Meller, A. *Biophys. J.* **2004**, *87*, 3205–3212.
(26) Moeller, K. E.; Lee, K. C.; Kissack, J. C. *Mayo Clin. Proc.* **2008**, *83*, 66–76.
(27) Kawano, R.; Osaki, T.; Sasaki, H.; Takeuchi, S. *Small* **2010**, *6*, 2100–2104.