

Structural Dynamics of Hairpin RNA Measured by 2D Fluorescence Lifetime Correlation Spectroscopy

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Introduction

RNA folding kinetics has attracted much interest because it provides information about the complex energy landscape on which multiple misfolding structures appear in competition with the most stable native structure. However, the study of structural dynamics of RNA is still difficult, especially for the secondary structure formation. In this work, we investigated a FRET-labeled hairpin RNA and compared it with the DNA analogue to understand the formation dynamics of a hairpin structure of RNA. Furthermore, we applied the 2D fluorescence lifetime correlation spectroscopy (2D FLCS)¹⁻³ to clarify the origins of this difference and tried to observe specific lifetime components and their interconversion.

Experiment

The hairpin RNA that we used is a single-stranded RNA labeled with 6-FAM and TAMRA (6-FAM-5'-UUUAACC(U)₁₈GGUU-3'-TAMRA). The DNA analogue has the same labeled dye molecules and bases as RNA but uracil is replaced by thymine. They were prepared in buffer solutions (10 mM Tris-HCl, 1 mM EDTA, BSA 0.01 %, pH 8.0) with various concentration of NaCl. 2D FLCS was measured by a home-built system.¹⁻³ Each photon was collected with absolute arrival time determined from the start of experiment (T) and the relative arrival time determined from the excitation laser pulse (t). The 2D emission-delay correlation map $M(\Delta T; t', t'')$ was generated at a characteristic time interval ΔT between two photons emitted at t' and t'' . The maximum entropy method (MEM) was utilized to convert 2D emission-delay correlation maps to 2D lifetime correlation maps so as to obtain the correlation of isolated species.

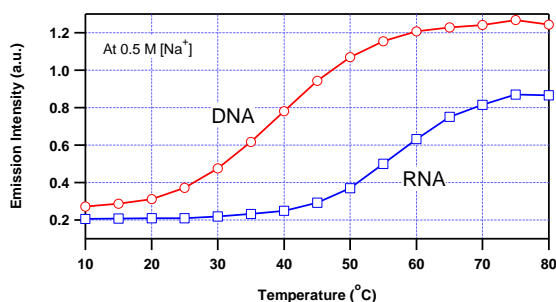


Fig. 1. Melting profiles of RNA and DNA. The emission intensity at 520 nm is plotted against temperature.

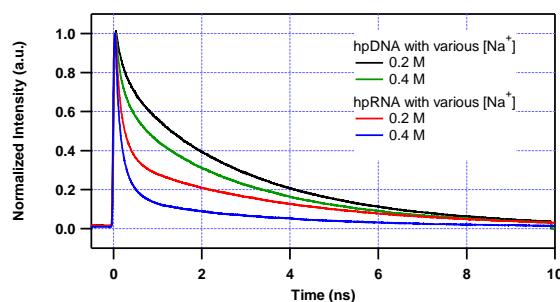


Fig. 2. Fluorescence lifetimes of RNA and DNA at various $[\text{Na}^+]$.

Results and Discussion

Fig. 1 shows the melting profiles described by the emission intensity at 520 nm for RNA and DNA at 0.5 M $[\text{Na}^+]$ plotted against temperature, reflecting the quenched fluorescence of donor due to FRET. The curves present the difference melting temperature of RNA and DNA. For RNA, it is 15 °C higher than DNA even with same $[\text{Na}^+]$, suggesting that there is a substantial difference in their structural change. The difference also emerged in lifetime measurements shown in Fig. 2, where the lifetime of RNA is much shorter than DNA at same $[\text{Na}^+]$, indicative of the different response to Na^+ between them.

Fig. 3(a) shows the lifetime distribution of RNA with 0.4 M $[\text{Na}^+]$. Three isolated species (**S1**, **S2**, and **S3**) were observed. They correspond to the diagonal peaks in 2D lifetime correlation maps at $\Delta T=20\text{-}30\ \mu\text{s}$ shown in Fig. 3(b). Because **S3** was also observed in donor-labeled RNA, it is assigned to acceptor-missing RNA. **S1** shows short-lifetime components and is assigned to the folded (F) form; moreover, the fast reaction among its components is observed, which suggests the formation and dissociation of the stem of RNA. **S2** is assigned to the unfolded (U) form. This is because the lifetimes of components are strongly affected by Na^+ , implying a flexible structure such as a random coil. In the range of measurable delay time, the off-diagonal peaks between **S1** and **S2** were not observed, indicating that the transition time between U and F forms of RNA is longer than ms, possibly due to the rigid backbone which restricts the structural change.⁴ DNA, by contrast, shows a shorter transition time around 100 μs between U and F forms.³

In summary, the investigation of hairpin RNA shows the response to Na^+ for the dynamics of RNA is different from DNA. Furthermore, the results of 2D FLCS show that the transition time between F and U forms for RNA is much longer than DNA, contributed from the difference in geometry.

Reference

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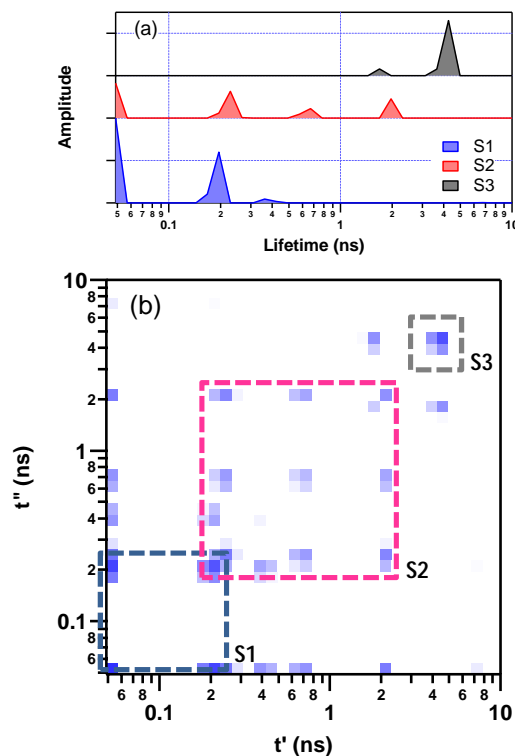


Fig. 3. 2D FLCS measurement of RNA with 0.4 M $[\text{Na}^+]$. The fluorescence lifetime distributions (a) and 2D lifetime correlation map (b) were evaluated at $\Delta T=20\text{-}30\ \mu\text{s}$. Dashed squares corresponded to the isolated species **S1**, **S2**, and **S3**.