Characterization of TectoRNA Assembly with Cationic Conjugated Polymers

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Supplementary Information

Materials & Methods

General Details.

The UV-Vis absorption spectra were recorded on a Shimadzu UV-2401 PC diode array spectrometer. Photoluminescence spectra were obtained on a Spex Fluorolog 2 spectrometer, using 90 degree angle detection for solution samples. ODs of all the RNAs were determined using a Thermo Spectronic Biomate 3. The water-soluble conjugated polymer (**P**) was prepared as previously reported.[i]

DNA Template and RNA Synthesis

RNA molecules were prepared in vitro by run-off transcription of DNA templates using T7 RNA polymerase. DNA templates for in vitro transcription were generated by amplifying a synthetic DNA molecule (RNAn.mat; n: 1 to 4), coding for the antisense sequence of the desired RNA molecule, with a forward primer (RNAn.fwd) containing

the T7 RNA polymerase promoter, and a reverse primer (RNAn.Rev). RNA**1** DNA template was PCR generated by amplifying RNA1.mat (AAGGAGCGTGGGGTAGAACCAAGTTTCCCCGGAACTACCCTTCTCCC) with RNA1.rev (AAGGAGCGTGGGGTAGA) and RNA1.fwd (TTCTAATACGA CTCACTATAGGGAGAAGGGTAG TTC). RNA**2** DNA template was PCR generated by amplifying RNA2.mat (AAGGACTTAGGAAGAACCAAGTTCCCCCGGAAC TTCCATATCCCTATAGTGAGTCGTATTAGAA) with RNA2.rev (AAGGACTT AGGAAGAACC) and RNA2.fwd (TTCTAATACGACTCACTATAGGGATA TGGAAGTTCC). RNA**3** DNA template was PCR generated by amplifying RNA3.mat (AAGGACTTAGGAAGAACCAAGTTACCCCGGAACTTCCATATCCCTATAGTGA GTCGTATTAGAA) with RNA2.rev and RNA2.fwd. RNA**4** DNA template was PCR generated by amplifying RNA4.mat (AAGGACTTAGGAAGAACCAAGCGAACC CGGAACTTCCATATCCCTATAGTGAGTCGTATTAGAA) with RNA2.rev and RNA2.fwd.

The forward and reverse primers were designed to hybridize to the template sequence (RNAn.mat) with T_m ~54 °C. Typical PCR reactions were carried out in a total volume of 200 µL and contained 2 mM $MgCl_2$, 50 mM KCl, 10mM Tris pH 8.9, 0.5% NP-40, 1 mg/mL gelatin, 0.5 mM of each dNTP, 2 nM of RNAn.mat, and 1 µM RNAn.fwd and RNAn.rev. The reactions were calibrated to produce 150 pmol of DNA template after 20 cycles (94 °C for 1 min 15 s; 53 °C for 1 min 15 s; 72 °C for 1 min 15

s). After purification of the PCR products using the QiaQuick PCR purification kit, 90 pmol of each DNA template was incubated for 4 h at 37 °C with T7 RNA polymerase (4 U/μL) in a buffer containing 10 mM $MgCl_2$, 2 mM spermidine, 50 mM Tris pH 7.5, 2.5 mM of each NTP, 1 mM DTT, 0.01 U /μL inorganic pyrophosphatase and 0.8 U/μL RNasin. After the reaction, the DNA templates were degraded by incubating with FPLC pure RQ1 DNase (0.4 U/μL) for 30 min at 37 °C. The RNA products were purified using denaturing polyacrylamide gel electrophoresis (10% acrylamide, 8 M urea). After elution overnight at 4°C in (200 mM NaCl, 10 mM Tris pH 7.5, 0.5 mM EDTA), the RNA was ethanol precipitated, rinsed twice with 90% ethanol, dried and dissolved in water. GMP RNA (5') was synthesized in a similar way: 90 pmol of each DNA template was incubated for 4 h at 37 °C with T7 RNA polymerase (4 U/μL) in a buffer containing 10 mM $MgCl_2$, 2 mM spermidine, 50 mM Tris pH 7.5, 2.5 mM of each A/U/CTP, 1 mM DTT, 0.01 μg/μL inorganic pyrophosphatase, 5 mM Li_2-GMP(S), 0.2 mM GTP, and 0.8 U/μL RNasin, with 2.5 μL of GTP (10 mM) added to the mixture in every 25 min. Labeling of 500 pmol of GMP RNA was done during 1 h at 30 °C in dark with 8 mM of 5-iodoacetamidofluorescein in a buffer containing 50 μM DTT, 2 mM Na EDTA (pH 8), 50 mM Tris (pH 8.3). RNA labeling was quenched by adding 1μL of DTT (100 mM) and the labeled RNA was purified in 10% acrylamide bisacrylamide (19:10)/8 M urea and washed in a similar way as for the RNA and RNA-GMP(S).

