 doi:10.20944/preprints201904.0297.v1

Article

Wet-dry Cycling Delays the Gelation of Hyperbranched Polyesters: Implications to the Origin of Life

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Received: date; Accepted: date; Published: date

Abstract: In extant biology, biopolymers perform multiple crucial functions. The biopolymers are synthesized by enzyme-controlled biosystems that would not have been available at the earliest stages of chemical evolution and consist of correctly sequenced and/or linked monomers. Some of the abiotic "messy" polymers approximate some functions of biopolymers. Condensation polymers are an attractive search target for abiotic functional polymers since principal polymers of life are produced by condensation and since condensation allows for the accurate construction of high polymers. Herein the formation of hyperbranched polyesters that have been previously used in the construction of enzyme-like catalytic complexes is explored. The experimental setup compares between the branched polyesters prepared under mild continuous heating and the wet-dry cycle conditions. The results reveal that period wetting during which partial hydrolysis of the polyester occurs, helps control the chain growth and retards the gel transition. It is significant to the origin of life studies that environmental, prebiotically plausible conditions could achieve such control without enzymes or a skilled chemist. As expected in marginally controlled systems, the identification of each component of the heterogeneous system has proved challenging, but it is not crucial for drawing the conclusions.

Keywords: hyperbranched polyester; functional polymer; chemical evolution; wet-dry cycle; gelation prevention; condensation polymer; origin of life

1. Introduction

In modern biological systems, biopolymers perform a multitude of functions. The major biopolymers (i.e., polypeptides, nucleic acids, polysaccharides) are characterized by a specific monomer sequencing and folding to achieve the necessary functions. In biochemistry, the synthesis of biopolymers is accomplished through intricate enzyme-controlled processes [1], that

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would not have been available at the earliest stages of chemical evolution. The abiotic synthesis of biopolymers is an active area of research. It has long been recognized that abiotic reactions can produce many monomers of biopolymers. For example, the Miller−Urey experiment showed that some of the simpler amino acids (e.g., glycine and alanine) are readily formed in a model prebiotic atmosphere [2,3]. Besides, amino acids used in life today have been found in carbonaceous meteorites that are more than 4 billion years old[4–6]. Different biopolymer blocks, including sugars and nucleobases, are also formed in a variety of model prebiotic reactions [7–10] and found in some meteoritic samples [11]. Several high-yielding abiotic processes of coupling of amino acids [12,13] and nucleotides [14–16] using have been proposed. These models, however, still lack mechanisms for a robust sequence and structure control of the products limiting the plausibility of functional biopolymer formation.

As an alternative approach to the abiotic synthesis of functional polymers chemically different from those of contemporary biopolymers has been considered [17–19] . It has been long appreciated that model prebiotic systems yield large amounts of intractable tarry polymeric material [20,21]. Some polymeric components of the tarry material with structures different from those of existing biopolymers are possibly capable of approximating some the biological functions. Examples of this approach include the catalytic function of proteinoid microsphere structures formed upon non-specific thermal condensation of certain amino acids [22–24].

Prebiotically plausible condensation polymers is a compelling system for the study of the chemical evolution of functional polymers. Firstly, principal polymers of extant life are synthesized via condensations reactions. Secondly, the step-growth mechanism of condensation allows for control over each step of polymerizations. This property allows for the construction of high polymer with accurately known structures [25]. Unlike the addition polymerization under which the chain elongation can occur only by the addition of a single monomer, under condensation reaction can occur between existing polymer species combining properties of different polymeric domains into one product. Furthermore, condensation reactions are often reversible; a polymeric product could break down into oligomeric building blocks that in turn could recombine into a polymer with novel properties.

