doi:10.1246/cl.2012.917
Published on the web September 1, 2012

Specific Amino Acid Sensing Using a Single Acridone-labeled DNA Aptamer

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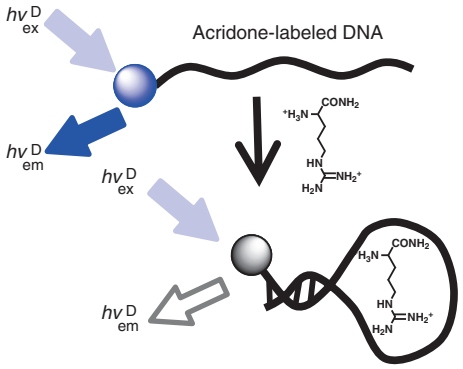
Acridone is an attractive fluorescent molecule for use in DNA probes. Sensing molecule, which has acridone as a signaling moiety and DNA aptamer as a recognition moiety, was prepared by postsynthetic modification method. The fluorescence of this sensing molecule changed depending on the DNA structure and L-argininamide could be detected by this molecule. This strategy could provide a quencher-free aptamer beacon.

Detection of specific small molecules involved in cellular processes is important for disease diagnosis and for studying these cellular processes. Aptamers are attractive candidates as recognition moieties because they have high affinities for specific molecules similar to those of antibodies. Also, similar to antibodies, they can be prepared for any type of molecule. Aptamers are single-stranded nucleic acids, which are prepared by in vitro selection.[1–3] Fluorescence labeling of aptamers provides signaling aptamers for specific molecules. Several signaling aptamers have been reported.[4,5] We have also reported a fluorescent DNA probe to detect L-argininamide, which is composed of an aptamer, a fluorophore, and a quencher.[6]

A fluorescent-labeled molecule is an essential tool for studying biomolecules. Fluorescent molecules such as fluorescein, pyrene, and cyanine dyes have been used in these studies.[7] Recently, acridone derivatives have been used by us and in other studies as fluorescent labels for nucleic acids.[8–10] Acridone is resistant to photobleaching and has a longer fluorescence lifetime (ca. 14 ns in ethanol) than fluorescein. In addition, acridone has a high quantum yield in a polar solvent. In a previous study, acridone-labeled DNA was used as a donor for a fluorescence resonance energy transfer (FRET) to detect target DNA strands.[9] A dabcyl group of dabcyl-labeled DNA quenched the acridone emission when it was close to the acridone moiety. Thus, it was found that the pair of acridone and dabcyl functioned as a FRET pair. In addition, acridone emission was quenched by guanine, which was close to acridone, on a complementary strand. This result suggested that acridone could be used as a quencher-free probe because guanines are present in native DNA. Also, Saito et al. reported G-quenched molecular beacon using an acridone-labeled DNA.[11]

Based on this concept, we synthesized a 5′-acridone-labeled aptamer because it had been reported that a 5′-acridone-labeled aptamer could specifically detect L-argininamide without an additional quencher.[12] Scheme 1 shows the concept of the acridone-labeled aptamer. In this study, we studied the 3′-terminal sequence of the 5′-acridone-labeled aptamer. This improved acridone-labeled aptamer exhibited suitable properties as a quencher-free aptamer beacon.



Scheme 1. Detection system for L-argininamide using an acridone-labeled aptamer.

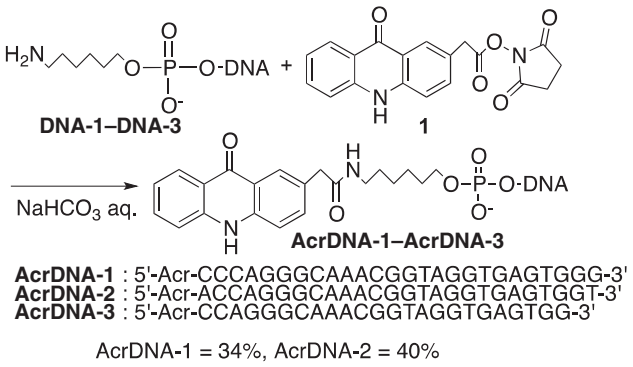


Figure 1. Synthesis of acridone derivatives and acridone-labeled DNAs. Synthesis of **AcrDNA-3** has been reported previously.[12]

