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Fluorescence quenching properties of multiple pyrene-modified RNAs

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ABSTRACT

RNA molecules with multiple pyrenylmethyl substituents on the 2′-*O*-sugar residues can form duplexes with complementary RNA sequences without losing thermal stability. In the RNA duplexes, covalently incorporated pyrenes can assemble in a helical manner along the minor grooves of the duplex. These helically assembled pyrene arrays exhibit intense excimer emissions that are efficiently quenched with methyl viologen.

1. Introduction

Many chromophores attached to nucleic acid sequences have been used as site-specific fluorescent probes for molecular diagnostics of nucleic acid sequences, such as single nucleotide polymorphysm.[1,2] The incorporation of multiple chromophores into nucleic acid sequences is a good way to amplify and modulate the fluorescence response[3–5] because multichromophoric nucleic acids absorb light more effectively and may exhibit excimer or exciplex fluorescence. In addition, the multichromophores can become arranged in defined spaces and distances upon hybridization with a complement.[6–16] Since spatially arranged chromophores can promote efficient and vectorial transfer of excitation energy and electrons, it should be possible to use them in many applications, such as in molecular photonic and electronic devices.

Among various aromatic chromophores attached to nucleic acid sequences, pyrene derivatives are widely used as fluorophores because they show structured absorption and emission peaks that are extremely sensitive to their environments. Due to the polarizability and hydrophobicity of pyrenes, they can form stable aggregates, such as dimers and excimers, which show broad excimer fluorescence. We have shown that RNA molecules with multiple pyrenylmethyl substituents on the 2′-*O*-sugar residues can form duplexes with complementary RNA sequences without losing thermal stability.[17,18] In the RNA duplexes, covalently incorporated pyrenes can arrange in a helical manner along the minor grooves of the duplex. These helically assembled pyrene arrays exhibit intense excimer emissions.

On the basis of our observations, we feel that excimer-emissive pyrene arrays on RNA duplexes can be used as fluorescent probes and in photonic/electronic devices. Since the efficiency of nucleic acid detection and of energy or electron transfer depends on the quenching properties, determination of the quenching properties of the photoexcited states is necessary. Kool and co-workers have reported that excimer emissions from DNA-scaffolded oligometric pyrenes are efficiently quenched.[7] Therefore, we thought that efficient fluorescence quenching of pyrene arrays would take place on RNA duplexes. Here, we report that excimer fluorescence from pyrene arrays on RNA duplexes is efficiently quenched with methyl viologen (MV^{2+}).

2. Results and discussion

Pyrene-modified RNAs (**P*n***, $n = 1$–4) and their complements (**rA$_{20}$**s) employed in this work are shown in Chart 1. The melting profiles for the pyrene-modified duplexes, **P*n*-rA$_{20}$**s ($n = 1$–4), monitored at 260 nm, are shown in Figure 1. In all of the melting curves, single-phase transitions similar to those of naturally occurring RNA duplexes were observed. Melting temperature (T_m) values obtained from the first derivatives of the melting curves are summarized in Table 1. **P*n*-rA$_{20}$**s ($n = 1$–4) had T_m values ranging from 45 to 46 °C, meaning that they were stable under the experimental conditions and could be used for the subsequent spectroscopic studies.

In the absorption spectra of single-stranded **P*n*s** ($n = 1$–4), the absorptions of the nucleobase and pyrene overlapped below 300 nm, and the 0–0 absorption bands for the ^1L_a transition of pyrene appeared in the region from 300 to 370 nm. No shifts in the ^1L_a transition absorption bands were observed for **P*n*s** (Fig. 2a). In contrast, the pyrene absorption bands for **P*n*-rA$_{20}$**s

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P1: 5'- rUUU UUU UUU XUU UUU UUU UU -3'
P2: 5'- rUUU UUU UUU XXU UUU UUU UU -3'
P3: 5'- rUUU UUU UUU XXX UUU UUU UU -3'
P4: 5'- rUUU UUU UUX XXX UUU UUU UU -3'
rA20: 5'- rA20 -3'

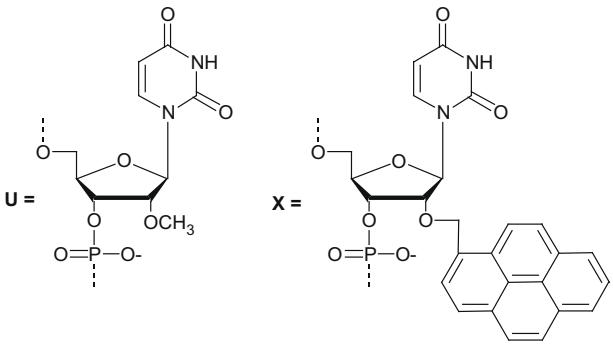

Chart 1. Sequences of pyrene-modified RNAs.