The bonds linking the monomers of many condensation biopolymers are characterized by the positive free energy of formation in the aqueous environment of the cytoplasm. For example, the energy of the amide bond in a polypeptide backbone ranges from +2 to +4 kcal/mol in aqueous solution [26]. One exception is the ester bond found in naturally occurring polymers, i.e., cutin and polyhydroxybutyrate, is characterized by slightly negative bond energy (~1 kcal/mol under physiological conditions [27]). As polyester synthesis is more thermodynamically favorable than that of a polypeptide, polyesters have been hypothesized to have preceded peptides by Orgel [28], and this notion is perhaps supported by the demonstrated ability of the ribosome to catalyze α-hydroxy acid coupling [29,30]. Glyceric acid has been previously shown to polymerize and form polyester chains of up to 25 residues under acidic conditions and moderate heating. Poly-glyceric acids prepared from optically pure and racemic glyceric acid have different solubilities suggesting the possibility of chiral selection [31]. We have shown that poly-malic acid can be

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formed under mild dry heating and maintained in water solution while undergoing wet-dry cycling [32].

Specific sequencing is not the only means to derive function form biopolymers. In polysaccharides, different stereochemistry of the glycosidic bonds, as well as linear or branched nature of the biomacromolecule, dictates its function. The polymers comprised solely of glucose monosaccharides are a compelling case in point. Cellulose, the major structural component of cell walls, is a linear polymer composed of repeated glucose units bonded by beta 1-4 glycosidic bond. Cellulose is insoluble in water [33] and possesses high tensile strength [34].In branched polysaccharides used as energy reserves, glycogen in animals and amylopectin in plants, the glucose units are linked in alpha 1,4 glycosidic bonds with branching alpha 1,6 bonds. Both polymers are water soluble.

Highly or hyperbranched polymers (HBPs) have attracted significant attention from industrial, synthetic and biomedical communities due to their unique properties, such as low viscosity and solubility. HBPs are intrinsically globular and have a high propensity towards ligand binding either inside internal pockets with suitable environments or at the polymer surface that is characterized by a large number of potentially functional end groups [35]. Biomimetic catalytic properties of HBP and dendrimers, a regular subclass of HBP, are well documented [35–38]. We have previously demonstrated that straightforwardly synthesized mixture of amine-bearing hyperbranched polyesters (HBPEs) is capable of catalyzing the Kemp elimination process by modulating the polarity of the environments in the internal pockets of HBPEs [19]. Furthermore, HBPs, and HBPEs, in particular, can be prepared in the one-pot process [39–41] and under prebiotically plausible conditions [42].

HBP synthesis commonly involves the coupling of monomers of AB_x type, where A and B refer to different mutually reactive functional groups. Flory's statistical theory of mass distributions of three-dimensional polymers suggests that polymerization of monomers of AB_x type can proceed infinitely without the occurrence of gelation due to the formation of crosslinked networks [43]. Condensation of AB_x monomers has been used to prepare a wide range of polymeric products including polyphenylenes [44,45] and polyesters [46–48]. Alternatively, HBPs can be synthesized starting from homofunctional monomers, some of which are more readily available commercially and are prebiotically relevant [49]. The present study focuses on a model polyesterification yielding branched polyesters synthesized from homofunctional monomers, glycerol and citric acid (**Scheme 1**).