The synthetic scheme and DNA sequences of acridone-labeled DNA are shown in Figure 1 along with synthesis of AcrDNA-1 reported previously.[12] The DNA sequence was based on the DNA aptamer bound to L-argininamide reported by Harada et al.[13] **DNA-3** was two bases shorter than **DNA-2**, and **DNA-1** had a terminal CG pair that replaced the AT pair of **DNA-2**. **DNA-1–DNA-3** were either synthesized by a standard phosphoramidite method or purchased from Japan Bio Services Co., LTD. (Saitama, Japan).

An *N*-hydroxysuccinimide ester of 2-acridoneacetic acid (compound **1**) was synthesized by a previously described method.[8,14] Compound **1** was reacted with 5′-amine-modified **DNA-1–DNA-3** under basic conditions. Isolation of acridone-attached DNA (**AcrDNA-1–AcrDNA-3**) was performed by a reversed-phase HPLC and characterized by ESI-MS.[15] The

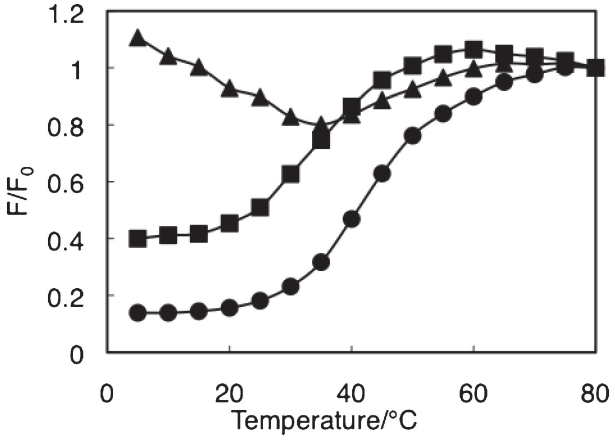


Figure 2. Temperature-dependent changes in the fluorescence intensity at 423 nm. **AcrDNA-1** (circles), **AcrDNA-2** (triangles), and **AcrDNA-3** (squares). Concentration of DNA, 0.2 μM; excitation wavelength, 388 nm; buffer, 10 mM sodium phosphate (pH 7.0). F is the fluorescence intensity at each temperatures and F_0 is the fluorescence intensity at 80 °C.

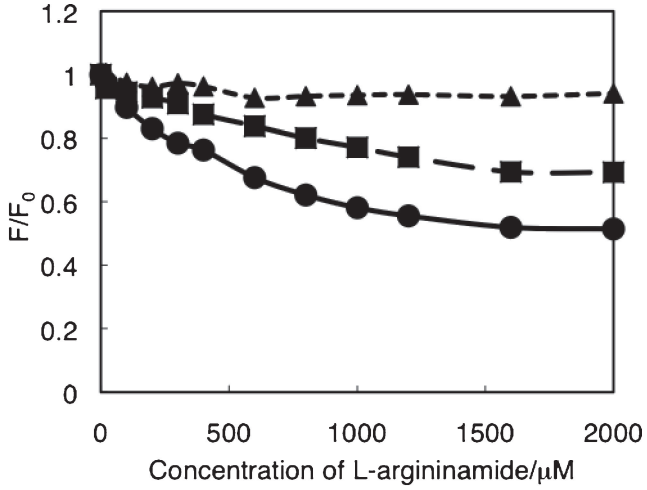


Figure 3. Fluorescence titrations of **AcrDNA-1** (circles), **AcrDNA-2** (triangles), and **AcrDNA-3** (squares). Concentration of AcrDNA, 0.2 μM; temperature, 45 °C; excitation wavelength, 388 nm; emission wavelength, 423 nm; buffer, 10 mM sodium phosphate (pH 7.0). F is the fluorescence intensity at each concentration of L-argininamide, and F_0 is the fluorescence intensity in the absence of L-argininamide.

photophysical properties of acridone derivatives have been previously described.[9] Briefly, the absorption maxima of 2-acridoneacetic acid were 388 and 407 nm in water, and the emission maxima were 422 and 448 nm. These properties were dependent on the solvents used. The fluorescent intensity decreased with decreasing solvent hydrophobicity. The fluorescent intensity was also dependent on the neighboring base. In particular, fluorescence was quenched by guanine. This property of acridone will result in a quencher-free probe.

