Turbidimetric detection of ATP using polymeric micelles and DNA aptamers†

Daisuke Miyamoto,‡ Zhonglan Tang, Tohru Takarada* and Mizuo Maeda

Received (in Cambridge, UK) 27th June 2007, Accepted 20th August 2007
First published as an Advance Article on the web 4th September 2007
DOI: 10.1039/b709775a

Two types of turbidimetric detection of adenosine 5′-triphosphate (ATP) by the naked eye were achieved through a combination of non-cross-linking aggregation of DNA-linked polymeric micelles and molecular recognition of ATP by a DNA aptamer.

DNA aptamers are single-stranded deoxyribonucleic acids (ssDNAs), which are capable of binding with strong affinity and high selectivity to a wide variety of target molecules, such as small organic dyes, ribonucleotides, carbohydrates and proteins.[1] Various analytical methods have been developed to exploit molecular recognition by aptamers,[2] based on a cantilever sensor,[3] mass spectrometry,[4] surface plasmon resonance imaging,[5] an electrochemical device,[6] affinity capillary electrophoresis[7] and fluorescence signaling.[8] In addition, a colorimetric detection assay was reported using cross-linking[9] or non-cross-linking[10] of gold nanoparticles. Here, we describe a facile method for naked-eye detection of complex formation of a DNA aptamer with its target molecule as a turbidity change in micellar dispersion.

We recently found that a polymeric micelle with a dehydrated poly(*N*-isopropylacrylamide) core surrounded by an ssDNA corona had drastically lowered colloidal stability when complementary DNA was added to the dispersion of polymeric micelles to form the fully-matched double helix.[11] Similar hybridization-induced colloidal destabilization was observed with ssDNA-linked gold[12] or polystyrene[13] nanoparticles, suggesting that the destabilization was independent of the properties of the hydrophobic core. The destabilization is at least partially attributed to a decrease in entropic repulsion between the micelles,[14] which is caused because duplex formation stiffens the DNA strands. In this study, two ATP detection methods were devised through an effective combination of hybridization-induced colloidal destabilization and specific recognition of ATP by a DNA aptamer.

We prepared an ssDNA-linked polymeric micelle according to the previous reports.[15] Poly(*N*-isopropylacrylamide) grafted with ssDNA (PNIPAAm-*g*-DNA) was synthesized by radical copolymerization between *N*-isopropylacrylamide and an 11-base DNA (**DNA1**, Fig. 1) modified with a methacryloyl group at the 5′-end (DNA macromonomer). The cloud-point temperature of PNIPAAm-*g*-**DNA1** (the molecular weight (M_n): 4.5 × 10^4; the distribution (M_w/M_n): 1.8; DNA macromonomer fraction: 0.33mol%) in 20 mM Tris-HCl buffer (pH 8.3) containing 300mM NaCl and 5 mM $MgCl_2$ was determined to be 34 °C, based on a slight decrease in transmittance at 500 nm.[11b] The concentration of **DNA1** in 0.12 mg ml^{-1} PNIPAAm-*g*-**DNA1** was determined to be 3.2 μM. When the same buffer solution of PNIPAAm-*g*-**DNA1** was heated at 40 °C, the copolymers immediately self-assembled to form a polymeric micelle (**PM1**) with a PNIPAAm core surrounded by **DNA1**. The hydrodynamic radius of **PM1** was determined to be 46 nm from dynamic light scattering measurement. The colloidal stability of the polymeric micelles was evaluated by the critical coagulation concentration (CCC) of the supporting electrolyte (NaCl). The CCC was determined from measurement of the transmittance at 500 nm of the micellar dispersion with a UV-vis spectrometer at 40 °C. The measurement temperature was chosen to be above the cloud-point of PNIPAAm-*g*-**DNA1** (34 °C) but below the lowest melting temperature (57 °C) of double-stranded DNAs (dsDNAs) formed on the micellar surface (*vide infra*).[16]

