Abiotic Synthesis of Nucleic acids: Hypochromicity and future research

K.Glass[1], M. Oye[2], D.Deamer[2], W. Vercoutere[3]

[1] University of California, San Diego [2] University of California, Santa Cruz [3] NASA Ames Research Center

Abstract

The earliest forms of life would likely have a protocellular form, with a membrane encapsulating some form of linear charged polymer. These polymers could have enzymatic as well as genetic properties. We can simulate plausible prebiotic conditions in the laboratory to test hypotheses related to this concept. In earlier work we have shown that mononucleotides organized within a multilamellar lipid matrix can produce oligomers in the anhydrous phase of dehydration-rehydration cycles (Rajamani, 2008). If mononucleotides are in solution at millimolar concentrations, then oligomers resembling RNA are synthesized and exist in a steady state with their monomers DeGuzman, 2014). We have used conventional and novel techniques to demonstrate that secondary structures stabilized by hydrogen bonds may be present in the condensation products produced in dehydration- rehydration cycles that simulate hydrothermal fields that were present on the early Earth. Gel electrophoresis data corroborates the presence of up to 200-base pair length RNA fragments in products of Hydration-Dehydration experiments. Furthermore, hypochromicity measurements demonstrate a degree of hypochromicity found in single RNA strand of known sequence, as well as results that indicate this is true also for a sample of complementary strands of RNA. Analysis of ionic current signatures of known RNA hairpin molecule as measured using a nanopore detector indicate a significant variability in pattern, different from the signatures produced by DNA hairpin molecules. This informs how we may interpret nanopore data gathered from prebiotic simulations.

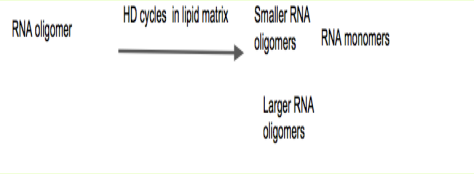
Background

Previous studies have shown that RNA oligomers can form using lipid bilayers as an organizing matrix when subject to dehydration-rehydration cycles(Rajamani et al, 2008) and exist in equilibrium with their nucleic acid counterparts in simulated early earth conditions.

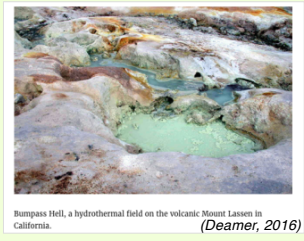
Furthermore, when complementary nucleotides were used, the resulting oligomers exhibited hyperchromicity and stained in ethidium bromide, both indicators of a double stranded structure.(Toppozini et al. 2013).

Additionally, directed evolution of RNA can occur in an environment in which there is a mechanism of recombination and mutation(Joyce 2004).

One can see that from dehydration-rehydration cycles, one obtains a mechanism of mutation (polymerization and equilibrium with monomers) and recombination (random polymerization of differing RNA strands).



Analog environments exist today:



Bumpass Hell, a hydrothermal field on the volcanic Mount Lassen in California. *(Deamer, 2016)*

Materials and Methods

• Samples were tested for secondary structures using precast 4% and 2% Gels containing an ethidium bromide alternative.
• Products yields are measured by Nanodrop spectrophotometry, which is also used to test for secondary structures or duplex strands.

• Hypochomicity was tested using the device shown above

•To analyze the samples using a gel, complimentary palindromic RNA samples, Deamer 001 and Deamer 002 were added to a 4% precast gel and run, in addition to a ladder and controls for both.

Seq. for Deamer 001 and 002 were AUUAUAAAUUUAUAUUAAUA and UAUUAAUAUAAAUUUAUAU

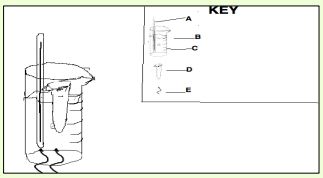


Figure 1. Shown above is the setup for obtaining the hypochromicity data. Beaker and thermometer(A) filled with water(C) was heated(E) in order to evenly heat a microcentrifuge tube(D) that contained an RNA Sample being sampled at a given temperature. (D) in initial experiments had a hole poked in the top

Results

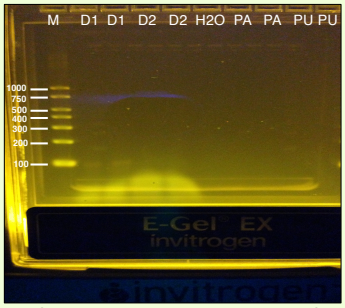


Figure 2. Shown above is a precast gel containing a RNA Century ladder (M), Deamer 001 (D1), Deamer 002 (D2) and control sequences (PA, PU) that do not show up. Note that D2 bands indicate molecule sizes that are up to 100 bases. Deamer 001 and 002, are 20mers.