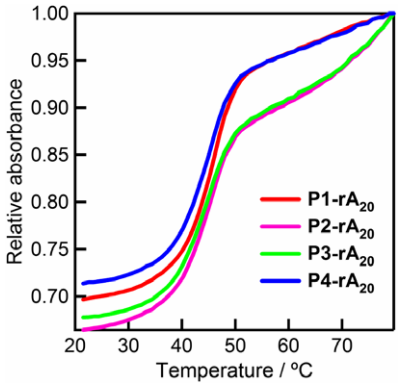

Figure 1. Melting profiles of **P*n*-rA$_{20}$** at 260 nm in a pH 7 buffer containing 0.01 M sodium phosphate, 0.1 M NaCl, and 1 mM EDTA·2Na.

(n = 1–4) shifted to shorter wavelengths with an increase in the number of pyrenes (Fig. 2b). Furthermore, peak-to-valley intensity ratios (P_As), which can be used for evaluating the interaction between pyrenes, were calculated from the absorption intensity of the red edge band and that of the adjacent minimum on the shorter wavelength side.[19] The P_A values for **P*n*-rA$_{20}$**s (n = 2–4), which decreased with an increase in the number of incorporated pyrenes, were 1.25, 1.14, and 1.10, respectively. Therefore, these data strongly suggest that the pyrenes of **P*n*-rA$_{20}$**s aggregate via π-interactions in the ground state.

Fluorescence spectra of **P*n*-rA$_{20}$**s (n = 1–4) are shown in Figure 3. **P1-rA$_{20}$** exhibited only a pyrene monomer emission at ~380 nm, whereas **P*n*-rA$_{20}$**s (n = 2–4) showed pyrene excimer emissions at ~450 nm as the major fluorescence. The fluorescence quantum yields (Φ) (Table 1) for **Pn-rA$_{20}$**s (n = 2–4) increased with an increase in the number of incorporated pyrenes. Figure 4 shows the excitation spectra of **P*n*-rA$_{20}$**s (n = 1–4). The excitation spectra for the excimers of **P*n*-rA$_{20}$**s (n = 2–4) (450 nm excitation) appeared at longer wavelength and were broader than those for the monomers of **P*n*-rA$_{20}$**s (n = 2–4) (386 nm excitation). These results also suggest that the pyrenes are aggregated in the ground state.

Fluorescence decay profiles for **P*n*-rA$_{20}$**s (n = 1–4) monitored in the region from 370 to 600 nm are shown in Figure 5. **P1-rA$_{20}$** exhibited two exponential decay curves with short (τ_1 = 4.37 ns) and long (τ_2 = 98.6 ns) lifetimes. For **P*n*-rA$_{20}$**s (n = 2–4), which exhibited excimer emissions, the decay profiles were deconvoluted into three components having short (τ_1 <10 ns), long (τ_2≈100 ns), and medium (τ_3 = 30–40 ns) lifetimes (Table 1). The values of τ_2 and τ_3 of the present systems were similar to the values of τ of the monomer (~100 ns) and the excimer (20–50 ns) of bispyrene-modified deoxyoligonucleotides reported by other research groups.[4,5] τ_1 of **P1-rA$_{20}$**, which did not exhibit excimer emission, was ascribed to background (buffer) species. However, the τ_1 values of **Pn-rA$_{20}$**s (n = 2–4) probably contain the background and partially overlapped excimer emissions. Separating the two species is difficult because they have similar τ.[4,5]

