Catalytic Synthesis of Polyribonucleic Acid on Prebiotic Rock Glasses

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Abstract

Reported here are experiments that show that ribonucleoside triphosphates are converted to polyribonucleic acid when incubated with rock glasses similar to those likely present 4.3–4.4 billion years ago on the Hadean Earth surface, where they were formed by impacts and volcanism. This polyribonucleic acid averages 100–300 nucleotides in length, with a substantial fraction of 3′-5′-dinucleotide linkages. Chemical analyses, including classical methods that were used to prove the structure of natural RNA, establish a polyribonucleic acid structure for these products. The polyribonucleic acid accumulated and was stable for months, with a synthesis rate of $2 \times 10^{-3}$ pmoles of triphosphate polymerized each hour per gram of glass (25°C, pH 7.5). These results suggest that polyribonucleotides were available to Hadean environments if triphosphates were. As many proposals are emerging describing how triphosphates might have been made on the Hadean Earth, the process observed here offers an important missing step in models for the prebiotic synthesis of RNA. Key Words: Impact glasses—RNA world—Origin of life—Prebiotic chemistry—Nucleoside triphosphates—Mafic rocks. Astrobiology 22, 629–636.

1. Introduction

A n early episode of life on Earth likely used RNA in both genetic and catalytic roles (the “RNA World”) (White, 1976; Gilbert, 1986). This suggests, but does not require (Hud et al., 2013), an “RNA-First Model” for life’s origin on Earth, proposed 60 years ago by Alexander Rich (Rich, 1962).

However, the structure of RNA, once called “a prebiotic chemist’s nightmare” (Joyce and Orgel, 1999), is seen by many to be too complex to have emerged spontaneously (Shapiro, 2007). Thus, a persuasive case for the RNA-First Model requires, at a minimum (Robertson and Joyce, 2012), an experimental demonstration of an abiological process that forms oligomeric RNA molecules with lengths sufficient to support Darwinian evolution (Krishnamurthy, 2015), perhaps 50–5000 nucleotides (Joyce, 2012). Furthermore, this process must work without human intervention in an environment likely to have been found during the Hadean.

Rocks available on the Hadean surface were likely driven by the redox state of the Hadean mantle, which was likely not far from modern values (Harrison, 2009; Trail et al., 2011). The surface was likely reworked by bombardment that remelted its mostly mafic (basaltic and diabasic) materials (Pierazzo and Melosh, 2000; Arndt and Nisbet, 2012; Mojzsis et al., 2019). This, in turn, generated air- and liquid water-quenched cryptocrystalline silicates (in common language, glasses) having the diverse composition of the early crust (Melosh, 1989).

In light of this geological context, we asked whether mafic glasses could convert ribonucleosides 5′-triphosphates into polyribonucleic acid. We report here that they can.
2. Results

Standard glass samples, obtained from pure oxides by methods used at the United States Geological Survey (USGS), had the composition of andesite (AGV), basalt (BRP), gabbro (GSM), diabase (MRM), and nephelinite (NKT) (Le Maître et al., 1989). Supplementary Tables S1–S5 summarize their compositions. Fused quartz powder was used as a reference.

Glass samples (22 mg) were incubated (pH 7.5, 25°C, 20 h) with water containing a mixture of all four ribonucleoside triphosphates (NTPs 3 μM final each, radiolabel introduced as [α-32P]GTP). Supernatants and eluates obtained by treatment with urea were then collected. Analysis of materials dissolved in both, done by denaturing polyacrylamide gel electrophoresis (PAGE), showed two classes of products (Fig. 1):

(a) Rapidly migrating products, which were delivered by all glasses. These resisted RNase ONE degradation for 18 h (Supplementary Fig. S18), excluding a 3′,5′-linked linear polyribonucleic acid structure for these rapidly migrating species. Their possible structure is not discussed here, but similar species have been seen in other pioneering work (Rajamani et al., 2008; Costanzo et al., 2009, 2016; Sponer et al., 2016).

