Boosting of Activity Enhancement of K^+-Responsive Quadruplex Hammerhead Ribozyme

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Supplementary Information

Materials

QHR (5'-GCGGUCUGAUGAUUUGGAGGA GGAGGUUGAAACAGG-3'); subunits of split QHR, 5'-spQHR (5'-GCGGUCUGAUGAUUUGGAGGA-3') and 3'-spQHR (5'-GGAGGUUGAAACAGG-3'); various complementary RNAs (CSs); and substrate RNA (5'-CCUGUCACCGC-3'), labelled with FITC, were synthesized, purified, and de-salted by FASMAC Co., Ltd. (Kanagawa, Japan), were purchased.

Cleavage reaction

Firstly, spQHRs (final concentration of 10 μM for both 5'- and 3'-spQHRs) or QHR (10 μM), either alone or mixed with various CSs (30 μM), were dissolved in a solution comprising 50 mM Tris-HCl buffer (pH 8.0) and either 0 or 100 mM KCl. Each obtained solution was heated at 95 °C for 5 min and then gradually cooled to 25 °C. Next, $MgCl_2$ was added to the final concentration of 50 mM. Then, the cleavage reaction was started by adding substrate RNA, which was labeled with fluorescein-5-isothiocyanate (FITC) (1 μM). The temperature of the solution was kept at 25 °C throughout the reaction. A small aliquot was taken from the reaction solution at various time points, and the reaction was stopped by adding EDTA and urea to final concentrations of 83 mM and 7.5 M, respectively. These small aliquots were applied to a denaturing 20% polyacrylamide gel, uncleaved and cleaved substrates being separated during electrophoresis. The amounts of FITC-labeled substrate RNAs were determined by means of fluorescence using a Pharos FX™ Molecular Imager (BIO-RAD).

The cleaved percentage was defined as the amount of cleaved substrate divided by the total amount of the substrate, namely the sum of the amounts of uncleaved and cleaved substrates. The first-order rate constant, k_{obs}, was determined by fitting the time-course experiment data to the equation: $P(t) = P_{max} - (P_{max} - P_0) \exp(-k_{obs} t)$, where $P(t)$ is the cleavage percentage at time t, P_{max} the cleavage percentage at infinite time, and P_0 the extrapolated cleavage percentage at t = 0.[1, 2] Error bars are standard deviation calculated from 3 trials.