The fluorescence of **P*n*-rA$_{20}$**s (n = 1–4) was quenched with MV^{2+} in an aqueous buffer solution. Figure 3a shows the fluorescence quenching of **P1-rA$_{20}$** with MV^{2+}. In the Stern–Volmer plot at 386 nm, quenching of the fluorescence showed a linear correlation (inset Fig. 3a), and the Stern–Volmer constant ($k_q\tau$) was determined to be 410 M^{-1}. For **P*n*-rA$_{20}$**s (n = 2–4), fluorescence in the monomer and excimer region was also quenched with MV^{2+}, as shown in Figure 3b–c, and the $k_q\tau$ values of the excimer (450 nm) increased nonlinearly from 200 to 1300 M^{-1} with an increase in the number of incorporated pyrenes (Table 1). The value of $k_q\tau$ for the excimer emission of **P2-rA$_{20}$** was smaller than that for the monomer emission of **P1-rA$_{20}$**.[20] On the contrary, the values of $k_q\tau$ for the excimer emissions of **P3-rA$_{20}$** and **P4-rA$_{20}$** were larger than that for monomer emission of **P1-rA$_{20}$**. In other words, the excimer emissions of **P3-rA$_{20}$** and **P4-rA$_{20}$** were more sensitive to quenching than the monomer emission of **P1-rA$_{20}$** and the excimer emission of **P2-rA$_{20}$** were. It is likely that highly efficient quenching of the excimer is due to delocalization of the exciton along the pyrene arrays, which is supported by the exciton coupling in the circular dichroism spectra.[17] From the $k_q\tau$ and τ values, the

Table 1
Values of melting temperature (T_m) and photophysical parameters of pyrene-modified RNA duplexes[a]

	T_m[b] (°C)	Φ[c] (10−2)	$k_q\tau$ (M−1)		Fluorescence lifetimes			
			Monomer	Excimer	τ_1[f] (ns)	τ_2[g] (ns)	τ_3[h] (ns)	χ^2
P1-rA$_{20}$	46.1	1.7	410[d]	–	4.37 (11.4%)	98.6 (88.6%)	–	1.15
P2-rA$_{20}$	45.1	0.7	88[d]	200[e]	3.13 (18.7%)	106 (15.9%)	38.8 (65.4%)	1.01
P3-rA$_{20}$	45.2	1.7	220[d]	790[e]	4.06 (44.3%)	120 (14.0%)	39.0 (41.6%)	1.04
P4-rA$_{20}$	45.2	3.6	265[d]	1300[e]	8.58 (13.0%)	105 (10.8%)	32.8 (76.1%)	1.21

[a] Measurements were carried out using a 2.5 μM solution of the RNA duplex in a pH 7 buffer containing 0.1 M NaCl, 0.01 M $NaHPO_4$, and 1 mM EDTA·2Na.
[b] Values of T_m were determined from first derivative of the absorption monitored at 260 nm with increase in temperature from 20 to 80 °C at a rate of 0.5 °C/min.
[c] Fluorescence quantum yield of the RNA duplexes were determined using quinine sulfate (Φ = 0.55) in 0.1 N sulfuric acid.
[d] Stern–Volmer constant for monomer emission (386 nm).
[e] Stern–Volmer constant for excimer emission (450 nm).
[f] Fluorescence lifetime of the component assigned to buffer background. The % intensity contributions are given in parentheses.
[g] Fluorescence lifetime of monomer emission. The % intensity contributions are given in parentheses.
[h] Fluorescence lifetime of excimer emission. The % intensity contributions are given in parentheses.