(b) Slowly migrating products, which were produced by BRP, GSM, and MRM glasses. The other glasses produced less of this material. Quartz produced none, and none was formed by incubating triphosphates in water without any glass (Supplementary Fig. S8 and others).

For subsequent analyses, we focused on products found in the supernatants of oligomerization reaction at equilibrium, leaving potentially strongly adsorbed species for future studies.

Products of type (b) were also formed from ATP, CTP, GTP, and UTP incubated separately (3 μM, alpha-32P-label; Supplementary Fig. S6). Again, MRM and BRP glasses gave these in substantial amounts. NKT and AGV glasses were less productive. Quartz was entirely unproductive (Supplementary Figs. S7–S10, right panels).

Focusing on the “productive” MRM glass, the amounts of slowly migrating radiolabeled products increased steadily with time, over days (Fig. 2). Incubation with larger amounts of NTPs (30–360 pmol) increased the rates of production (Supplementary Fig. S3), suggesting that the glasses act as true catalysts.

To establish catalysis, experiments were run for 20, 33, and 41 days (pH 7.5, 25°C) with MRM glass and all four NTPs (30–360 pmol) increased the rates of production (Supplementary Fig. S3), suggesting that the glasses act as true catalysts.

To establish catalysis, experiments were run for 20, 33, and 41 days (pH 7.5, 25°C) with MRM glass and all four NTPs (30–360 pmol) increased the rates of production (Supplementary Fig. S4). Even after synthesis, formation of the slowly migrating material [products of type (b)] continued. Similar accumulations were observed for BRP and GSM. Again, NKT and AGV glasses gave very little products of type (b), and more slowly. Quartz continued to produce no such products, even after months (Supplementary Fig. S5).

The linearity of the process over time and its dependence on the specific type of glass rule out “generalized surface catalysis.” These and other experiments also exclude other artifacts. Thus, the time progression excludes the possibility that the high molecular weight materials observed by PAGE are noncovalent aggregates. To exclude the possibility that glasses “leaked,” unknown species that artificially slowed electrophoresis of short RNA, 5′-labeled rC12, and 5′-labeled rA12 were incubated in supernatants of MRM glass previously incubated in water for 6 months, and electrophoresed. No slowly migrating products of type (b) were produced (Supplementary Fig. S24).

Several independent assessments of the slowly migrating products confirm that the nucleotides in type (b) products were covalently linked. Specifically, the complete product mixture from MRM glass was passed through Amicon™ ultrafilters with increasing size cutoffs. Most of the high molecular weight products [type (b)] were retained by 30–50 kDa filters. Approximately 25% were retained by 100 kDa filters (Fig. 3). This corresponds to what would be expected from polynucleotides that have a length of 90–300 nucleotides.

Noncovalent aggregates were also excluded by performing ultrafiltration in the presence of formic acid (0.005%,
pH 3), 15% ethanol, or both. Changing the pH and adding organic solvents would disrupt noncovalent aggregates joined by hydrogen bonds and/or hydrophobic forces (Table 1). This also rules out phosphoramidite linkages, where the phosphate is linked to a nitrogenous base; such linkages are not stable at low pH, and would not be available in any case to polyuridylic acid. Then, 10 separate fractions that represented all product bands were recovered from gels and separately rerun on PAGE. The electrophoretic mobility of each material was not changed (Supplementary Fig. S15). Furthermore, when oligomerization products were analyzed by PAGE having different percentages (Supplementary Fig. S11), the high molecular weight products behaved as expected, running faster on low percentage PAGE and slower on high percentage PAGE. In contrast, low molecular weight products followed the opposite trend: their migration slowed with increasing gel percentage. This nucleic acid type behavior further supports our structural assignment of the slow migrating products, but not of the fast migrating products, as covalently linked polyribonucleic acid chains.