Fig. 1 shows the colloidal stability of various polymeric micelles used in the present study. When the complementary 11-base DNA (**DNA2**) equimolar to **DNA1** (3.2 μM) was added to the dispersion of **PM1**, the resulting dsDNA-linked polymeric micelle (**PM2**) drastically decreased the colloidal stability. The aggregation occurred within 3 min after **DNA2** was added. In contrast, when the 25-base DNA (**DNA3**) was similarly hybridized with **DNA1** to form **PM3**, the colloidal stability of **PM3** remained unchanged. Protrusion of a flexible 14-base ssDNA presumably caused entropic repulsion between the micelles. Consistently, when the

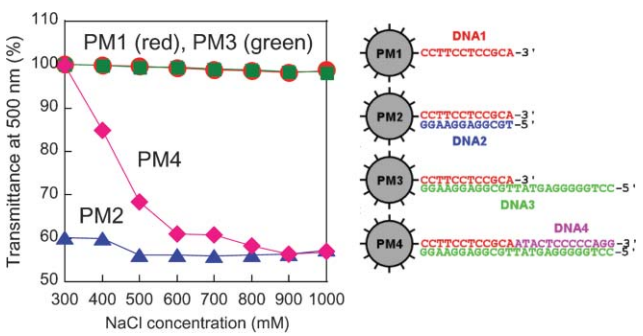

Fig. 1 Transmittance at 500 nm of the dispersion of DNA-linked polymeric micelles as a function of the concentration of NaCl in 20 mM Tris-HCl buffer (pH 8.3) containing 5 mM $MgCl_2$ at 40 °C. The concentration of polymeric micelles was 0.12 mg ml^{-1}, containing 3.2 μM of **DNA1**. The concentrations of **DNA2**, **DNA3** and **DNA4** were each 3.2 μM.

Bioengineering Laboratory, Discovery Research Institute, RIKEN, Wako, Saitama 351-0198, Japan. E-mail: ttkrd@riken.jp; Fax: +81-48-462-4658; Tel: +81-48-467-5489

† Electronic supplementary information (ESI) available: Experimental details and additional results. See DOI: 10.1039/b709775a

‡ Present address: Tsukuba Research Center for Interdisciplinary Materials Science (TIMS), University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan.

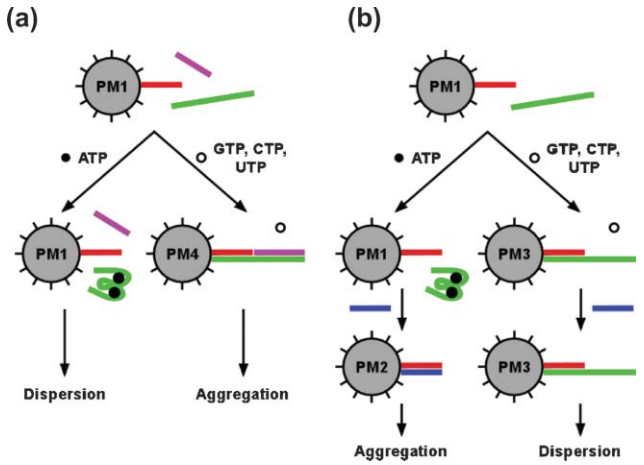

Fig. 2 Schematic representation of two methods for detecting ATP on the basis of (a) dispersion and (b) aggregation of the polymeric micelles. Red, blue, green and purple rectangles denote **DNA1**, **DNA2**, **DNA3** and **DNA4**, respectively.

14-base ssDNA complementary to the protruding part (**DNA4**) was added to the dispersion of **PM3** to form a singly nicked duplex on the surface, the resulting micelle (**PM4**) was remarkably destabilized again. Since **DNA3** is an anti-ATP DNA aptamer, which captures two ATP molecules,[17] ATP will inhibit competitively the hybridization of **DNA3** with **DNA1** on the surface and alter the colloidal stability of the micelles. Based on this working principle, we designed two ATP detection methods.

