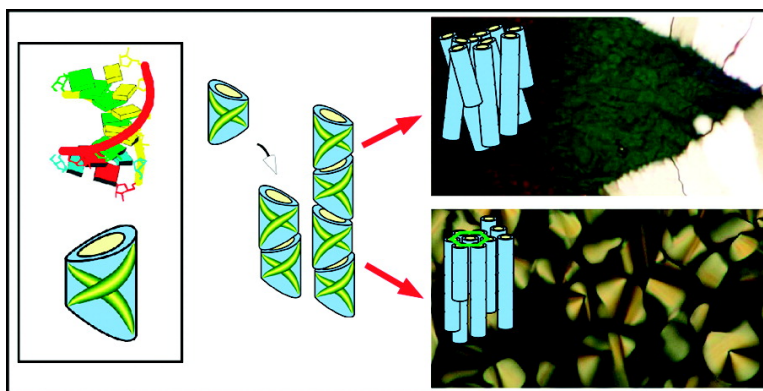Communication

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Physical Polymerization and Liquid Crystallization of RNA Oligomers

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Nucleic acids (NA) are flexible polymers as single strands but form rather rigid units when duplexed. Rod-shaped solutes with length $L > 4D$ (diameter) are expected to form the nematic liquid crystal (LC) phase in the Onsager regime, that is, at volume fractions above a critical value $\phi_c \approx D/L$, wherein the tradeoff of orientational and translational entropy favors alignment. Indeed, solutions of DNA chains larger than 100 base pairs (bp) exhibit chiral nematic (N*) ordering and, at higher concentration, columnar (COL) LC phases,[1] also theoretically predicted in systems of interacting repulsive rods. Experiments performed on RNA homopolymers, for example, PolyU + PolyA, suggest the same phase behavior for long double stranded (ds) RNA molecules.[1b] We have recently reported that, in solutions of very short B-DNA ("nanoDNA", 6−20 bp), LC phases form in the sub-Onsager regime, where the values of D/L cannot justify orientational ordering.[2] Such phases appear because base stacking forces promote end-to-end adhesion of the duplexes into elongated "physical polymers" with effective $L_{eff} \gg D$. Here we show that nanoRNA exhibits similar self-assembly into N* and COL phases, indicating that this newly observed form of spontaneous ordering is universal in NA. We have suggested that this staged self-assembly (duplexing, end-to-end adhesion, LC phase formation, and ultimately phase separation of LC-forming oligomers[1a]) may have been instrumental in early life as a means of templating the linear polymer structure of information carriers, since in the presence of appropriate chemistry end-to-end aggregation and phase separation would strongly promote the elongation of complementary oligomers. In this context it is essential to establish such a self-assembly motif in RNA—an earlier form of NA than DNA—especially since RNA exhibits A-form helices having shorter pitch and tilted bases, and with cohesion energies different from those of the DNA B-helices. We show here that the LC ordering is nevertheless maintained but with relevant differences in its structure and stability.

The terminal bases in A-helices are tilted ∼20° with respect to the normal to the helical axis, while in the B-form the tilt is ∼0°. This corresponds to a crucial difference between DNA and RNA since the straightness of the aggregate required for LC ordering implies for RNA the matching of the tilt of the terminals, as sketched in Figure 1A, in turn constraining the relative positions of the sugar−phosphate chains at their chemical discontinuities along the weakly aggregated physical polymer. This constraint appears particularly favorable for promoting ligation at the gaps of the phosphor chain, should the chemical environment be apt to catalyze such a reaction.

Concentrated solutions of self-complementary RNA oligomers, 6 to 22 bp,[3] and mutually complementary sequences ("Y−Z" mixture)[3] were loaded in thin glass cells and inspected by
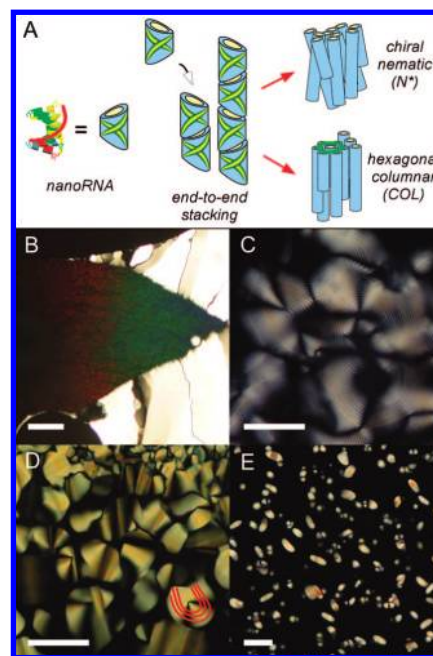
Figure 1. (A) Sketch of end-to-end stacking and LC ordering of nanoRNA. (B−E) DTLM images of LC textures observed in concentrated nanoRNA solutions. Size bars: 50 μm. (B) N* phase of 12 bp: colors indicate a pitch in the visible range, decreasing with increasing concentration from left to right. (C) N* "fingerprints" of 22 bp, revealing an average pitch of ∼2 μm. (D) Developable domains of the COL phase in a 10 bp sample: red lines show the orientation of columns of duplexes. (E) Phase coexistence of (bright) COL domains with (black) isotropic background in a 5:1 Y−Z mixture of mutually complementary sequences.

depolarized transmitted light microscopy (DTLM) to observe the LC textures; microinterferometry was used to measure local concentration and fluorescence microscopy to detect the duplex unbinding temperature T_U.[3]