First, temperature-dependent fluorescent spectra of acridone-attached DNA were measured (Figure 2).[18] Fluorescence intensities of **AcrDNA-3** at 423 nm increased with increasing temperature along a sigmoid curve. Because the three base pairs in the stem can form at a low temperature, the acridone moiety at 5′-terminus is close to the 3′-terminus in a stem–loop structure. Acridone fluorescence is quenched by guanine at the 3′-terminus. However, at a higher temperature, the acridone moiety is away from the 3′-terminus because the stem–loop structure is not formed. Addition of an AT pair at the **DNA-3** terminus disturbed the quenching of acridone fluorescence (**AcrDNA-2**), although the four base pairs could be formed in the stem–loop structure. This was consistent with a previous report, which indicated that acridone emission was quenched by a nearby guanine rather than other bases. This quenching by guanine could be because of its good electron-donating ability.[16,17] Addition of a GC pair at the **DNA-3** terminus, which resulted in three consecutive guanine residues at the 3′-terminus, showed large temperature-dependent changes in the fluorescence intensity (**AcrDNA-1**). These results indicated that acridone fluorescence was effectively quenched by the three consecutive guanine residues at the 3′-terminaus in the stem region of 5′-acridone-labeled DNA.

The DNA sequence used in this study was a DNA aptamer for L-argininamide. When L-argininamide was bound to DNA, the stem–loop structure was stabilized. At an appropriate temperature, the structure of the DNA–ligand complex changes from that of free DNA (Scheme 1). We previously reported a similar detection system using a fluorophore- and quencher-labeled aptamer.[6] In that study, the binding affinity of the labeled aptamer against L-argininamide was almost the same as that of the original aptamer.

Fluorescence titrations of acridone-labeled DNA (**AcrDNA-1** and **AcrDNA-2**) with L-argininamide are shown along with that of **AcrDNA-3** in Figure 3. The fluorescence titration experiments were performed at 45 °C, at which the ratio of stem–loop structure of **AcrDNA-1** in the absence of L-argininamide was ca. 50% considering the result shown in Figure 2. Fluorescence intensities of **AcrDNA-2**, which did not have guanine at its 3′-terminus, did not change much by addition of L-argininamide. In contrast, the fluorescence intensities of both **AcrDNA-1** and **AcrDNA-3** decreased with increasing concentration of the ligand. The binding of L-argininamide to each DNA resulted in formation of the stem–loop structure, and the acridone moiety at the 5′-terminus was close to the 3′-terminal nucleoside. The 3′-terminal guanines of **AcrDNA-1** and **AcrDNA-3** could quench acridone fluorescence, while the 3′-terminal thymine of **AcrDNA-2** did not quench much. In addition, **AcrDNA-1** bearing three consecutive guanine residues exhibited greater fluorescence changes according to the concentration of L-argininamide than **AcrDNA-3** bearing two consecutive guanine residues. The potent sensitivity of the present acridone–aptamer (**AcrDNA-1**) is ca. 1.5 times greater compared with the previous one (**AcrDNA-3**). As described above, the 3′-terminal consecutive guanine residues are essential for this L-argininamide detection system.

The binding affinities, which were estimated from the change in the fluorescence intensity, were 338 μM for **AcrDNA-1** and 568 μM for **AcrDNA-3**. This indicates that four base pairs are required in the stem region of this aptamer.

To investigate the specificity of this labeled aptamer, titrations with several ligands were performed. The results are shown in Figure 4. L-Arginine, L-glycine, L-lysine, and guani-

