Alignment of pyrene aromatics along RNA double helix

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ABSTRACT

Multiple pyrene modified RNA duplex exhibits pyrene excimer fluorescence. The pyrene excimer fluorescence is significantly enhanced with an increase in the number of incorporated pyrenes. The pyrenes in the RNA helically aggregate with partial π-stacking along outside of double stranded helical backbone.

INTRODUCTION

Aromatic π-arrays mimicking natural light-harvesting systems have attracted considerable interest because of several applications in molecular functional materials such as molecular conductor or wires. Double stranded DNA and RNA can be suitable building blocks for the construction of nano-scale functional materials, because they have the unique molecular recognition properties, the helical structure, and well-developed strategies to incorporation of a wide variety of π-aromatics.1 Asanuma et al. described the DNA conjugate involving multiple Methyl Red moieties in sequence formed Hπ aggregate in the single stranded state and the Methyl Red Hπ aggregate converted into another aggregated structure by hybridization with a complementary strand.2c In their system, however, the Methyl Red Hπ aggregate formed inside DNA duplex and need natural sequence both ends of the aggregate to hybridize. On the other hand, we have previously developed the method for incorporation of polycyclic aromatic hydrocarbons into the 2-sugar position of DNA or RNA, and found that the attached π-aromatic is projected toward outside from the modified RNA duplexes.2 In particular, incorporation of pyrene into RNA enhanced the pyrene monomer fluorescence upon binding to a complementary RNA, whereas no fluorescence enhancement observed in the pyrene modified DNA. Furthermore, when two pyrenes were introduced consecutively in RNA, pyrene excimer emission was observed with high quantum yield.3 We therefore expected that our strategy might be easily applicable to incorporate multiple pyrenes to generate π-aromatic arrays along outside of double helical RNA. Herein we report the significant enhancement of pyrene-excimer emission from the helical aggregation of pyrenes along outside of double stranded helical backbone.

RESULTS AND DISCUSSION

\[
P1; \ 5'- CCG CGG CGGGX CCG CGG CGG -3' \\
P2; \ 5'- CCG CGG CGGXX CCG CGG CGG -3' \\
P3; \ 5'- CCG CGG CGGXXX CCG CGG CGG -3' \\
P4; \ 5'- CCG CGG CGGXXXX CCG CGG CGG -3'
\]

Scheme 1. Sequences of pyrene modified RNAs.

Table 1. Melting points and absorption maximum of pyrene modified RNA duplexes.

<table>
<thead>
<tr>
<th></th>
<th>T_m (°C)</th>
<th>λmax (nm)</th>
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<tbody>
<tr>
<td>P1</td>
<td>47</td>
<td>340, 353</td>
</tr>
<tr>
<td>P2</td>
<td>46</td>
<td>334, 347</td>
</tr>
<tr>
<td>P3</td>
<td>46</td>
<td>334, 349</td>
</tr>
<tr>
<td>P4</td>
<td>42</td>
<td>334, 349</td>
</tr>
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\(^a\) Measurements were carried out at 260 nm for 1:1 mixture of oligonucleotides (6 μM) in a buffer containing 10 mM sodium phosphate and 100 mM NaCl, adjusted to pH 7.0.

The multiple pyrene modified RNA employed in the present work are listed in Scheme 1 and were synthesized according to the method described in earlier publication.2 Each pyrene-RNA formed a stable duplex with a complementary rA20 in pH 7.0 buffer solutions and the melting points of the pyrene-RNA duplexes are summarized in Table 1. Figure 1 shows the UV-visible spectra of the double stranded pyrene-RNAs. In all cases, the 0-0 absorption bands for the \( ^1L_0 \) transition of pyrene\(^c\) appeared in the region 300-350 nm and shifted to shorter wavelength with the number of incorporated pyrenes (Table 1). In contrast, no shift of the absorption bands occurred in single stranded pyrene-RNAs with the number of incorporated pyrenes. Thus the blue shift of the pyrene absorption band in duplexes suggests that the pyrene units form aggregation.
Figure 2 shows the fluorescence spectra of the pyrene modified RNAs in the double stranded state with excitation in the red-edge of the absorption band ($\lambda_{ex} = 350$ nm). In the case of P1, pyrene monomer emission was observed around 390 nm. When multiple pyrenes were incorporated, pyrene-excimer emission was observed as well as monomer emission. In particular, the 350 nm excitation for P4 resulted the excimer emission was extremely enhanced, but the monomer emission was barely observed. The emission maximum of the excimer shifted to the blue side with an increase in the number of pyrenes. These observations are indicative of ground state aggregation of pyrenes in Pn (n $\geq$ 2).

Biexponential fluorescence decays with short- and long-lived components were observed for P3 and P4. The content of short-lived component for P4 is larger than that for P3. Similar biexponential fluorescence decay have been reported for $\text{Ln}$-bis(1-pyrenyl)alkanes; the long- and short-lived components are ascribed to fully and partially overlapped excimer geometry, respectively. Therefore, our results indicate that the pyrenes helically aggregated with partial π-stacking and the conformation among pyrenes gradually regulated with an increase in the number of incorporated pyrenes, which resulted increase of the partially overlapped excimer (short-lived component).

The helical pyrene aggregation was supported by circular dichroism (CD) spectra. The negative induced CD signal for P1 duplex in the range of 300-380 nm. For P2, P3, and P4 duplexes, positive Cotton effects were observed in the CD signal. On the other hand, no exciton coupled CD signal observed for single stranded Pn. These observations indicate that the pyrenes helically aggregate along outside of double stranded helical backbone.

CONCLUSION

Alignment of pyrene aromatics was established by multiple pyrene modified RNA upon hybridisation with a complementary RNA. The excimer fluorescence was significantly enhanced with an increase in the number of incorporated pyrenes.

REFERENCES


