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Formation of supramolecular assemblies and liquid crystals by purine nucleobases and cyanuric acid in water: Implications for the possible origins of RNA

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Supporting Information

General Information

UV-vis analysis was carried out on an Agilent 8453 spectrophotometer equipped with an 89090A temperature controller. To maintain an optical density below 1.3, cells of different path lengths (0.1 and 0.01 mm) were used. ^{1}H NMR spectra were collected on a Bruker DRX-500 500 MHz NMR and were the sum of 32 transients in D_2O. All samples investigated by NMR were D_2O exchanged before addition of the internal standard trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (TSP) at 0.56 mM. TSP did not show any indication of interacting with the assemblies. Determination of CyCo6-DAP precipitate composition was performed in DMSO-d_6. OPM imaging was performed on a Leica DMRX light microscope at room temperature. CD analysis was carried out on a Jasco J-810 CD spectrometer equipped with a six-cell Quantum Northwest peltier temperature controller. Strain-free 0.01 mm demountable cells from Starna were used for CD analysis.

AFM imaging was performed on freshly cleaved mica that was pre-treated by adding 40 μL of 20 mM $MgCl_2$ with 40 min incubation. The mica was rinsed with water and dried under N_2 (*g*). A 3 to 10 μL aliquot of the assembly solution was deposited on mica and spread by spin coating or a stream of nitrogen, followed by washing with cold water to remove excess material. Samples were incubated on ice to promote assembly and were applied to the mica surface while still cold. AFM imaging was performed with a Nanoscope IIIa (Digital Instruments) in tapping mode in air, using Si tips from either Vistaprobes (48 N/m) for the DAP-CyCo4 system, or MicroMash (16 N/m) for the adenine-CyCo4 and AMP-Cy systems.

DAP-CyCo4 assemblies were prepared by adding DAP to a solution containing a molar equivalent of CyCo4 and 200 mM sodium phosphate buffer (pH 7), followed by heating the solution to fully dissolve DAP. The solutions were then allowed to cool on ice. Adenine-CyCo4 assemblies were prepared by adding a solution containing 100 mM CyCo4 and sodium phosphate buffer to a lyophilized sample of adenine in order to achieve adenine concentrations as high as 100 mM. AMP-Cy assemblies were formed by combining a solution of AMP and sodium phosphate buffer to a molar equivalent of lyophilized Cy.

Materials and Synthesis

CyCo4[1] and CyCo6[2] were synthesized according to previous reports. Adenine and adenosine 5'-monophosphate (AMP) were purchased from Sigma. The heterocycles were dissolved in unbuffered water with the concentration of stock solutions depending on the solubility of each compound. Stock solutions of CyCo4 and AMP were prepared at 100 mM and 200 mM, respectively. All assembly solutions were analyzed within 5 h of preparation.

CyCo4: ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.749 (m, 2H; CH_2), 2.243 (t, J = 7.5 Hz, 2H; CH_2CO), 3.675 (t, J = 7 Hz, 2H; CH_2N), 11.63 ppm (brm, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 23.23, 31.42, 40.47, 149.15, 150.44, 174.47 ppm; MS (m/z): $[M-H]^-$ 213.8.

CyCo6: ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.32 (m, 2H; CH_2), 1.64 (m, 4H; CH_2), 2.30 (t, ^{3}J=7.7 Hz, 2H; CH_2CO), 3.71 (t, ^{3}J=7.7 Hz, 2H; CH_2N), 11.63 ppm (brm, 2H; NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 24.6, 25.8, 27.3, 33.8, 39.1, 149.0, 150.3, 174.6 ppm; ESI-MS (m/z): 241.9 (M^-)