First, we developed an analytical system where the turbidity increased in the absence of ATP (Fig. 2a). The transmittance of the dispersion of **PM1** containing **DNA3** and **DNA4** (3.2 μM each) in the presence of ATP (1.0 mM) was almost 100%, and did not depend on the concentration of NaCl (Fig. 3a). This suggested that **DNA1** on the surface of **PM1** remained single-stranded due to the complex formation of the DNA aptamer (**DNA3**) with two ATP molecules, leading to the stable dispersion of **PM1** (see Fig. 1). In contrast, the transmittance of the dispersions of **PM1** containing **DNA3** and **DNA4** in the presence of guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP) or uridine 5′-triphosphate

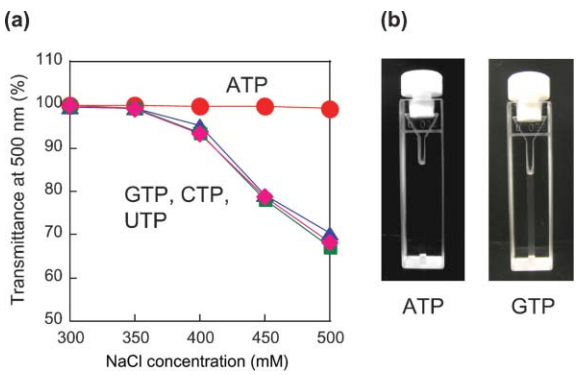

Fig. 3 (a) Transmittance of the dispersion of polymeric micelles containing **DNA3**, **DNA4** and NTP (ATP (red), GTP (blue), CTP (green) or UTP (purple); 1.0 mM each) as a function of NaCl concentration. The conditions were the same as those in Fig. 1. (b) Photograph of the micellar dispersion 1 min after the addition of ATP or GTP. The concentration of NaCl was 500 mM.

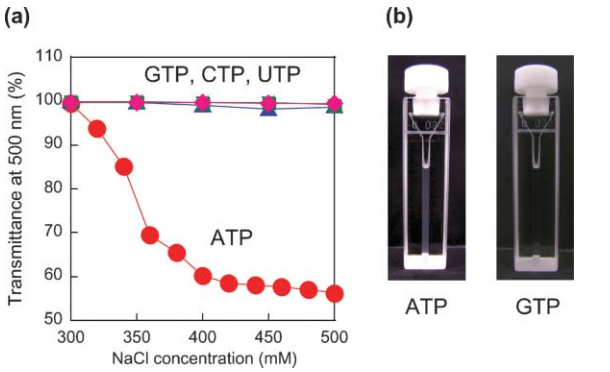

Fig. 4 (a) Transmittance of the dispersion of polymeric micelles containing **DNA2**, **DNA3** and NTP (ATP (red), GTP (blue), CTP (green) or UTP (purple); 1.0 mM each) as a function of NaCl concentration. The conditions were the same as those in Fig. 1. (b) Photograph of micellar dispersion 1 min after the addition of ATP or GTP. The concentration of NaCl was 400 mM.

(UTP) decreased gradually when the concentration of NaCl was increased. The CCC in each case was determined to be 350 mM (Fig. 3a). **DNA1**, **DNA3** and **DNA4** formed a singly nicked dsDNA on the micellar surface to give **PM4**, resulting in the salt-induced aggregation of the micelles (see Fig. 1). The difference in turbidity between the solution of ATP and that of GTP can be detected by the naked eye, as shown in Fig. 3b.

The inverse test can also be carried out (Fig. 2b). **DNA3** (3.2 μM) was added into the dispersion of **PM1** containing 1.0 mM ATP, GTP, CTP or UTP, followed by the subsequent addition of **DNA2** (3.2 μM). In the presence of ATP, the transmittance of the dispersion of the obtained micelles (**PM2**) decreased gradually as the concentration of NaCl increased: the CCC was determined to be 300 mM (Fig. 4a). **DNA1** on the surface was allowed to hybridize with **DNA2** because **DNA3** had already formed a complex with two ATP molecules. As a result, the salt-induced aggregation of **PM2** presumably occurred. In contrast, the transmittance of the micellar dispersion in the presence of GTP, CTP or UTP was constant at almost 100%, and did not depend on the concentration of NaCl (Fig. 4a). Since **DNA3** was unable to recognize GTP, CTP or UTP, **DNA3** was hybridized with **DNA1** to protrude its 14-base portion from the surface, thereby inducing the entropic repulsion between the micelles. The difference in turbidity between the solution of ATP and that of GTP was also

