Efficient Site-selective RNA Activation and Scission Achieved by Geometry Control of Acridine Intercalation in RNA/DNA Heteroduplex

Akinori Kuzuya, Yun Shi, Keita Tanaka, Kenzo Machida, and Makoto Komiyama*
Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904

(Received January 22, 2009; CL-090080; E-mail: komiyama@mkomi.rcast.u-tokyo.ac.jp)

Two kinds of new acridine–DNA conjugates were synthesized by attaching acridines with bulky substituents to the end of DNA. These conjugates were hybridized to complementary RNA in combination with another unmodified DNA that hybridize to the adjacent fraction of the RNA, and were used as site-selective RNA activators for site-selective RNA cleavage catalyzed by Lu^{III}. The site-selective hydrolyses by these combinations are several times as fast as that achieved with the use of commercially available acridine moiety.

As important roles of various small RNAs in biological systems have been widely recognized,[1] demands for versatile RNA manipulation, i.e. site-selective cutting and pasting of RNA strand, have been increasing not only for the purpose of traditional antisense chemistry but also for controlling RNAi.[2,3] Especially, development of site-selective artificial ribonuclease has been the main target for researchers because no natural protein-based ribonuclease recognizes their target sequence.[4] Recently, we have developed a quite effective system for site-selective RNA cleavage.[5] There, the target phosphodiester linkage in substrate RNA is activated through noncovalent interactions with complementary DNA conjugate bearing an acridine, and differentiated from the other linkages in terms of reactivity. Accordingly, this linkage is selectively hydrolyzed by free catalysts (e.g., lanthanide(III) ions) in the solution. The reaction is selective and efficient enough to achieve simultaneous tandem scission in close proximity as small as 10 nucleotides,[6] and this technique has been applied to new genotyping methods for single-nucleotide (SNP) or insertion–deletion (indel) polymorphisms.[7]

The two key factors in the mechanism of RNA activation by acridine are (1) general acid catalysis by protonated acridine and (2) rearrangement of RNA backbone conformation induced by acridine intercalation.[8] Control of the geometry of acridine intercalation is thus quite important to develop still more active systems.[9,10] In this study, we have examined how the size of the substituents on acridine affects its intercalation and RNA activation, especially focusing on the one at the 2-position. Figure 1 shows the structures of the oligonucleotides used in this study. The acridine–DNA conjugates **2a**–**2c** are complementary to the 5′-half of 36-mer substrate RNA **1**, and bear an acridine residue **X** at the 5′-end. The acridine in **2a**–**2c** has a nitro-group at the 6-position and a methoxy- (**2a**), ethoxy- (**2b**), or isopropoxy-group (**2c**) at the 2-position. When **2a**–**2c** is combined with unmodified **3** and hybridized to **1**, only the target site U19 in **1** remains unpaired, and the 5′-phosphodiester linkage of U19 is selectively activated.

All the oligonucleotides were synthesized with standard phosphoramidite chemistry on an automated synthesizer. Phosphoramidite monomers bearing acridines for the conjugates **2a**–**2c** were synthesized according to the route described in a

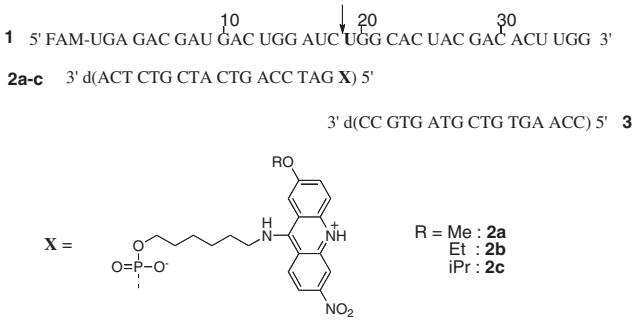

Figure 1. Structures of the acridine–DNA conjugates that bear an acridine at the 5′-end and oligonucleotides used in the present study. The target phosphodiester linkage for the site-selective scission (the 5′-side of U19) is indicated by the arrow.

previous report,[8] starting from the coupling between 2-chloro-4-nitrobenzoic acid and appropriate 4-substituted aniline. The products were purified by denaturing polyacrylamide gel electrophoresis (PAGE) and then by reversed-phase HPLC, and characterized by MALDI-TOF MS. The site-selective scission of substrate **1**, which is fluorescently labeled with 6-fluorescein amide (FAM) at the 5′-end, was performed with 100 μM $LuCl_3$ at pH 8.0 and 37 °C in the presence of 200 mM NaCl. After predetermined reaction time, the reactions were quenched with 10 mM EDTA–2Na and analyzed by denaturing PAGE. The scission efficiency was evaluated by a Fuji Film FLA-3000G fluorescent imaging analyzer.