Supplementary Figures

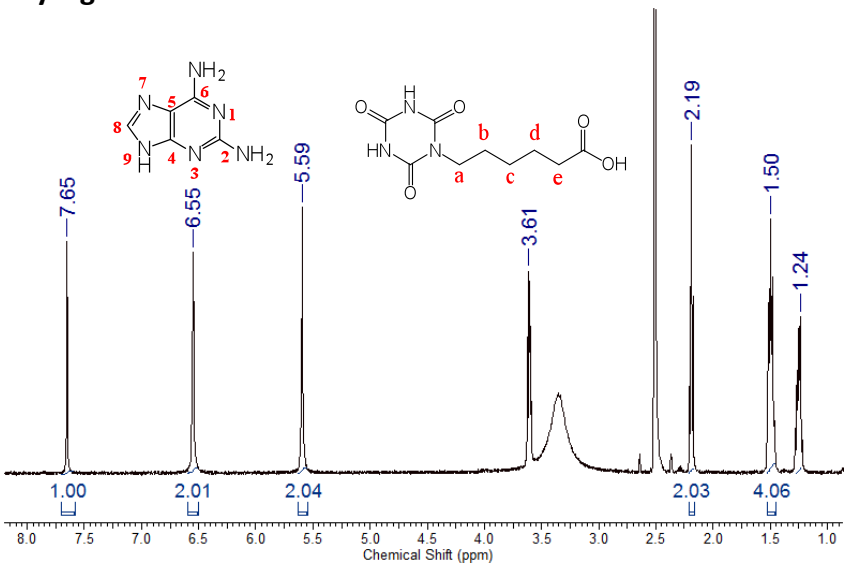

Fig. S1 ^{1}H NMR spectra (DMSO-d_6) of DAP and CyCo6 that coprecipitated from water. The peaks at 7.65, 6.55, and 5.59 ppm are assigned to H-8, 2-NH_2, and 6-NH_2 in DAP, respectively. The peaks at 3.61, 2.19, 1.50, and 1.24 ppm are assigned to H-a, H-e, H-b,d and H-c, respectively. The integrated intensity demonstrates a 1:1 stoichiometry of DAP and CyCo6 in the precipitate.

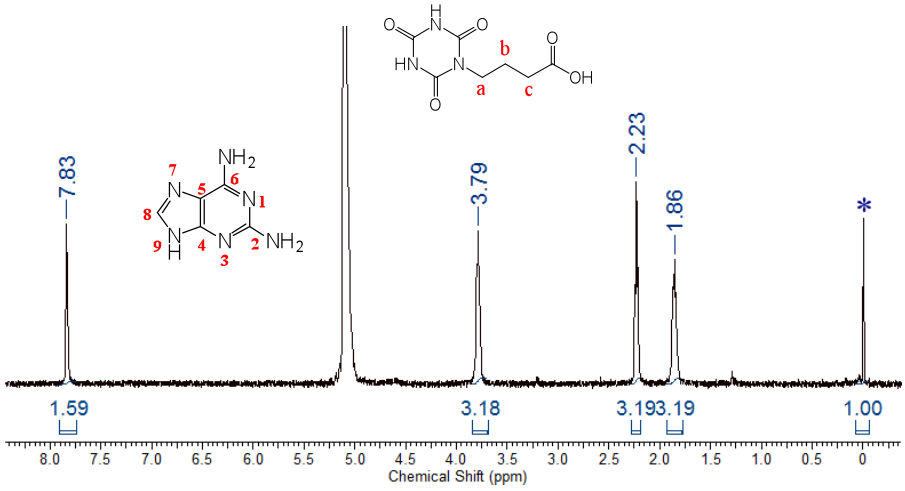

Fig. S2 ^{1}H NMR spectra of a solution containing a 1:1 mixture of DAP and CyCo4 (20 mM each) at 5 °C. The peak at 7.83 ppm is assigned to H-8 of DAP, which shows 7.95 mM DAP free in the solution (12.05 mM DAP assembled). The peaks at 3.79, 2.23, and 1.86 ppm are assigned to H-a, H-c, and H-b in CyCo4, respectively. The integrated intensity presents 7.95 mM CyCo4 free in the bimolecular system (12.05 mM CyCo4 assembled). TSP is labeled (*).

Fig. S3 Inverted-vial test of a solution of AMP and Cy at concentrations (from left to right) of 100, 80, 60, 50, 40, 20 mM in each molecule. All solutions contain 200 mM sodium phosphate buffer, pH 7.