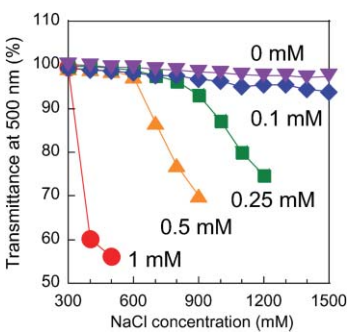

Fig. 5 Transmittance of the dispersion of polymeric micelles containing **DNA2**, **DNA3** and various amounts of ATP as a function of NaCl concentration. The conditions were the same as those in Fig. 1.

detectable by the naked eye (Fig. 4b). Fig. 5 shows that the detection limit of ATP with the second method was roughly 0.25 mM, since the turbidity with 80% transmittance at 500 nm can be detected by the naked eye. The sensitivity of the present method will be improved by utilizing a DNA aptamer that shows stronger affinity to ATP.

In summary, we developed an analytical system for detecting ATP based on non-cross-linking aggregation of polymeric micelles. This system possesses three remarkable features: (1) target molecules can be detected by the naked eye without any apparatus; (2) the structural change in the aptamer induced by binding the target molecule is not necessarily requisite; (3) both positive and negative tests can be simultaneously conducted to validate the results using common polymeric micelles. These features are especially suitable for on-site detection of environmental pollutants. Such an attempt is currently under way.

This work was supported by a Grant-in-Aid for Young Scientists (B) (KAKENHI 16700376) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to T. T.), and by a grant for Ecomolecular Science Research provided by RIKEN (to M. M. and T. T.).