Recognizing the importance of this result to the “RNA-First Model” for the origin of life, we then established that the high molecular weight material was a polymer of ribonucleotides. This structure proof used much the same tools as those used to originally prove the structure of natural RNA 70 years ago.

First, the high molecular weight material [type (b) products] was obtained in large amounts by an 8-month incubation with MRM glass with nonradioactive triphosphates. This was classically analyzed. Residual 5'-phosphorylated nucleoside precursors carried over from the incubation were first removed by treating samples with NaIO₄ (10 mM, 30 min, then pinacol to remove excess periodate; Muthukumaran et al., 2005). The materials were then digested with concentrated ammonia (see, e.g., analyses of liable linkages by alkaline hydrolysis in Supplementary Figs. S12–S14). The mixture of hydrolysis products was lyophilized and then resolved by reverse phase high performance liquid chromatography (HPLC). HPLC showed that this material contained nucleoside 2'- and 3'-monophosphates. These were quantitated by UV spectroscopy and authenticated by comparison with synthetic standards (Fig. 4; Supplementary Figs. 19–23; Supplementary Table S8).

Recovery of 2'- and 3'-nucleoside monophosphates from products whose only nucleotidic input was nucleoside 5'-phosphates establishes that the polymer involved a new
phosphodiester linkage between two nucleotides. No other product structure accounts for these data.

This linkage must be either a phosphodiester between the 5'-OH of one nucleotide (the original nucleoside-O-P linkage) and the 3'-OH of another, or between the 5'-OH of one nucleotide and the 2'-OH of another. This experiment does not distinguish which, as ammonia digestion of polyribonucleic acid proceeds through nucleoside 2',3'-cyclic monophosphates (Supplementary Fig. S12). Thus, the ratio of nucleoside 2'- and 3'-monophosphates is independent of the ratio of 2',5' and 3',5'-phosphodiester linkages in the digested polyribonucleic acid. However, those monophosphate

**Table 1. Ultrafiltration of Polyribonucleic Acids Made on Diabase**

| Sample | Treatment | Retained cpm | Eluted cpm | Total cpm | Retained % | Eluted % |
|--------|-----------|--------------|------------|-----------|------------|----------|
| Diabase | Water     | 23,329       | 66,179     | 89,508    | 26.06      | 73.94    |
|        | EtOH      | 6247         | 55,503     | 61,750    | 10.12      | 89.88    |
|        | pH 3.0    | 12,669       | 76,100     | 88,769    | 14.27      | 85.73    |
|        | EtOH + pH 3.0 | 12,968 | 77,327     | 90,295    | 14.36      | 85.64    |
| Quartz | Water     | 12,390       | 53,172     | 65,562    | 18.90      | 81.10    |
|        | EtOH      | 2984         | 65,773     | 68,757    | 4.34       | 95.66    |
|        | pH 3.0    | 1969         | 64,870     | 66,839    | 2.95       | 97.05    |
|        | EtOH + pH 3.0 | 821   | 67,109     | 67,930    | 1.21       | 98.79    |
| Water  | Water     | 12,756       | 36,764     | 49,520    | 25.76      | 74.24    |
|        | EtOH      | 1499         | 49,222     | 50,721    | 2.96       | 97.04    |
|        | pH 3.0    | 872          | 46,775     | 54,647    | 1.83       | 98.17    |
|        | EtOH + pH 3.0 | 939  | 47,900     | 48,839    | 1.92       | 98.08    |

*Bold values highlight material retained above filters.*

Radiation cpm and percentages (%) of radiation in retained and eluted material in diabase, quartz, or water-incubated NTPs after treatment with various deaggregating agents followed by ultrafiltration through 10 kDa MWCO filters.

**FIG. 3.** Ultrafiltration of polyribonucleic acid formed on diabase. Shown are starting material (S), filter eluates (E), and filter retentates (R) with increasing MWCO filters. K=kDa. NEPETop and 31RA: partial alkaline hydrolysis of 14-mer and 31-mer RNA molecules. Sizes in nucleotides are on the sides. Twenty percent PAGE, 7M urea. MWCO, molecular weight cutoff.
products cannot occur without the glass having catalyzed the attack of either the 2'-OH or the 3'-OH on the alpha phosphate of the starting triphosphate. This observation is significant regardless of which hydroxyl group was involved.