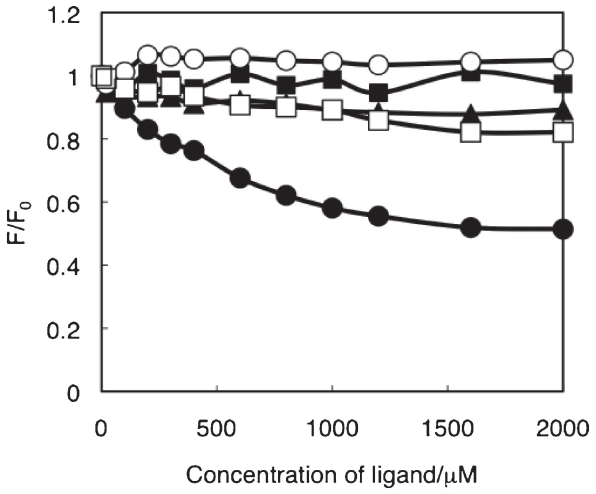


Figure 4. Fluorescence titration of **AcrDNA-1** with several L-amino acids and guanidine. L-Argininamide (closed circles), L-arginine (closed squares), L-glycine (closed triangles), L-lysine (open circles), and guanidine (open squares). Concentration of **AcrDNA-1**, 0.2 μM; temperature, 45 °C; excitation wavelength, 388 nm; emission wavelength, 423 nm; buffer, 10 mM sodium phosphate (pH 7.0). F is the fluorescence intensity at each concentration of L-argininamide, and F_0 is the fluorescence intensity in the absence of L-argininamide.

dine were used as similar ligands. Addition of L-argininamide decreased the fluorescence intensity from acridone depending on the concentration of L-argininamide. Because addition of other ligands did not result in changes in the fluorescence intensity, this modified aptamer maintained its selectivity for L-argininamide.

Compared with conventional doubly labeled aptamer beacon, this acridone-labeled aptamer was easily prepared from 5′-amine-modified DNA and a succinimide ester of 2-acridoneacetic acid. Three consecutive guanine residues in the stem region could effectively quench acridone fluorescence. The fluorescence intensity of this molecule changed depending on the concentration of L-argininamide, and its specificity for L-argininamide remained unchanged after labeling. Acridone labeling of DNA could thus be useful for detecting specific molecules because it produces a quencher-free aptamer beacon.

References and Notes

1 D. L. Robertson, G. F. Joyce, *Nature* **1990**, *344*, 467.
2 C. Tuerk, L. Gold, *Science* **1990**, *249*, 505.
3 A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.
4 R. Yamamoto, P. K. R. Kumar, *Genes Cells* **2000**, *5*, 389.
5 R. Nutiu, Y. Li, *J. Am. Chem. Soc.* **2003**, *125*, 4771.
6 H. Ozaki, A. Nishihira, M. Wakabayashi, M. Kuwahara, H. Sawai, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4381.
7 A. A. Martí, S. Jockusch, N. Stevens, J. Ju, N. J. Turro, *Acc. Chem. Res.* **2007**, *40*, 402.
8 A. Shoji, T. Hasegawa, M. Kuwahara, H. Ozaki, H. Sawai, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 776.
9 Y. Hagiwara, T. Hasegawa, A. Shoji, M. Kuwahara, H. Ozaki, H. Sawai, *Bioorg. Med. Chem.* **2008**, *16*, 7013.
10 Y. Saito, K. Hanawa, N. Kawasaki, S. S. Bag, I. Saito, *Chem. Lett.* **2006**, *35*, 1182.
11 Y. Saito, E. Mizuno, S. S. Bag, I. Suzuka, I. Saito, *Chem. Commun.* **2007**, 4492.
12 H. Ozaki, Y. Hagiwara, H. Asakura, M. Kuwahara, *Nucleic Acids Symp. Ser.* **2009**, *53*, 255.
13 K. Harada, A. D. Frankel, *EMBO J.* **1995**, *14*, 5798.
14 A. Szymańska, K. Wegner, L. Łankiewicz, *Helv. Chim. Acta* **2003**, *86*, 3326.
15 Isolated yields and ESI-MS data for **AcrDNA-1-2**: **AcrDNA-1**, 34%, ESI-MS; Found 8746.1 (Calcd for $[M\text{-}13H]^{13-}$ 8743.6); **AcrDNA-2**, 40%, ESI-MS Found 8595.6 (Calcd for $[M\text{-}12H]^{12-}$ 8596.6).
16 S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617.
17 I. Nazarenko, R. Pires, B. Lowe, M. Obaidy, A. Rashtchian, *Nucleic Acids Res.* **2002**, *30*, 2089.
18 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