Notes and references

1 (*a*) L. Gold, B. Polisky, O. Uhlenbeck and M. Yarus, *Annu. Rev. Biochem.*, 1995, **64**, 763; (*b*) D. S. Wilson and J. W. Szostak, *Annu. Rev. Biochem.*, 1999, **68**, 611.
2 S. D. Jayasena, *Clin. Chem. (Washington, DC, U. S.)*, 1999, **45**, 1628.
3 C. A. Savran, S. M. Knudsen, A. D. Ellington and S. R. Manalis, *Anal. Chem.*, 2004, **76**, 3194.
4 J. R. Cole, L. W. Dick, Jr., E. J. Morgan and L. B. McGown, *Anal. Chem.*, 2007, **79**, 273.
5 Y. Li, J. Lee and R. M. Corn, *Anal. Chem.*, 2007, **79**, 1082.
6 (*a*) A.-E. Radi, J. L. A. Sanchez, E. Baldrich and C. K. O'Sullivan, *J. Am. Chem. Soc.*, 2006, **128**, 117; (*b*) J. A. Hansen, J. Wang, A.-N. Kawde, Y. Xiang, K. V. Gothelf and G. Collins, *J. Am. Chem. Soc.*, 2006, **128**, 2228; (*c*) R. Polsky, R. Gill, L. Kaganovsky and I. Willner, *Anal. Chem.*, 2006, **78**, 2268; (*d*) F. L. Floch, A. H. Ho and M. Leclerc, *Anal. Chem.*, 2006, **78**, 4727; (*e*) R. Y. Lai, K. W. Plaxco and A. J. Heeger, *Anal. Chem.*, 2007, **79**, 229; (*f*) S. Centi, S. Tombelli, M. Minunni and M. Mascini, *Anal. Chem.*, 2007, **79**, 1466; (*g*) K. Maehashi, T. Katsura, K. Kerman, Y. Takamura, K. Matsumoto and E. Tamiya, *Anal. Chem.*, 2007, **79**, 782; (*h*) L. Shen, Z. Chen, Y. Li, P. Jing, S. Xie, S. He, P. He and Y. Shao, *Chem. Commun.*, 2007, 2169.
7 (*a*) D. D. Buchanan, E. E. Jameson, J. Perlette, A. Malik and R. T. Kennedy, *Electrophoresis*, 2003, **24**, 1375; (*b*) M. Berezovski, R. Nutiu, Y. Li and S. N. Krylov, *Anal. Chem.*, 2003, **75**, 1382; (*c*) M. A. Rehder-Silinski and L. B. McGown, *J. Chromatogr., A*, 2003, **1008**, 233; (*d*) C.-C. Huang, Z. Cao, H.-T. Chang and W. Tan, *Anal. Chem.*, 2004, **76**, 6973; (*e*) J. Ruta, C. Ravelet, C. Grosset, J. Fize, A. Ravel, A. Villet and E. Peyrin, *Anal. Chem.*, 2006, **78**, 3032.
8 (*a*) M. N. Stojanovic and D. M. Kolpashchikov, *J. Am. Chem. Soc.*, 2004, **126**, 9266; (*b*) C. J. Yang, S. Jockusch, M. Vicens, N. J. Turro and W. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 17278; (*c*) E. J. Merino and K. M. Weeks, *J. Am. Chem. Soc.*, 2005, **127**, 12766; (*d*) M. Levy, S. F. Cater and A. D. Ellington, *ChemBioChem*, 2005, **6**, 2163; (*e*) J. Wang, Y. Jiang, C. Zhou and X. Fang, *Anal. Chem.*, 2005, **77**, 3542; (*f*) N. K. Navani and Y. Li, *Curr. Opin. Chem. Biol.*, 2006, **10**, 272; (*g*) J. H. Choi, K. H. Chen and M. S. Strano, *J. Am. Chem. Soc.*, 2006, **128**, 15584; (*h*) B. Li, H. Wei and S. Dong, *Chem. Commun.*, 2007, 73.
9 (*a*) C.-C. Huang, Y.-F. Huang, Z. Cao, W. Tan and H.-T. Chang, *Anal. Chem.*, 2005, **77**, 5735; (*b*) J. Liu, D. Mazumdar and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 7955.
10 W. Zhao, W. Chiuman, M. A. Brook and Y. Li, *ChemBioChem*, 2007, **8**, 727.
11 (*a*) T. Mori and M. Maeda, *Polym. J. (Tokyo, Jpn.)*, 2002, **34**, 624; (*b*) T. Mori and M. Maeda, *Langmuir*, 2004, **20**, 313; (*c*) Z. L. Tang, T. Takarada, Y. Sato and M. Maeda, *Chem. Lett.*, 2004, **33**, 1602; (*d*) M. Maeda, *Polym. J. (Tokyo, Jpn.)*, 2006, **38**, 1099.
12 (*a*) K. Sato, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2003, **125**, 8102; (*b*) K. Sato, K. Hosokawa and M. Maeda, *Nucleic Acids Res.*, 2005, **33**, e4; (*c*) K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa and M. Maeda, *Anal. Biochem.*, 2006, **350**, 162; (*d*) K. Sato, K. Hosokawa and M. Maeda, *Anal. Sci.*, 2007, **23**, 98.
13 K. Sato, M. Sawayanagi, K. Hosokawa and M. Maeda, *Anal. Sci.*, 2004, **20**, 893.
14 D. H. Everett, *Basic Principles of Colloid Science*, The Royal Society of Chemistry, London, 1988.
15 (*a*) T. Mori, D. Umeno and M. Maeda, *Biotechnol. Bioeng.*, 2001, **72**, 261; (*b*) T. Mori and M. Maeda, *Polym. J. (Tokyo, Jpn.)*, 2001, **33**, 830.
16 The melting temperature values of the dsDNA used in this study are shown in the electronic supplementary information (ESI)†.
17 D. E. Huizenga and J. W. Szostak, *Biochemistry*, 1995, **34**, 656.