Electronic Supporting Information (ESI) for

Development of an RNA aptamer that acquires binding capacity against HIV-1 Tat protein via G-quadruplex formation in response to potassium ions

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Table of Contents

Materials and Methods

Materials

All RNAs were synthesized, purified, and de-salted by FASMAC Co., Ltd. (Kanagawa, Japan). All peptides, including fluorescence-labeled ones, were synthesized and purified by Toray Research Center, Inc. (Tokyo, Japan).

QTAp (5′-r(GGGAGCUUGAUCCCUUUGGAGGAGGAGGUUGGUCGAUCGCU CCC)-3′) contains three and two uracil residues (underlined) at 5′- and 3′-neighbours of R11, respectively, as linkers. The length of the linkers was determined on the basis of our previous study.[1] Target-binding activity of QTAp was compared with that of QTAp lacking the linker. QTAp turned out to exhibit more preferable character than that lacking the linker.

Nuclear magnetic resonance (NMR) measurements

QTAp was dissolved at the final concentration of 8 μM in 50 mM Tris-HCl (pH 7.4) containing 100 mM KCl (or NaCl), 5% D_2O and 10 μM 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS). DSS was used as an internal chemical shift reference. Each sample solution was incubated at 95 °C for 5 min and then cooled to 37 °C in 10 min using a thermal cycler. NMR spectra were recorded at 37 °C with Selective Optimized-Flip-Angle Short-Transient (SOFAST)[2] technique for water suppression using Bruker BioSpin DRX 600 and AVANCE III HD 600 spectrometers equipped with a cryogenic probe and a Z-gradient.

Fluorescence resonance energy transfer (FRET)-based QTAp-binding assay

F-Tat-R (FAM-AAARKKRRQRRRAAAC-TMR) was dissolved at the final concentration of 100 nM in 50 mM Tris-HCl (pH 7.4) containing 100 mM KCl (or NaCl). Fluorescence spectra were recorded at 37 °C using a FP-8500 spectrofluorometer (JASCO, Japan).

In the titration experiments, QTAp was dissolved at the final concentration of 10 or 100 μM in 50 mM Tris-HCl (pH 7.4) containing 100 mM KCl (or NaCl). Each sample solution was incubated at 95 °C for 5 min and then cooled to 37 °C in 10 min using a thermal cycler.

Fluorescence anisotropy-based QTAp-binding assay

FITC-Tat (FITC-AAARKKRRQRRR) was dissolved at the final concentration of 450 nM in 50 mM Tris-HCl (pH 7.4) containing 100 mM KCl. Fluorescence anisotropy were recorded at 37 °C using a FP-8500 spectrofluorometer (JASCO, Japan).

In the titration experiments, QTAp was dissolved at the final concentration of 10 or 100 μM in 50 mM Tris-HCl (pH 7.4) containing 100 mM KCl (or NaCl). Each sample solution was incubated at 95 °C for 5 min and then cooled to 37 °C in 10 min using a thermal cycler.

Repetitive ON/OFF switching of QTAp activity

F-Tat-R (FAM-AAARKKRRQRRRAAAC-TMR) was dissolved at the final concentration of 100 nM in 50 mM Tris-HCl (pH 7.4) containing 500 nM QTAp. This sample solution was incubated at 95 °C for 2 min and then cooled to 37 °C in 2 min using a thermal cycler. Fluorescence spectra were recorded at 37 °C using a FP-8500 spectrofluorometer (JASCO, Japan).

In the repetitive ON/OFF switching experiments, KCl and 18-crown-6 ether was added alternately (I→II→III→IV→V in Fig. 4B). In Fig. 4B, the concentration of KCl and 18-crown-6 ether are 48.8 mM and 0 mM at point I, 32.4 mM and 90.5 mM at point II, 149.5 mM and 85.1 mM at point III, 125.9 mM and 142.6 mM at point IV, and 199.5 mM and 137.0 mM at point V. The concentration of the K^+:18-crown-6 complex was calculated on the basis of the dissociation constant (8.9×10^{-3} M)[3]. The FRET fluorescence intensity of F-Tat-R was corrected by considering dilution during the procedure.