To prove that glass-synthesized polyribonucleic acid had many 3',5'-phosphodiester linkages, the polyribonucleic acid produced by glass catalysis was digested with various ribonuclease enzymes that are specific for 3',5' linkages. Treatment with Ribonuclease ONE® (Promega) left no high molecular weight material (Fig. 5). This established that the type (b) polyribonucleic acid contains a substantial proportion of 3',5' linkages between ribonucleotides in a linear structure. These data do not, of course, exclude some 2',5' linkages or some branching.

Expanding on this result, each sample of gel-purified polyribonucleic acid, as well as the low molecular weight materials, was separately incubated with RNase ONE (Supplementary Figs. S16 and S18), RNase A (CTP, UTP, and mixed NTPs samples; Supplementary Fig. S17, left panel), and RNase T1 (GTP and mixed NTPs samples; Supplementary Fig. S17, right panel). All RNases digested the high molecular weight products, but not the low molecular weight products.

We then asked how fast MRM glass catalyzed the formation of polyribonucleic acid (detailed methods in Supplementary Data). Radiolabeled polyribonucleic acid was collected at time intervals and resolved by PAGE (Fig. 2). Scintillation counting quantitated in bands cut from the gel (counts per minute [cpm] normalized for total cpm loaded, specific activity of the starting triphosphate, and adjusted for decay). Products were analyzed in nine bands, covering the eight rapidly migrating products (not polyribonucleic acid) and the slowly migrating products.

**FIG. 4.** 3D-Diode Array HPLC of products obtained by ammonia hydrolysis of polyribonucleic acid synthesized on diabase glass. (A) Hydrolyzed products of oligomerization for polyribonucleic acid made from all four standard nucleotides. (B) Control mixture of 2' and 3'-nucleotide monophosphate. Peak assignments were confirmed by HPLC runs of treated and nontreated NMPs and by matching spectrum profiles for controls and samples (Supplementary Figs. S19 and S20, and data not shown). The major peak in [A] running at ~6 min, resulting from the degradation of ATP input material, masks the peaks from 2' and 3'UMP. The presence of lines corresponding to UMP peaks can be detected in the 3D view (Supplementary Fig. S22) and was confirmed with the analysis of single-nucleotide UTP-oligomerization reactions (Supplementary Fig. S23). Far-left peaks in [A] come from residual open-ring nucleosides from previous HPLC (Methods section in Supplementary Data; Supplementary Fig. S19). Right-tilted and front views are shown in Supplementary Figure S21. Color shading is added for emphasis.
The rate of incorporation of \(^{32}\text{P}\)-GTP into polyribonucleic acid (band 1) was \(2 \times 10^{-3}\) pmoles/hour per gram of MRM glass; the rate for incorporation into band 8 (not polyribonucleic acid) was \(4.9 \times 10^{-3}\) pmoles/hour per gram of glass. GSM and BRP glasses showed similar rates of incorporation for band 1 (\(\approx 0.6\) and \(1.8 \times 10^{-3}\) pmoles/hour per gram of glass). NKT and AGV glasses produced rates <10\(^{-5}\) pmoles/hour per gram of glass. Pure quartz powder produced no detectable polyribonucleic acid.

The diversity of polyribonucleic acid sequences was then assessed. Products of incubations with MRM glass were analyzed with different combinations of triphosphates: \([\text{z-}^{32}\text{P}]\text{CTP}\) alone, with ATP, with UTP, with GTP, with ATP and with UTP in binary mixtures, with ATP and GTP, GTP and UTP as ternary mixtures, and with all four nucleoside triphosphates. Analogous combinations were examined with \([\text{z-}^{32}\text{P}]\text{GTP}\), \([\text{z-}^{32}\text{P}]\text{ATP}\), and \([\text{z-}^{32}\text{P}]\text{UTP}\). All nucleotides appear to incorporate well (Supplementary Fig. S10). GTP and CTP may be modestly preferred as substrates for MRM-catalyzed formation of polyribonucleic acid sequences. However, this preference is not large.