As shown in lanes 1–3 of Figure 2, the substrate RNA was site-selectively and quite efficiently hydrolyzed by the **2**/**3**/Lu^{III} systems, mainly at the 5′-phosphodiester of U19 as the target site (a minor scission is perceived at its 3′-side). The conversions of the cleavage at the 5′-side after 4 h were 70% (for the **2c**/**3** combination in lane 2), 56% (**2b**/**3**, lane 3), and 47% (**2a**/**3**, lane 4), respectively. Conjugate bearing a more bulky substituent at the 2-position showed higher RNA activation. Quite contrary, cleavage at the 3′-side of U19 was only 1/10 compared to the 5′-side for the **2a**/**3** combination, and was not much affected by the difference of the substituent. Observed rate constants of the cleavage estimated from the time course of the reaction are shown in Table 1.[11] The conjugate **2c** bearing 9-amino-2-isopropoxy-6-nitroacridine is ca. 80% more active than **2a** that bears 9-amino-2-methoxy-6-nitroacridine. Our previous study has shown that **2a** is more than twice as active as a commercially available acridine monomer that bears 9-amino-6-chloro-2-methoxyacridine.[8] Thus, **2c** is nearly 4 times more active than this most popular acridine derivative used in oligonucleotide chemistry. The significant role of the substituent at the 2-position on site-selective RNA scission has been revealed.

According to the previous study,[8] it is suggested that protonated species of the acridine acts as an general acid catalyst in

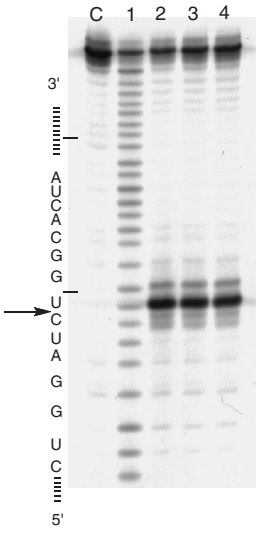

Figure 2. Site-selective scission of RNA **1** by combining **2**, **3**, and Lu^{III} at 37 °C and pH 8.0 (10 mM Tris-HCl) for 4 h. The selective-scission site (the 5′-side of U19) is indicated by the arrow. Lane 1, treatment with Lu^{III} alone; lane 2, **2c**/**3**/Lu^{III}; lane 3, **2b**/**3**/Lu^{III}; lane 4, **2a**/**3**/Lu^{III}. C, control reaction in buffer solution. $[\mathbf{1}]_0 = 5$, $[\mathbf{2}] = [\mathbf{3}] = 10$, and $[LuCl_3] = 100\,\mu M$.

Table 1. Observed first-order rate constants (k_{obs}), pK_a of the acridine, and melting temperatures (T_m) of the conjugates/**1** hetero-duplexes

	k_{obs}/h^{-1}	pK_a	T_m/°C
2a	0.13	8.8	68.0 ± 1.0
2b	0.16	8.9	69.3 ± 1.0
2c	0.23	9.1	69.5 ± 1.0

the mechanism of site-selective RNA activation and subsequent selective cleavage by Lu^{III}. Thus, more acidic acridine promotes RNA cleavage more efficiently. To see whether the present difference of the activity is because of different acidity of the acridines, we examined pK_a values of the acridines in **2c** and **2b**, as well as the melting temperatures of **2a**–**2c**/**1** hetero-duplexes (Table 1). By fitting theoretical curves to the pH dependence of the absorption of the acridines, pK_a values of **2c** and **2b** were estimated to be 9.1 and 8.9, respectively (Figure 3). Considering that the pK_a of 9-amino-2-methoxy-6-nitroacridine in **2a** is 8.8, the acridine in **2a** is slightly more acidic than those in **2c** or **2b**, and thus the high activity of **2c** cannot be ascribed to the difference of efficacy of acid catalysis. In addition, T_m values of the complexes of **2c**/**1**, **2b**/**1**, and **2a**/**1** are 69.5 ± 1.0, 69.3 ± 1.0, and 68.0 ± 1.0 °C, respectively. The strength of stacking interaction between the acridines and adjacent base pairs as well as electrostatic interaction between positive acridines and negative RNA phosphodiester backbone are not much different among

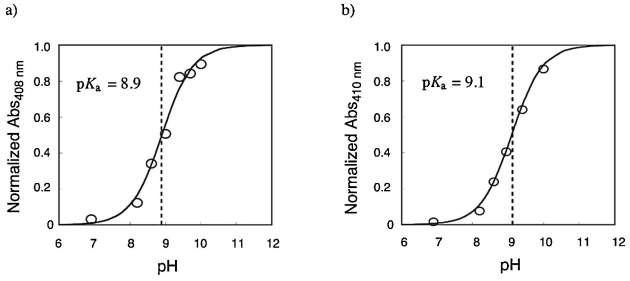

Figure 3. Dependence of the absorption of the acridines in **2b** (a) and **2c** (b) on pH.

these complexes. We thus conclude that the present acceleration of the reaction can be simply ascribed to steric effects of the substituent alone. The bulky substituents at the 2-position of the acridines in **2c** and **2b** may slightly change the geometry of intercalation in RNA/DNA heteroduplex, and make the conformation of RNA backbone more preferable for intramolecular nucleophilic attack of phosphate by 2′-OH.

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