Determination of the K_d value

The K_d value was obtained by curve fitting using the following equation,[4] the binding stoichiometry of QTAp (or TAp) and the Tat peptide being assumed on the basis of the titration experiment results.

$$F = F_0 + \Delta F \left([\mathrm{RNA}]_0 + [\text{F-Tat-R}]_0 + K_d \right.$$
$$\left. - \left(\left([\mathrm{RNA}]_0 + [\text{F-Tat-R}]_0 + K_d \right)^2 - 4 [\mathrm{RNA}]_0 + [\text{F-Tat-R}]_0 \right)^{1/2} \right) / 2$$

where F and F_0 are the fluorescence intensities of the TMR in the presence and absence of the QTAp (or TAp), respectively. ΔF is the difference in the fluorescence intensity of F-Tat-R between in the presence of an infinite concentration of the QTAp (or TAp) and in the absence of QTAp (or TAp). $[\mathrm{RNA}]_0$ is the concentration of total QTAp (or TAp) at each titration point. $[\text{F-Tat-R}]_0$ is the concentration of total F-Tat-R.

Reference

1. T. Nagata, Y. Sakurai, Y. Hara, T. Mashima, T. Kodaki and M. Katahira, *FEBS J.*, 2012, **279**, 1456-1463.
2. P. Schanda, E. Kupce and B. Brutscher, *J. Biomol. NMR*, 2005, **33**, 199-211.
3. R. M. Izatt, J. S. Bradshaw, S. A. Nielsen, J. D. Lamb, J. J. Christensen and D. Sen, *Chem. Rev.*, 1985, **85**, 271-339.
4. C. Matsumoto, K. Hamasaki, H. Mihara and A. Ueno, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1857-1861.

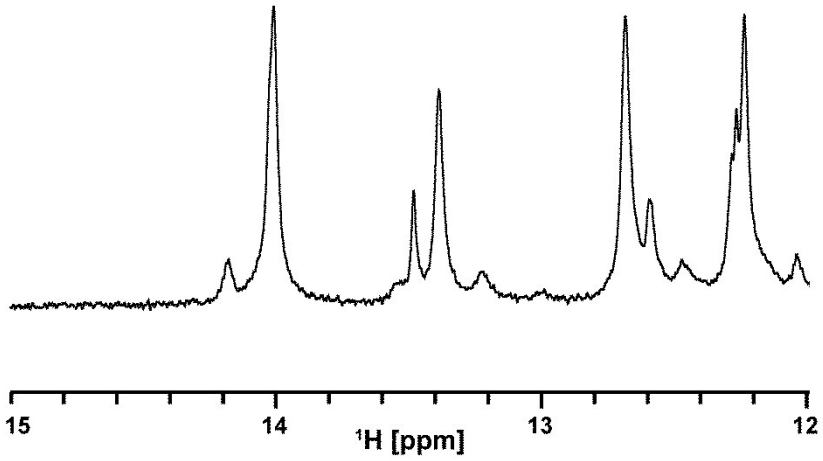

Fig. S1 Imino proton region of ^{1}H NMR spectrum of QTAp at 37 °C. The spectrum was measured with the sample solution of 50 μM of QTAp in 20 mM potassium phosphate buffer (pH 6.5) containing 80 mM KCl. Appearance of imino proton resonances at 12.0-14.2 ppm (Watson-Crick base pair region) indicates the formation of the duplex stem by 5′- and 3′-TAp subunits of QTAp.

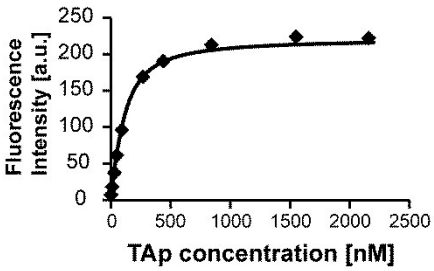

Fig. S2 TMR fluorescence intensity of F-Tat-R in the course of titration with TAp in the presence of 100 mM KCl at 37 °C.