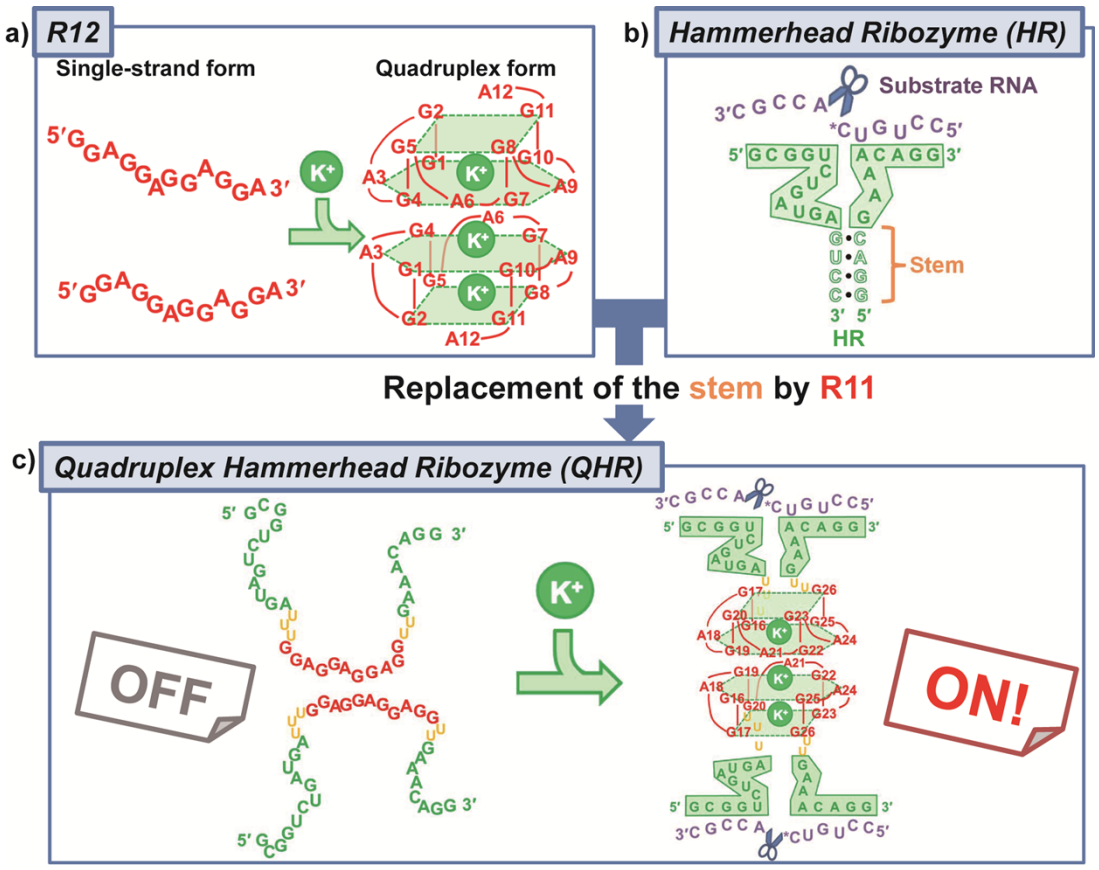

Fig. S1 Architecture of Quadruplex Hammerhead Ribozyme (QHR) and the concept of its activity switching in response to K^+. (a) The sequence of R12 and its quadruplex structure in the presence of K^+. (b) The sequences of Hammerhead Ribozyme (HR) (green), in which the 5'-HR and 3'-HR domains (shaded), and stem regions (outlined) are highlighted, and substrate RNA (purple). (c) The sequence of QHR and its expected structure in the presence of K^+. In QHR, the stem of HR is replaced by the R11 sequence, r(GGAGGAGGAGG) (red), and uridine-linker sequences (yellow).

Table S1.

	$k_{obs}^{0\ mM}$ [min^{-1}]	$k_{obs}^{100\ mM}$ [min^{-1}]	$[k_{obs}^{100\ mM}] / [k_{obs}^{0\ mM}]$
QHR without CS	3.91×10^{-2}	9.05×10^{-2}	2.3
QHR with CS1	1.38×10^{-2}	8.10×10^{-2}	5.9
QHR with CS2	1.66×10^{-2}	1.53×10^{-2}	0.9
QHR with CS3	2.46×10^{-3}	1.82×10^{-2}	9.3
QHR with CS4	9.52×10^{-4}	1.06×10^{-2}	11.8
QHR with CS5	3.39×10^{-4}	4.47×10^{-3}	17.9
QHR with CS6	4.77×10^{-3}	8.57×10^{-2}	21.4