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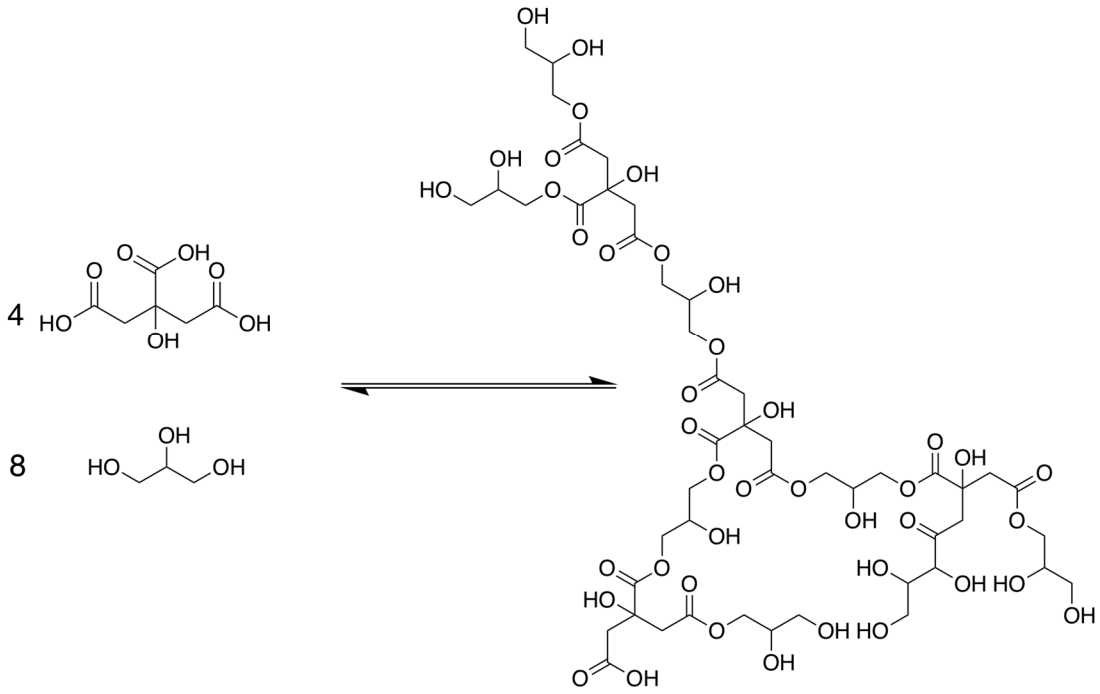

Scheme 1. Schematic representation of citric acid and glycerol polyesterification. The formation of one of the possible oligomers comprised from four citric acid and eight glycerol monomers is depicted.

HBP tend to undergo the process of gelation. Gelation occurs when a branched polymer forms an extensive network between strands through cross-linking [50].The gel transition is associated with a drastic change in polymer properties, such as viscosity and solubility [49].When the goal is to synthesize soluble catalysts, gelation is undesirable. Gelation is sharply dependent upon the degree of polymerization [50]. In synthetic chemistry, many methods exist for controlling and preventing gelation, such as a rigorous control over monomer stoichiometry [49,51] and the degree of polymerization [46,52], branching core inclusions [48], etc. The focus of this study is to explore a more straightforward prebiotically reasonable conditions of wet-dry cycling associated with day-night or seasonal cycle as a method of HBPE chain growth control.

2. Materials and Methods

Reagent grade citric acid, glycerol were purchased from Sigma-Aldrich and used without further purification.

A typical polyesterification reaction was conducted starting with 5ml of an aqueous solution containing 330mM of citric acid and 660mM glycerol. The pH of the solution was not adjusted; the pH was measured at 2 and remained unchanged throughout the polymerization process. The samples undergoing wet-dry cycle were allowed to air dry for 48 hours at 85˚C, reconstituted and incubated for 48 hours at 75˚C. Sample tubes were removed at the end of first, second, fourth and eighth periods of both wetting and drying. Continuously dried samples were allowed to air dry