Non-Denaturing Gel Electrophoresis Characterization

RNA at various concentrations were heated at 90 °C for 1 min, immediately cooled on ice, and allowed to dimerize for 30 min at 30 °C in 89 mM Tris borate buffer (pH = 8.3), 15 mM of $Mg(OAc)_2$ and 5% glycol. Monomers and dimers were then separated by electrophoresis at 15 °C on 9% (30:1) non-denaturing polyacrylamide gels. PAGE electrophoresis experiments were performed in a cold room at 4°C for better temperature control and to avoid gel overheating. The K_d corresponds to the RNA concentration at which half of the RNA is dimerized.

RNA Concentration Measurements and RNA Sample Preparation

ODs of all the RNAs were determined by the 260 nm absorbance measurements done on a 1/70 diluted sample in a 70 μL quartz cuvette. Once the concentration of all the RNAs was established, a 1:1.2 mixture of RNA-F* and the target RNA strand was mixed for supra-molecular assembly. The mixtures were first heated at 90 ^{o}C for 1 min, immediately cooled on ice for 3 min, and then incubated at 30 ^{o}C for 2 min. RNAs were assembled at 30 ^{o}C for 30 min in buffer (25 mM HEPES (pH = 7.5), 15 mM $Mg(OAc)_2$), and then cooled on ice.

Fluorescence Measurements

Fluorescence intensities were determined from the integrated areas under emission spectra of both the donor (**P**) and the acceptor fluorescein. The differences in

energy transfer were compared by measuring the fluorescence intensity of the acceptor in the presence of the same concentration of the donor.

Fluorescent spectra were collected in buffer (15 mM $Mg(OAc)_2$ + 25 mM HEPEs pH 7.5) using a 150 μL cuvette. Measurements, performed at temperatures between 15 and 18°C in a room with air conditioning, were taken 30 s after polymer addition to the RNA solution equilibrated at 15°. CCP solutions were added to annealed RNA mixtures at once. For the mixture of 100 μL RNA**1**-F* and RNA_T at [RNA**1**-F*] = 5 nM, 3 μL of [**P** = 5×10^{-6} M] solution were added. Similarly, 4 μL of [**P**] = 1×10^{-4} M solution were added to 100 μL RNA-F* and RNA_T at [RNA**1**-F*] = 100 nM, and 30μL of [**P**] = 1×10^{-4} M solution were added to 100 μL RNA**1**-F* and RNA_T at [RNA**1**-F*] = 1000 nM. The excitation wavelength was 380 nm, which selectively creates **P** excited states. The emission intensity is normalized relative to polymer emission.

For experiments using FRET to estimate solution K_d values, different concentrations of RNA**1**-F*/RNA_T (1:1.2) and RNA**1**-F*/RNA_{NB} (1:1.2) mixtures were prepared. 4 μL of [**P**] = 1×10^{-4} M solution were added to 100 μL of different concentrations (3 nM, 5 nM, 6nM, 10 nM, 30 nM, 60 nM, 100 nM) of RNA**1**-F*/RNA (1:1.2) respectively for K_d measurement) of RNA**1**-F*/RNA_T (1:1.2) and RNA**1**-F*/RNA_{NB} (1:1.2). The emission spectra for both RNA pairs at each concentration were measured and the difference in dye emission intensities was calculated (after subtraction

of residual polymer emission). A plot of $(I_T-I_{NB})/I_{NB}$ against [RNA], shows the concentration range wherein the increased fluorescence is the result of dimerization. The K_d was thus calculated to be 6±2 nM.

[i] Liu, B.; Wang, S.; Bazan G. C.; Mikhailovsky, A. *J. Am. Chem. Soc.* **2003**, 125, 13306.