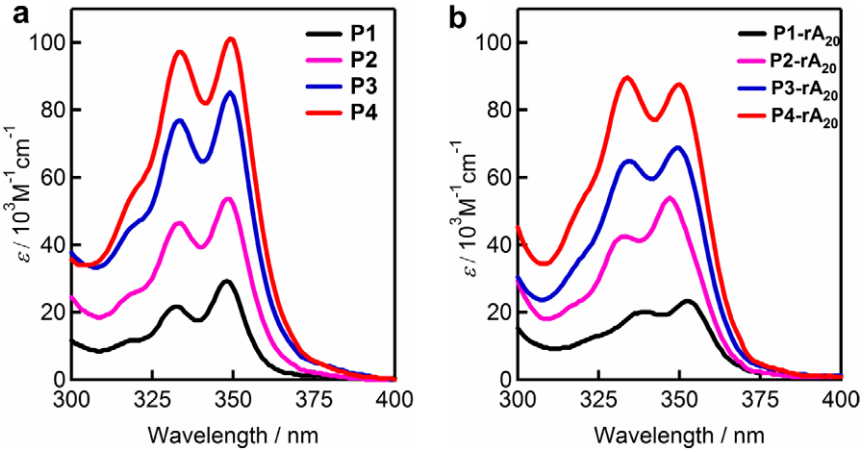

Figure 2. Absorption spectra of (a) **P*n*** (n = 1–4) and (b) **P*n*-rA$_{20}$** (n = 1–4) in a pH 7 buffer containing 0.01 M sodium phosphate, 0.1 M NaCl, and 1 mM EDTA·2Na.

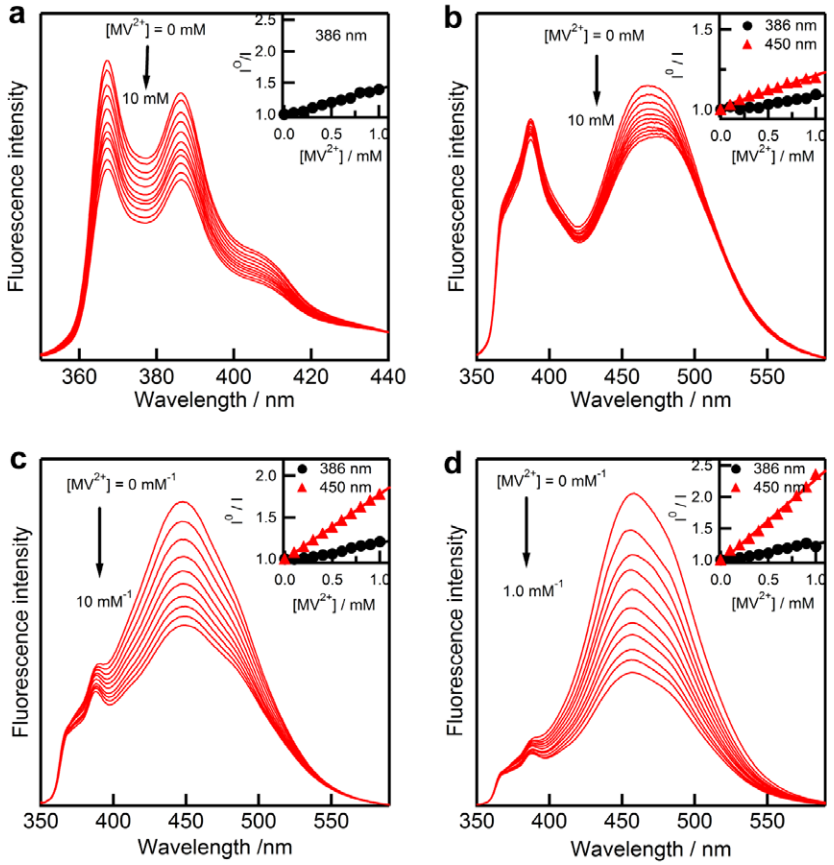

Figure 3. Fluorescence quenching of (a) **P1-rA$_{20}$**, (b) **P2-rA$_{20}$**, (c) **P3-rA$_{20}$**, and (d) **P4-rA$_{20}$** with MV^{2+} in a pH 7 buffer containing 0.01 M sodium phosphate, 0.1 M NaCl, and 1 mM EDTA·2Na. Insets are the Stern–Volmer plots of the fluorescence quenching.

quenching rate constants were estimated to be $\sim 10^{10}\ s^{-1}\ M^{-1}$, which is close to the diffusion rate constant in water,[21] indicating that dynamic quenching (diffusion-controlled quenching) occurred in the present systems. Since MV^{2+} can collide with the exciton delocalized pyrene array at any point, the quenching of the excited state is pronounced.

3. Conclusion

The pyrenes of multiple pyrene-modified RNA duplexes, **P*n*-rA$_{20}$**s (n = 2–4), form arrays via π-interaction in the ground state. The pyrene arrays, which exhibit strong excimer emissions, were effectively quenched with MV^{2+} because of the delocalization of the exciton. The incorporation of several pyrenes into RNA is useful for enhancing the quenching efficiency due to the delocalization of the exciton as well as the amplitude and modulation of the fluorescence response.