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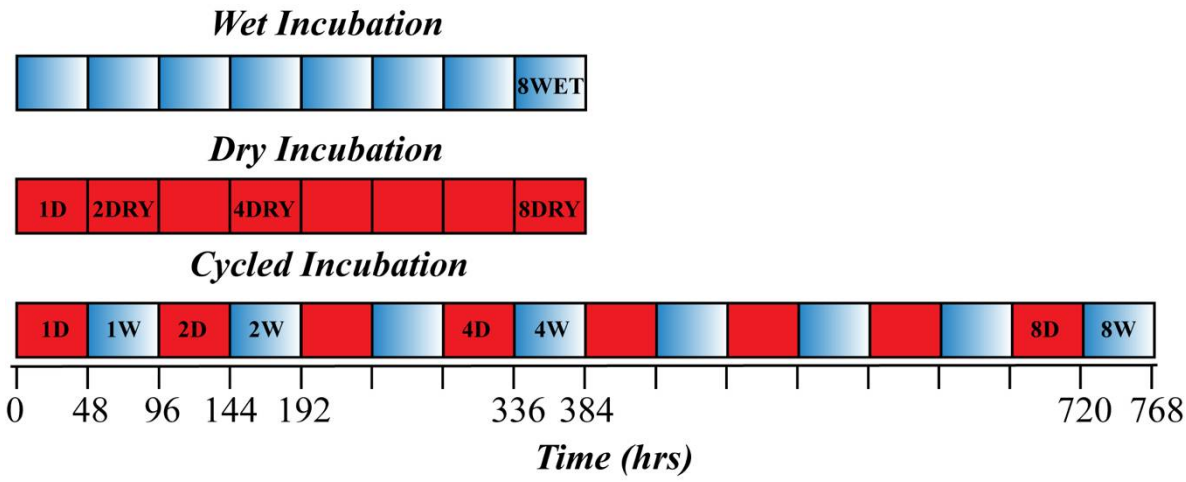
and incubated at 85˚C and sampled after 48, 96, 192 and 384 hours. An additional sealed sample was incubated for 384 hours. Schematic representation of the incubation and sampling schedule is depicted in scheme 2. Prior to analysis the dry samples were reconstituted and stirred at room temperature for 12 hours. Aliquots were collected from all solutions, freeze dried and dissolved in appropriate solvents.

For size exclusion chromatography (SEC) analysis, the reconstituted dry and wet samples were diluted 1:1 in deionized water. The analysis was performed on an Advanced Polymer Chromatography system (Waters Corporation, Manchester, UK) interfaced with a UV-vis detector equipped with Acquity APC AQ column [125 Å, 2.5 µm, 4.6 mm Å~ 30 mm, Waters Corp] and Acquity APC XT column (125 Å, 2.5 µm, 4.6 mm Å~ 30 mm, Waters Corp). An isocratic flow of 0.500 mL min−1 using H2O or 80%/20% THF/Methanol was utilized. The column temperature was set at 40 °C.

MALDI-MS spectra were collected on an ultrafleXtreme Bruker Daltonics MALDI-TOF-MS (Bruker Corporation, Billerica MA, USA) in positive ion mode. External mass calibration was conducted using standard peptide mixtures. Sample preparation matrix (trans-2-(3-(4-tert-butylphenyl)-2-methyl-2-propenylidene)malononitrile or (DCTB)) was dissolved 80%/20% THF/Methanol. Subsequently, the freeze-dried samples and the matrix (DCTB) were mixed at a 1:10 [v/v] ratio in advance and then the mixture was applied to the plate before analysis. ESI-MS spectra were collected on a Bruker micrOTOF II (Bruker Corporation, Billerica MA, USA) in positive ion mode. The samples were dissolved in 80%/20% THF/Methanol.

1H Nuclear Magnetic Resonance [NMR] spectra were recorded on a Bruker Avance 400 spectrometer (Bruker Corporation, Billerica MA, USA) at 25°C. The spectra were collected employing 30° inversion pulses with 11s acquisition time, and 1s recycle delay.



Scheme 2. Schematic representation of experimental setup and the sampling schedule. Red squares represents the dry heating period, blue-shaded squares represent the wet incubation period. The samples were collected at the end of labeled periods.