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### 3. Discussion

This study shows that various mafic rock glasses almost certainly present on the surface of the Hadean Earth catalyze the formation of polyribonucleic acid in water starting from nucleoside triphosphates. Both gel electrophoresis and ultrafiltration show that this polyribonucleic acid is, on average, 90–150 nucleotides in length. RNA molecules of this length are sufficiently long to participate in various laboratory processes that are reminiscent of RNA-based Darwinism (Attwater et al., 2013; Horning and Joyce, 2016; Joyce and Szostak, 2018; Horning et al., 2019; Wachowius and Holliger, 2019).

The enzymatic digestion experiments prove that a substantial fraction of the linkages in the “prebiotic” polyribonucleic acid are 3’\(,\)5’. However, these experiments cannot exclude the presence of 2’\(,\)5’ linkages, nor some amount of branching. Most astonishing would be products wherein the linkages were not mixed.

This notwithstanding, the robustness of function in polyribonucleotides with both linkages remains an important
point of discussion. Thus, Rohatgi et al. (1996) reported that 2',5' linkages in single-stranded RNA hydrolyze at pH 7 ~ 3 times faster than 3',5' linkages. Others have suggested that the 2',5' linkages might "cure" to 3',5' linkages, or be formed selectively due to pH differences (Usher and McHale, 1976; Englehart et al., 2013; Mariani and Sutherland, 2017).

In any case, the process is catalytic. Polyribonucleic acid synthesis continues over time, products accumulate over months, and the process does not consume the glass. Furthermore, the process occurs under conditions wherein polyribonucleic acid is stable, especially against depurination (Mungi et al., 2019). Kinetic data suggest that a small impact region on the Hadean surface containing just a few metric tons of fractured and water-permeated glass could have had the ability to produce close to a gram of RNA per day, limited (of course) by the supply of triphosphates.

Thus, the prebiotic relevance of this result very much depends on whether nucleoside triphosphates were present to Hadean impact fields. Models to create parts of, and bonds within, those nucleosides, as well as complete nucleoside triphosphates, are now advancing in many laboratories (Kim et al., 2011, 2016; Neveu et al., 2013; Xu et al., 2018; Becker et al., 2019; Benner et al., 2019; Castaneda et al., 2019; Kawai et al., 2019; Kim and Kim, 2019; Kim and Benner, 2021). If triphosphates were available, mafic glasses on the surface of the Hadean Earth (and Noachian Mars) may provide a piece missing in the "RNA First" puzzle.

Authors' Contributions

E.B. was involved in conceptualization, methodology, validation, formal analysis, investigation, original draft preparation, review and editing, visualization, supervision, and project administration. S.A.B. was involved in conceptualization, methodology, formal analysis, original draft preparation, review and editing, visualization, supervision, project administration and funding acquisition. S.J.M. was involved in conceptualization, resources, and review and editing. H.I.K. was involved in methodology and formal analysis. C.A.J. was involved in investigation and formal analysis.

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Data and Materials Availability

There were no restrictions on materials or data. All data are available in the main text or Supplementary Data.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data
Supplementary Figures S1–S24
Supplementary Tables S1–S8

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**Abbreviations Used**

| Abbreviation | Definition |
|--------------|------------|
| AGV          | andesite   |
| BRP          | basalt     |
| cpm          | counts per minute |
| GSM          | gabbro     |
| MRM          | diabase    |
| MWCO         | molecular weight cutoff |
| NKT          | nephelinite |
| NTPs         | ribonucleoside triphosphates |
| PAGE         | polyacrylamide gel electrophoresis |