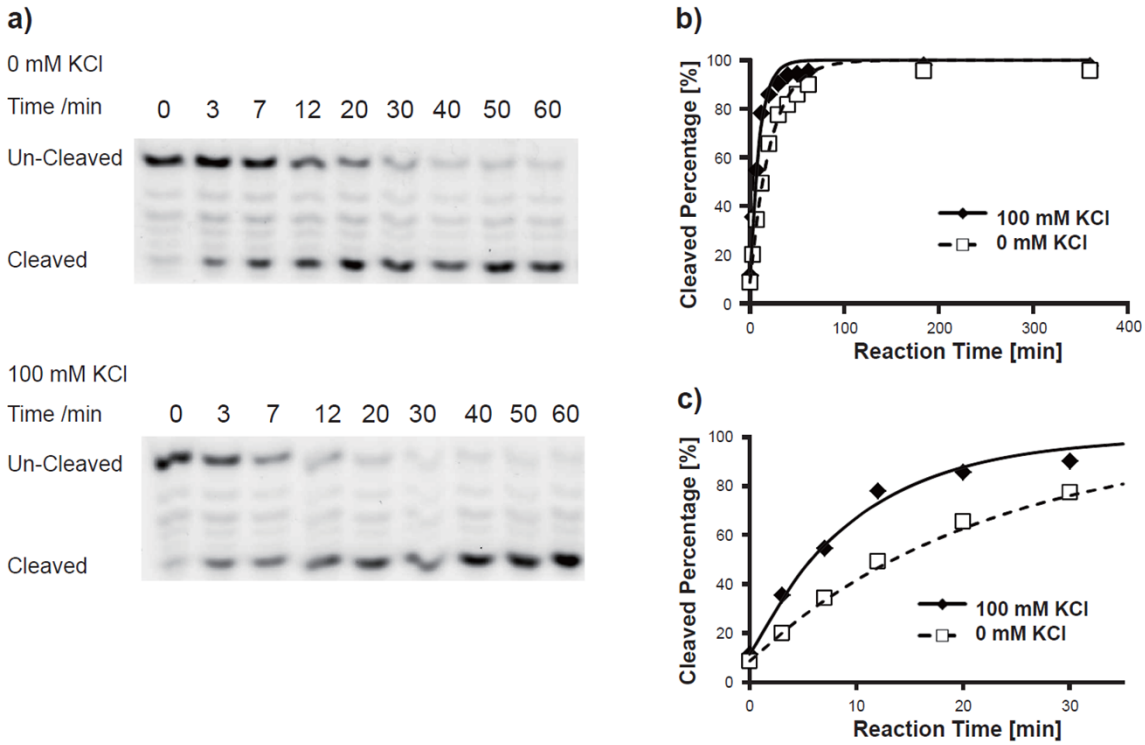

Fig. S2 Activity of Quadruplex Hammerhead Ribozyme (QHR). Polyacrylamide gel electrophoresis of the products produced on cleavage of the substrate with QHR. The top and bottom panels show cleavage in the absence of K^+ and presence of 100 mM K^+, respectively. (b) Time course of the increase in cleaved substrate with QHR in the absence of K^+ (open diamonds, dashed line) or presence of 100 mM K^+ (filled diamonds, solid line). (c) The magnification of the first 35 minutes.

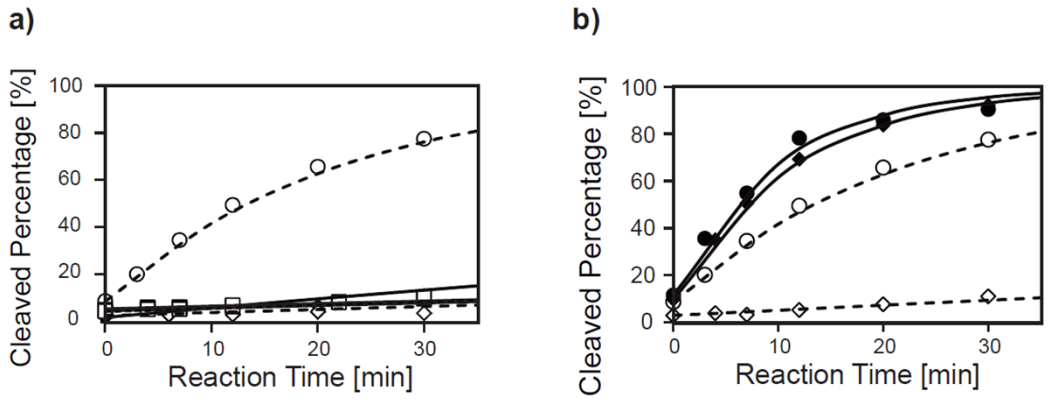

Fig. S3 (a) The magnification of the first 35 minutes of Fig. 1b. Time course of the increase in cleaved substrate with QHR in the absence of K^+ (open diamonds, dashed line) or presence of 100 mM K^+ (filled diamonds, solid line). (b) The magnification of the first 35 minutes of Fig. 2d. Time course of the increase in cleaved substrate with QHR in the absence of K^+ (open circles, dashed line) or presence of 100 mM K^+ (filled circles, solid line); and with QHR and CS6 in the absence of K^+ (open diamonds, dashed line) or presence of 100 mM K^+ (filled diamonds, solid line).

1 T. Nagata, Y. Sakurai, Y. Hara, T. Mashima, T. Kodaki, M. Katahira, *FEBS J.* **2012**, *279*, 1456-1463.

2 T. Sakamoto, M. H. Kim, Y. Kurihara, N. Sasaki, T. Noguchi, M. Katahira, S. Uesugi, *J Biochem* **1997**, *121*, 288-294