3. Results

3.1. Polyesterification

Since this study intends to approximate prebiotically plausible conditions, the polymerization of citric acid and glycerol was conducted without an active water removal or catalyst addition. The starting solution for all polymerizations contained 2:1 ratio of glycerol to citric acid. Excess of glycerol has been previously shown to favor more soluble less cross-linked product [42,51]. Dry heating of the glycerol citric acid solution for the first 48 hours (cycle 1D, **Scheme 2**) produced a clear gelatinous product. Similar clear glass like products formed after 96, 192 and 384hours of continuous drying (cycles 2DRY, 4DRY, 8DRY, **Scheme 2**). These products swelled in water but did not fully dissolve. For the wet-dry cycling experiment, the regime of 48 hours of dry heating at 85˚C followed by 48 hours of wet incubation at 75˚C for additional 48 hours was selected. The citric acid-glycerol polymer has proven to be more resistant to hydrolysis than the previously investigated poly-malic acid system [32].Whereas poly-malic acid polymer would fully hydrolyze after several hours of heating at 75˚C, substantial amounts of citric acid-glycerol polymeric material were still present after 48 hour period of incubation. The hydrolysis of branched polymers is often retarded compared to linear polymers as dendritic spatial structures of the former limit the accessibility of water [53–56]. The systems that were subjected to periods of wet-dry cycled products remained fully soluble by the end of 384 hours. The last dried sample was slow to dissolve the solid did not fully dissolve following the typical sample workup, stirring for 12 hrs. The solid fully dissolved during the 48-hour wet incubation at 75˚C. The observations suggest that continuously dried polymerization systems have undergone gelation much faster than the cycled ones. Little to no polymer was formed in the sample that have undergone continuous wet incubation suggesting that condensation only takes place during the drying periods.

3.1. Size-exclusion chromatography (SEC)

SEC is a chromatographic technique yielding separation based on the hydrodynamic volume of analytes. The separation is triggered by the disparity in the interactions of the eluting particles within pores of the stationary phase unlike the surface interactions in other chromatographic methods. In principle, high molecular weight molecules, with large hydrodynamic volumes elute quickly, whereas smaller molecules experience more interactions with the stationary phase and elute later. In the case of linear polymers, the hydrodynamic volume is directly correlated to the molecular weight; thus, the molar masses of unknown linear polymers may be estimated relative to standards. In the case of branched and dendritic polymers, such correlation is challenging to draw [47]. Moreover, the nature of the terminating groups of branched polymers is expected to have a substantial effect on the polymer interaction with the solvent that, in turn, determines the hydrodynamic volume. The hydrodynamic volume of carboxylic acid terminated dendrimers in aqueous solution, for example, is strongly pH-dependent and can change up to 50 % by merely changing the pH of the solution [57]. The SEC separation, in this case, is therefore based mainly on a complex relationship between the oligomer chemistry and architecture rather than the molar mass. The chromatographs shown in **Figure 1** were analyzed using aqueous mobile phase. Better separation of the low molecular weight products was achieved using normal phase THF-methanol mobile phase [Figure S1], however the products only marginally dissolved in THF-methanol and

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194 caused on-column precipitation. Only small number of samples was analyzed using normal phase
195 SEC.

196 The chromatographs in **Figure 1** represent the polymeric samples produced during different
197 experimental cycles (**Scheme 2**). Panel 1A depicts the chromatographs of the samples that have
198 undergone one cycle of drying (1D or DRY) and one cycle (1W) of wet incubation. The cycle 1D
199 sample is represented by one broad peak at ~2.18 min and smaller broad shoulders at ~2.3 and
200 ~2.4 min probably due to high dispersity of the sample. The cycle 1W sample shows an
201 additional shoulder at ~2.10 min that is due to monomeric citric acid confirmed by co-injection.
202 It is unusual in SEC for the monomer to elute faster than the polymer. In this case, the
203 phenomenon is probably due to the formation of extensive hydration shell around the citric acid
204 molecule or the formation of non-covalent complexes. Glycerol monomer was not detected as it
205 is invisible to the UV at 220nm. **Figure 1**B shows the chromatographs of the wet-dry cycled
206 samples collected at the end of the wet cycles [series W]. While the chromatogram 1D (**Figure**
207 **1**A) showed no peak due to the citric acid monomer, about 50% of all product in 1W samples is
208 the citric acid monomer. Citric acid monomer does not appear in samples 2, 4 and 8W. In
209 samples 2 and 4W the major peak is shifted compared to the cycle 1D sample from ~2.18 min to
210 ~2.14 min possibly due to some polymer degradation. In the sample 8W the major peak remains
211 at 2.18 min. **Figure 1**C compares between the samples belonging to DRY series. Qualitatively,
212 all chromatograms look the same with major peak at ~2.18 min with shoulders at ~2.3 and ~2.4
213 min. The intensity of the peaks gradually diminishes with every successive sample due to partial
214 precipitation. Only water soluble fraction was analyzed. Finally, **Figure 1**D compares between
215 the samples of the D series. Samples 1,2 and 4D appear roughly similar. Sample 8D here was
216 analyzed after the normal workup of stirring the sample in water at room temperature for 12 hrs,
217 therefore a very small fraction is visible in the chromatogram. The sample was fully dissolved
218 after 48 hours incubation at 75˚C (sample 8W).

