Supporting Information

pH-Driven RNA strand separation under prebiotically plausible conditions

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MATERIALS AND METHODS

Materials. Reagents and solvents were obtained from *Fisher Scientific*, *Severn Biotech Ltd.*, *Sigma-Aldrich*, or *VWR*. All the reactions with RNA oligonucleotides were performed in nuclease-free water, not DEPC treated (*Ambion*). A *Mettler Toledo* SevenEasy pH Meter S20 was used to monitor the pH of the solutions, adjusted with either NaOH or HCl solutions, as appropriate. RNA oligonucleotides (**Table S1**) were purchased in HPLC-purified Na^+ forms from *Integrated DNA Technologies*. Oligonucleotide concentrations were determined by UV absorbance at 260 nm using NanoDrop® ND-1000 spectrophotometer.

NMR spectroscopy. ^{1}H NMR spectra were acquired using a *Bruker* Avance-III spectrometer operating at 400.1 MHz. Samples consisting of H_2O/D_2O mixtures were analyzed using HOD suppression to collect ^{1}H NMR data. The yields of conversion were determined by relative integration of the signals in the ^{1}H NMR spectra. Data analysis was performed using MestReNova (version 7.0).

Gel electrophoresis. 20% polyacrylamide, 8 M urea gels (0.75 mm thick, 20 cm long) were typically run at 15 W in TBE buffer. Fluorescence imaging was performed using an Amersham Typhoon imager (*GE healthcare*) and quantified using ImageQuant TL software (version 7.0).

UV melting measurements. UV thermal melting curves were acquired using a Varian Cary 6000i UV-Vis-NIR spectrophotometer. Unless otherwise stated, measurements were carried out in sodium citrate buffer (10 mM, pH 3.1-7.1) and NaCl (final Na^+ 100 mM), as detailed in each experiment, using 5 μM of each complementary strand. The pH of the resulting solution was confirmed by mixing sodium citrate and NaCl at the appropriate concentration and pH, in the absence of the oligonucleotide strands. Prior to UV measurements, samples were degassed by heating at 95 °C for 2 min followed by brief sonication. Measurements were made in 1 mm quartz Starna cuvette. The temperature was ramped at a rate of 0.5 °C/min, with absorbance measurements at 260 nm taken at 0.5 °C intervals. The oligonucleotides were annealed and equilibrated by the first heat-cool cycle, holding for 2 min at the maximum temperature. UV melting heat-cool runs were then conducted in triplicate. T_m were determined from the average of the three heating cycles and data analysis was performed using GraphPAD Prism (version 7.0b) and Microsoft Excel (Mac 2011) as previously described.[1] The T_m of duplex **1** at pH 3.1 and of duplex **2** at pH 3.6 were calculated from the first derivative maximum of the thermal melting curves, using the Cary WinUV thermal application software (version 3.0).

CD melting measurements. CD thermal melting curves were acquired using a Jasco J-815 CD spectrometer. Measurements were carried out in sodium citrate buffer (10 mM, pH 3.1-7.1) and NaCl (final Na^+ concentration 0.1 M), as detailed in each experiment, using 15 μM of each complementary strand. The pH of the resulting solution was confirmed by mixing sodium citrate and NaCl at the appropriate concentration and pH, in the absence of the oligonucleotide strands. Prior to CD measurements, samples were annealed by heating at 95 °C for 2 min followed by slow cooling (0.1 °C/sec) at 20 °C. Measurements were made in 1 mm quartz Starna cuvette. The temperature was ramped at a rate of 1 °C/min, with measurements at 260 nm taken at 0.5 min intervals. Accumulated CD spectra were collected in the 200-300 nm range at the initial and final temperature (16 scans) and at 5 °C intervals (4 scans). T_m were determined from the thermal melting curves and data analysis was performed using GraphPAD Prism (version 7.0b) and Microsoft Excel (Mac 2011) as previously described.[1]

General procedure to study adenosine depurination at different pH and temperature values. Adenosine (0.005 mmol) and sodium citrate monobasic (0.025 mmol) were dissolved in in H_2O/D_2O (9:1, 0.5 mL) and the pH was adjusted to the reported value (either 3.5 or 7.1) with NaOH/HCl. The reactions were incubated at the

reported temperature (either 60 °C or 90 °C) and their progress was monitored over time by ^{1}H NMR spectroscopy.