Self-assembly of RNA duplexes into LC structures was readily recognized for all fully complementary sequences, while, as in DNA, added unpaired tails were observed to suppress the LC ordering.[2,3] Figure 1B shows a typical pattern of the N* phase in a 12mer sample: selective Bragg reflection indicates that the pitch P of the N* phase, equal to the wavelength of the reflected light in the material, is in the optical range. As the concentration c grows, P ranges from being larger than visible wavelengths to $P \approx 260$ nm. Longer oligos display a larger P, showing fingerprint texture[4] in 22bp RNA (Figure 1C, $P \approx 2$ μm). The dependence of P on the oligo length and c could not however be cleanly determined since P is also found to depend on the cell preparation. Overall P is shorter in nanoRNA than in nanoDNA for any oligo length and, remarkably, it displays an opposite dependence on c. While the shorter P could simply reflect the shorter helical pitch of the A-form
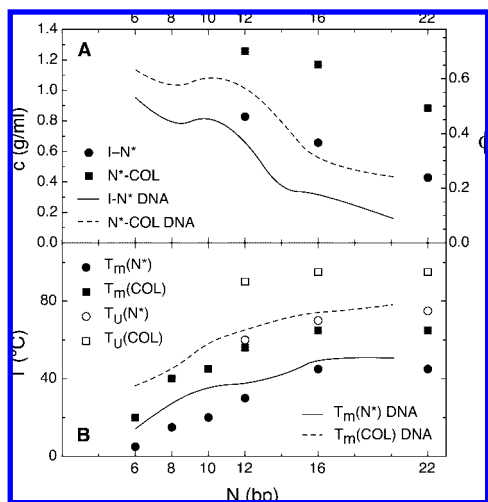
Figure 2. Phase behavior of various RNA oligomers, compared with the data obtained for DNA.[2] (A) Concentration (c) and volume fraction (ϕ) for the I−N* and N*-COL transitions measured for $T = 20$ °C. (B) Melting temperatures T_m and duplex unbinding temperatures T_U.

structure with respect to B-form, its concentration dependence cannot be intuitively interpreted. However, experiments on long DNA chains have revealed a nonmonotonic dependence of P on concentration, and theoretical results indicate that P critically depends on the Fourier components of the helical charge distribution with the potential for P vs c dependence of either signs.[5] This notion is supported by the fact that P is found to depend on oligo length for both RNA and DNA.[2] Hence the observed dependence could just reside in the differences between the A- and B-helix forms and in the azimuthal continuity of the phosphate chains along the aggregated physical polymers.

At higher concentration the solution organizes into the COL phase, as testified by the developable domains[4] shown in Figure 1D: birefringence indicates that the RNA stacks are aligned as for DNA, concentrically around the vertices of the domains.

Experiments on concentrated Y−Z mixtures with $Y > Z$ molar concentration show that nanoRNA, as nanoDNA, phase separates, nucleating COL droplets of Y−Z duplexes in an isotropic fluid rich in single stranded Y (Figure 1E), a behavior interpreted as the manifestation of an entropically driven separation between flexible and rigid polymer chains.[2]

Figure 2 displays c_{IN} and c_{NCOL}, the concentrations at the I−N* and N*-COL phase boundaries at temperature $T = 20$ °C (Figure 2A), and T_m, the largest T at which N* and COL phases are found (Figure 2B), as a function of the length of the RNA oligos. c_{NCOL} is independent of T [for $T < T_m(N^*)$]. Hence, while at 20 °C the N* phase is found for $c_{IN} < c < c_{NCOL}$, upon heating it progressively melts, the last portions of N* being found at $T_m(N^*)$ in the most concentrated regions ($c \approx c_{NCOL}$). In the figure we also report the nanoDNA results for comparison. It appears that the RNA phase diagram is characterized by larger c at which the LC phases are found and by lower T_m. Moreover, in RNA we find that $T_m(N^*) \ll T_U$ (Figure 2B), indicating that the melting of the N* phase takes place independently from the melting of the duplexes. This observation was not possible with nanoDNA because of its lower T_U,[6] close to T_m. We interpret the N* phase boundaries, in T and

in c, as the condition at which the linear aggregation of oligoRNA yields the minimum L_{eff}/D required by the Onsager theory for the onset of nematic ordering.[3] On this basis, we can evaluate the duplex end-to-end stacking free energy ϵ_S from c_{IN}, while $T_m(N^*)$ enables us to estimate its T dependence. We obtain for all oligomers $\epsilon_S \approx 3.8 \pm 0.2$ kcal/mol (~ 6.3 $k_B T$),[3] bigger but of the same order as in nanoDNA. The same evaluation performed at c_{NCOL}, the largest concentration at which the N* phase is found, yields smaller values for ϵ_S, indicating that the duplex stacking free energy decreases with increasing T. Again, the various oligos yield a similar value for the T dependence of ϵ_S: $d\epsilon_S/dT \approx -0.13 \pm 0.04$ kcal mol^{-1} K^{-1}. These figures can be compared with the evaluation of base-stacking energy obtained by studying stability and conformation of dsDNA with nicks or dangling ends.[7] These yield a wide range of values, between one-half and one-fifth of those determined from our observations. Given the spread of base-stacking values obtained by the different approaches on DNA and RNA,[7,8] and given the fact that we are studying inter-NA stacking while all studies are performed for intra-NA interactions, we consider this partial agreement as a further confirmation that the forces inducing the reversible aggregation are indeed base-stacking forces. Since the nanoRNA duplexes are held together only by stacking interactions, our value of ϵ_S represents an estimate free of the ambiguities intrinsic for interactions among bases within oligomers, wherein the chemical connection between bases provides additional constraints.

The present study establishes the connection between the supramolecular organization of RNA oligomers and their degree of complementarity, opening the way for studying whether and how macroscopic phase behavior could have influenced molecular evolution in prebiotic environments.

Supporting Information Available: RNA sequences, experimental methods, LC textures, and procedure for the extraction of stacking free energy. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA804718C