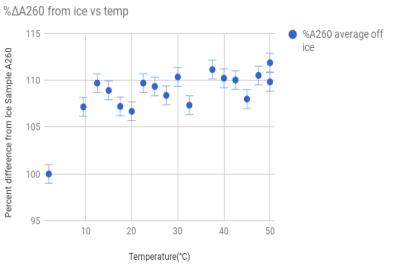


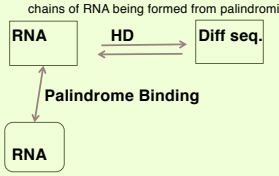
Figure 3. A graph showing the percentage difference in absorbance between samples taken from 0 to 55° C. As one can see, this show an approximate 10% increase in absorbance.

Summary and Conclusions

From our experiment we can observe ~10% hypochromicity from our mixed RNA compliments. Furthermore, from our gel data, we can observe monomers of palindromic RNAs, Deamer001 and Deamer002 spontaneously binding to form longer chains. Together these indicate that RNA when combined with its complimentary sequence can bind and as a result, can spontaneously form more lengthy RNA fragments, suggesting a possible protocellular utility to having palindromic RNA. As palindromes might occur with some frequency in random recombination of RNA segments by Hydration Dehydration cycles, this is a phenomena that could occur in early earth protocells.

Future Research

- Further hypochromicity and electrophoresis testing, with a different gel for a shorter period of time and higher concentration to get clearer bands around the 100 base line
- Testing the effect of the addition of palindromic RNA to the Products of HD cycles
- From the results of our experiment we propose to test to see if palindromic RNA has an impact on the products of hydration dehydration reactions in a simulated protocell, to determine if it creates a trend towards RNA molecules with a specific palindromic sequence.
- The reason for this being that palindromic sequences could allow for two other RNA sequences with matching corresponding sequences to attach onto the same RNA palindrome, and that due to it being palindromic, the RNA could attach to copies of itself, possibly suggested from longer chains of RNA being formed from palindromic 20mers.



This is significant because the HD cycled RNA has the correct experimental setup for directed evolution of RNA, both acting as a tool of recombination and mutation for RNA sequences. Thus, the protocell might have underwent a similar process with palindromic RNA providing a stabilization fact that would allow for it to be conserved over time.

Literature cited

Deguzman V, Vercoutere W, Shenasa H, Deamer D. Generation of oligonucleotides under hydrothermal conditions by non-enzymatic polymerization. J Mol Evol. 2014;78(5):251-62.

Joyce GF. Directed evolution of nucleic acid enzymes. Annu Rev Biochem. 2004;73:791-836.

Rajamani S, Vlassov A, Benner S, Coombs A, Olasagasti F, Deamer D. Lipid-assisted synthesis of RNA-like polymers from mononucleotides. Orig Life Evol Biosph. 2008;38(1):57-74.

Toppozini L, Dies H, Deamer DW, Rheinstädter MC (2013) Adenosine Monophosphate Forms Ordered Arrays in Multilamellar Lipid Matrices: Insights into Assembly of Nucleic Acid for Primitive Life. PLoS ONE 8(5): e62810. https://doi.org/10.1371/journal.pone.0062810

Singer, E, Deamer. D. "In Warm, Greasy Puddles, the Spark of Life?" *Quanta Magazine*. Quanta Magazine, 17 Mar. 2016.

Acknowledgements

This work was supported by the NASA Ames Research Center CIF (Center Innovation Fund).

Thanks go to the late Edward Lam for mentoring and helping me get involved with the research process. I would not have done this research or obtained these results without his help.