General procedure to study oligonucleotides hydrolysis at different pH and temperature values. Hydrolysis reactions were carried out on oligonucleotide **5a** (10 μM) in sodium citrate buffer (10 mM, either pH 3.6 or 7.1) and NaCl (100 mM), as detailed in each experiment. The pH of the resulting solution was confirmed by mixing sodium citrate and NaCl at the appropriate concentration and pH, in the absence of the oligonucleotide. The reactions were incubated at the reported temperature (either 60 °C or 90 °C, mineral oil cover) and their progress was monitored over time. Aliquots of 1 μL were taken at the appropriate time point, diluted with 4 μL of loading buffer (95 % (v/v) formamide, 5 % (v/v) glycerol, 50 mM EDTA, Orange G) and analysed by gel electrophoresis, as described above. Experiments were repeated in triplicates, and quantification was performed by relative integration of the FAM fluorescence of oligonucleotide **5a** and of the fragments derived therefrom upon hydrolysis. Data analysis was performed using GraphPAD Prism (version 7.0b). The average half-life ($t_{1/2}$) of a phosphodiester bond was calculated by multiplying the half-life of oligonucleotide **5a** by the number of its phosphodiester bonds. The average hydrolysis rate constant (k_{obs}) of a phosphodiester bond was calculated by dividing the rate constant of oligonucleotide **5a** by the number of its phosphodiester bonds.

General procedure to study oligonucleotides hydrolysis at different pH and temperature values, in the presence of Mg^{2+}. Hydrolysis reactions were carried out on oligonucleotide **5a** (10 μM) in sodium citrate buffer (10 mM, either pH 3.6 or 7.1), NaCl (100 mM) and $MgCl_2$ (10 mM), as detailed in each experiment. The pH of the resulting solution was confirmed by mixing sodium citrate, NaCl and $MgCl_2$ at the appropriate concentration and pH, in the absence of the oligonucleotide. The reactions were incubated at the reported temperature (either 60 °C or 90 °C, mineral oil cover) and their progress was monitored over time. Aliquots of 1 μL were taken at the appropriate time point, diluted with 4 μL of loading buffer (95 % (v/v) formamide, 5 % (v/v) glycerol, 50 mM EDTA, Orange G) and analysed by gel electrophoresis, as described above. Experiments were repeated in triplicates, and quantification was performed by relative integration of the FAM fluorescence of oligonucleotide **5a** and of the fragments derived therefrom upon hydrolysis. Data analysis was performed using GraphPAD Prism (version 7.0b). The average half-life ($t_{1/2}$) of a phosphodiester bond was calculated by multiplying the half-life of oligonucleotide **5a** by the number of its phosphodiester bonds. The average hydrolysis rate constant (k_{obs}) of a phosphodiester bond was calculated by dividing the rate constant of oligonucleotide **5a** by the number of its phosphodiester bonds.

Procedure to assess the reversibility of the system to pH oscillations. Oligonucleotide **5a** (10 μM) and its complementary strand **5b** (10 μM) were dissolved in sodium citrate buffer (10 mM, pH 3.5) and NaCl (final Na^+ concentration 1 M). The pH of the resulting solution was confirmed by mixing sodium citrate and NaCl, in the absence of the oligonucleotide strands. The mixture was degassed by heating at 95 °C for 2 min followed by brief sonication, and then subjected to a single UV melting heating ramp as detailed above. Aliquots of 2 μL were taken before and after the melting, diluted with 8 μL of loading buffer (95 % (v/v) formamide, 5 % (v/v) glycerol, 50 mM EDTA, Orange G) and analysed by gel electrophoresis, as described above. Quantification revealed that 0.8% of oligonucleotide **5a** degraded following the UV melting measurement at pH 3.5. Hence, 10 μL of HEPES buffer (final concentration 200 mM, final pH 6.8) were added to the solution, and the mixture was subjected to a new UV melting measurement. In parallel, a control experiment was performed taking into account the 0.8% degradation, as follows. Oligonucleotide **5a** (9.92 μM) and its complementary strand **5b** (9.92 μM) were dissolved in sodium citrate buffer (10 mM, pH 3.5) and NaCl (final Na^+ concentration 0.1 M). The mixture was degassed by heating at 95 °C for 2 min followed by brief sonication. Prior to UV measurement, 10 μL of HEPES buffer (final concentration 200 mM, final pH 6.8) were added to the solution, and the mixture was subjected to a UV melting heating ramp as

detailed above. The pH 6.8 melting curves of the two samples were analyzed and compared using GraphPAD Prism (version 7.0b).