4. Experimental

Solutions of the RNA duplexes (2.5 μM) were prepared using a pH 7 buffer solution containing 0.1 M NaCl, 0.01 M $NaHPO_4$, and 1 mM EDTA·2Na without removing the dissolved oxygen.

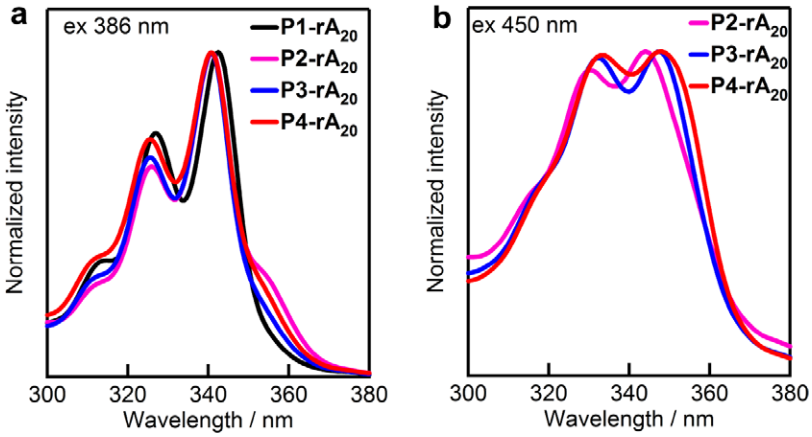

Figure 4. Excitation spectra of (a) monomer and (b) excimer fluorescence for **P*n*-rA$_{20}$** (n = 1–4) in a pH 7 buffer containing 0.01 M sodium phosphate, 0.1 M NaCl, and 1 mM EDTA·2Na.

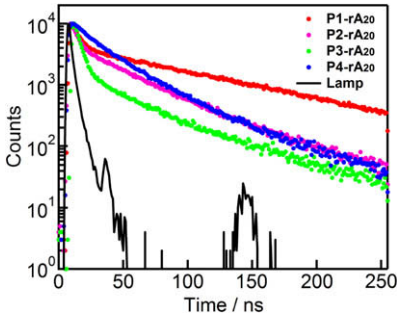

Figure 5. Fluorescence decay profiles (λ_{ex} = 340 nm) of **P*n*-rA$_{20}$** (n = 1–4) in the region from in a buffer of pH 7 containing 0.01 M sodium phosphate, 0.1 M NaCl, and 1 mM EDTA·2Na. The decay profiles were monitored in the region from 370 nm to 600 nm.

4.1. Melting temperature measurement

Absorbance versus temperature profiles were measured at 260 nm using a Beckman Coulter DU 800 spectrophotometer in the temperature range from 20 to 80 °C at a rate of 0.5 °C/min. Values of the melting temperature (T_m) were determined from the first derivative of the melting curves.

4.2. Fluorescence measurement

Fluorescence spectra were obtained using a Hitachi F-2500 spectrofluorometer. Excitation wavelength was 340 nm. The fluorescence quantum yields of the RNA duplexes were determined using quinine sulfate (Φ = 0.55) in 0.1 N sulfuric acid.[22]

Fluorescence quenching experiments were carried out by adding appropriate amounts of a MV^{2+} solution (0.1 M) to 1 mL of a solution of the RNA duplexes. The $k_q\tau$ values were determined using the following Stern–Volmer equation:

$$I^0/I = 1 + k_q\tau[MV^{2+}]$$

where I is the fluorescence intensity, k_q is the quenching rate constant, and τ is the fluorescence lifetime. In this study, the equilibrium between monomer and excimer was neglected.

4.3. Fluorescence lifetime measurement

Fluorescence decay was measured by using a time-correlated single photon-counting method on a Horiba NAES-550 nanosecond fluorometer. The excitation light (pulse width = 4 ns) from a flash lamp (Horiba NFL-111A) was passed through a band pass filter (Asahi Spectra MZ0340), and the emission was detected by using a photomultiplier (Horiba SSU-112A) with a cut-off filter (L38). The decay data were fitted with sums of a function that were deconvoluted from the instrument's response function by using a nonlinear least-squares method.

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