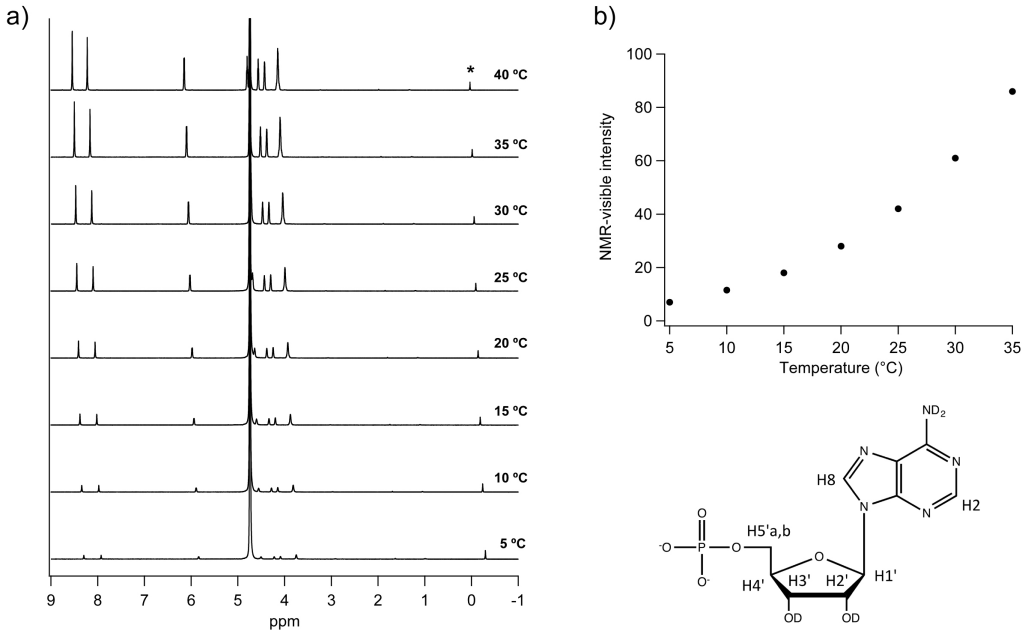

Fig. S4 (a) ^{1}H NMR spectra of a solution of AMP and Cy (100 mM each) at various temperatures. (b) Plot of the apparent solution-phase concentrations (equivalent to the MAC) of AMP vs temperature from spectra shown in (a). Assignments of the non-exchangeable protons shown in (a) at 40 °C: ^{1}H NMR (500 MHz, D_2O): 8.60 (H2), 8.28 (H8), 6.20 (H1'), 4.86 (H2'), 4.62 (H3'), 4.49 (H4'), 4.20-4.19 (H5'a,b). Solution contained 200 mM sodium phosphate buffer, pH 7. TSP is labeled (*).

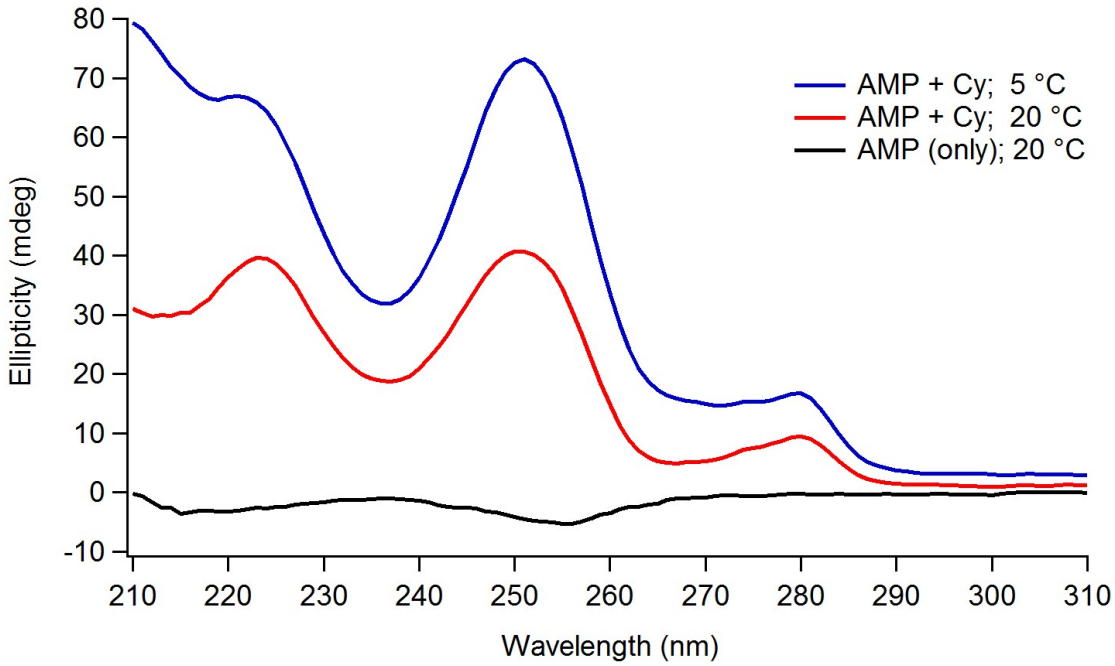

Fig. S5 Plot of CD spectra acquired from solutions containing AMP and Cy at 70 mM each at 5 °C (blue line) and 20 °C (red line), and spectrum of 70 mM AMP in the absence of Cy at 20 °C (black line). Each solution contained 200 mM sodium phosphate buffer, pH 7.

Supplementary References

(1) Cafferty, B. J.; Avirah, R. R.; Schuster, G. B.; Hud, N. V. *Chem. Sci.* 2014, 5, 4681.
(2) Hager, K.; Franz, A.; Hirsch, A. *Chem. Eur. J.* 2006, 12, 2663.