REFERENCES

1) Xu, J., Duffy, C. D., Chan, C. K. W., and Sutherland, J. D. (2014) *J. Org. Chem. 79*, 3311-3326.

SUPPLEMENTARY FIGURES AND TABLES

Table S1. Sequences of the oligonucleotides employed in this study.

Duplex	RNA oligonucleotides	GC content
1	**1a.** 5'-CCAGUAGGUUCUC-3' **1b.** 5'-GAGAACCUACUGG-3'	53.8%
2	**2a.** 5'-CUAGUAUGUUAUC-5' **2b.** 5'-GAUAACAUACUAG-3'	30.8%
3	**3a.** 5'-CCAGUCGGUCCUC-3' **3b.** 5'-GAGGACCGACUGG-3'	69.2%
4	**4a.** 5'-GAAGUCAGUACGCCAUUCGAGAUCCUCAUG-3' **4b.** 5'-CAUGAGGAUCUCGAAUGGCGUACUGACUUC-3'	50.0%
5	**5a.** 5'-FAM-UGUGCCAGUA-3' **5b.** 5'-UACUGGCACA-3'	50.0%

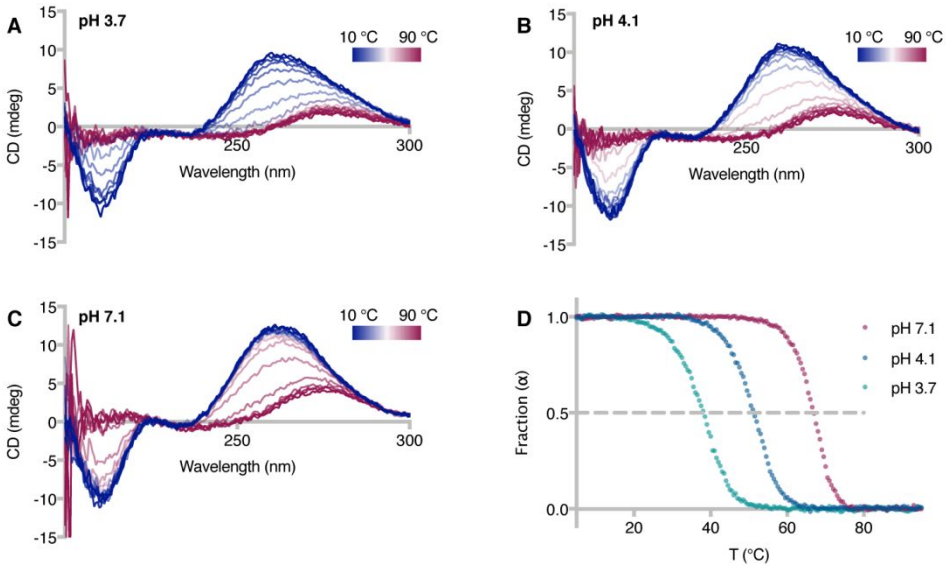

Figure S1. CD thermal melting experiments on duplex **1**. (A) CD spectra (200-300 nm) collected at 5 °C intervals in the 10-90 °C temperature range, at pH 3.7. (B) as (A) at pH 4.1. (C) as (A) at pH 7.1. (D) CD thermal melting curves of duplex **1** at different pH values. Fraction of duplex RNA (α) versus temperature (T): α = 1 double stranded RNA, α = 0 single stranded RNA (T_m = 38 °C, 51°C and 67 °C, at pH 3.7, 4.1 and 7.1, respectively).

