Hammerhead ribozyme activity and oligonucleotide duplex stability in the mixed solutions of water and organic compounds

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Supporting information



Fig. S1. (A) Rate constants of the hammerhead ribozyme reaction with different NaCl concentrations. The log-log plot is shown in the inset. (B) Kinetic traces of the RNA cleavage reactions with 1.5 M NaCl in the absence (black) and presence of PEG8000 (purple), EtOH (brown), FA (red), or DMF (cyan) at 20 wt%. The correlation coefficients of an exponential fit are greater than 0.992. (C) The NaCl concentration dependence of *k* in the absence (black) and presence of MeOH (purple), EtOH (brown), FA (red), NMF (blue), DMF (cyan), or AcAm (green) at 20 wt%. The correlation coefficients of a linear fit are greater than 0.993. (D) Plots between the slope of the log *k* vs. log [NaCl] plot and *k* with 1.5 M NaCl. The data using PEG and EG are indicated by black symbols, and those using the amide compounds and other compounds are indicated in green and red, respectively. The data using the solution without cosolutes are indicated in gray.



Fig. S2. (A) CD spectra of the 11-mer RNA duplex in the 20 wt% cosolute solutions. The signal of RNA shorter than the wavelength 220–240 nm could not be accurately obtained when using the solutions containing the amide compounds or DMSO because of their strong absorption. (B) Comparison of the $-\Delta G^{\circ}$ of the duplex in the mixed solutions containing 1 M NaCl. ND indicates that the measurements could not be performed because of a phase separation or evaporation at high temperature.



Fig. S3. Plots of the $-\Delta G^{\circ}$ of the RNA duplex stability at 1 M NaCl against the logarithm of water activity a_w (A) or viscosity (B). Plots of the *k* of the ribozyme reaction with 1.5 M NaCl against the logarithm of water activity (C) or viscosity (D). Plots between the values of *k* and $-\Delta G^{\circ}$ (E). The data using PEG and EG are indicated by black symbols, and those using the amide compounds and other compounds are indicated in green and red, respectively. The data using the solution without cosolutes are indicated in gray.



Fig. S4. Comparison of the relative dielectric constant of the solutions with and without 20 wt% cosolutes, used in this study.



Fig. S5. Plots of the slope of the log *k* vs. log [NaCl] plot against ε_r^{-1} (A), the logarithm of water activity (B), or viscosity (C). The corresponding plots for log *K* of the RNA duplex are indicated in panels (D)-(F). The data were obtained using PEG200 (blue), PEG8000 (purple), Glyc (red), EtOH (brown), and AcCN (cyan) at 5, 10, or 20 wt%. The data using the solution without cosolutes are indicated in gray.