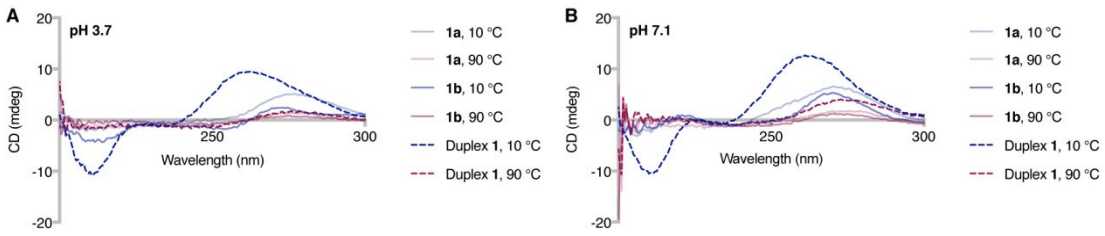

Figure S2. Comparison of the CD spectra of duplex **1** and single stranded RNA **1a** and **1b**. (A) CD spectra (200-300 nm) collected at 10 °C or 90 °C at pH 3.7. (B) as (A) at pH 7.1.

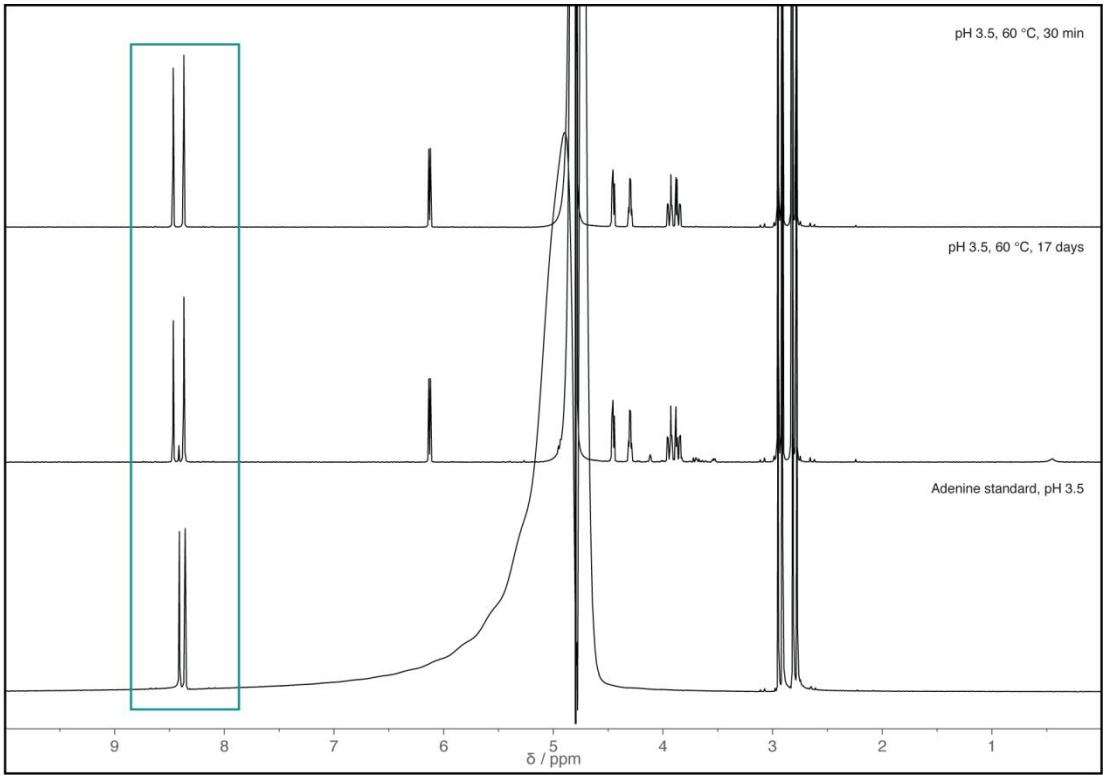

Figure S3. Full ^{1}H NMR spectra from **Figure 2A**, showing the extent of adenosine degradation at pH 3.5, 60 °C.

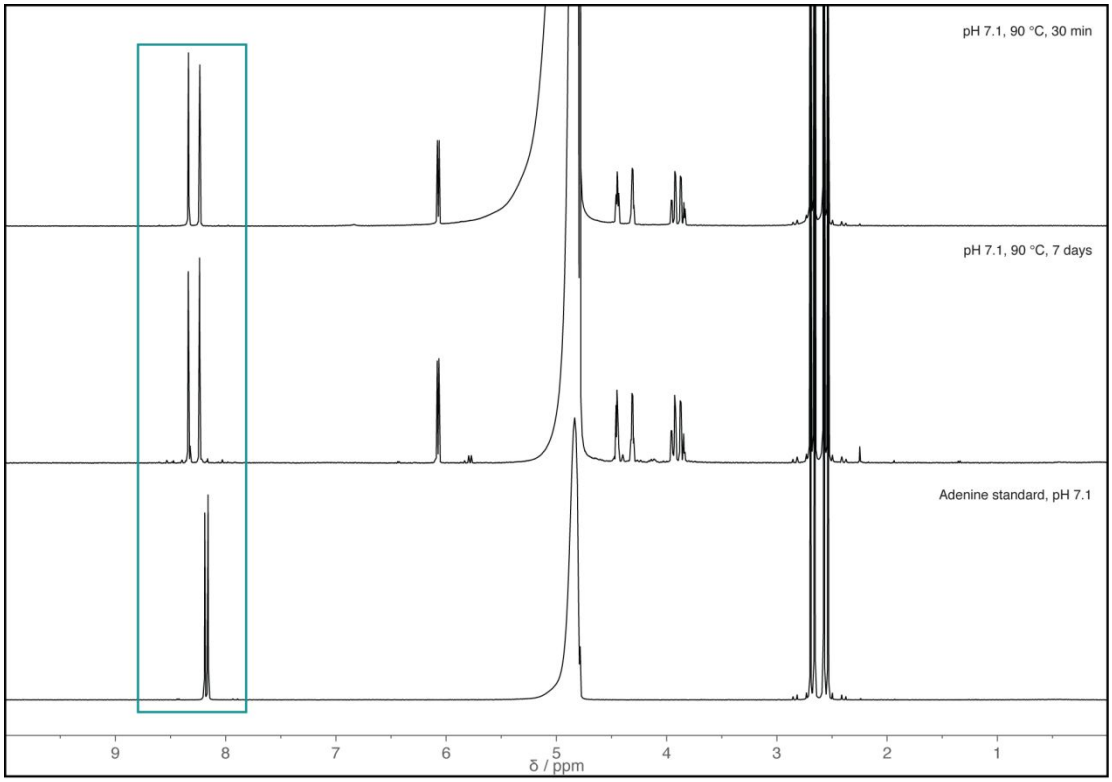

Figure S4. Full ^{1}H NMR spectra from **Figure 2B**, showing the extent of adenosine degradation at pH 7.1, 90 °C.

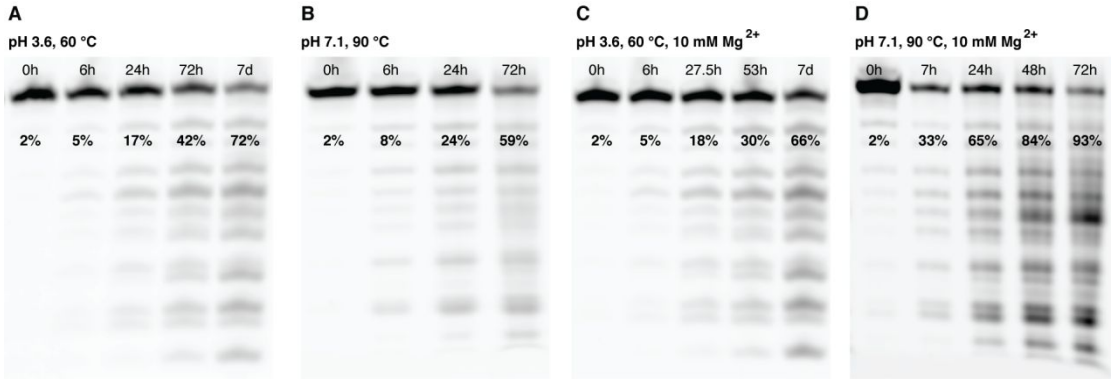

Figure S5. Degradation % of oligonucleotide **5a**, detected by gel electrophoresis and fluorescence imaging. (A) pH 3.6, 60 °C. (B) pH 3.6, 60 °C, 10 mM Mg^{2+}. (C) pH 7.1, 90 °C. (D) pH 7.1, 90 °C, 10 mM Mg^{2+}. To avoid deviations due to evaporation (despite the mineral oil cover), samples at pH 7.1 were incubated to 90 °C up to 72 hours.