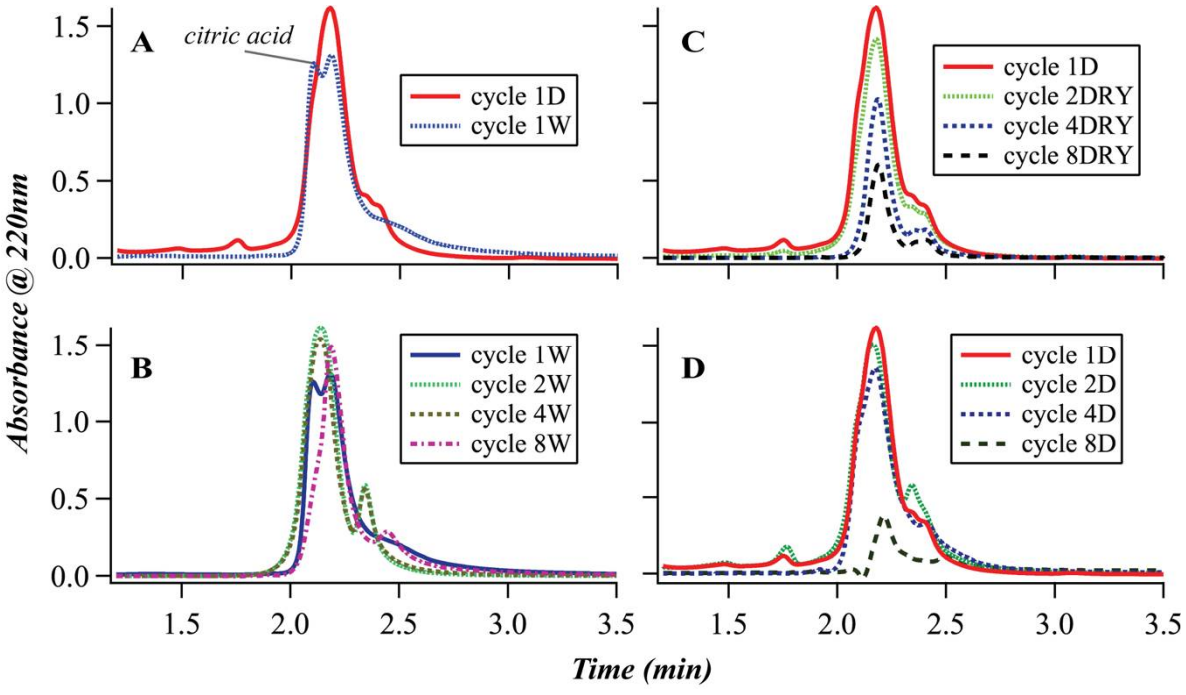

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Figure 1. SEC analysis of the citric acid glycerol polymerization products (**a**) Comparison between the samples collected at the end of the first dry and wet cycle; (**b**) Comparison between the samples collected at the end of wet cycles (W series); (**c**) Comparison between samples of the DRY series; (**d**) Comparison of the samples collected at the end of dry period in the cycled series (D series).

3.1. Mass spectrometry

Figure 2 shows the MALDI mass spectra of (a) water-soluble fraction of the polymeric sample collected after a period of continuous drying for 384 hours (cycle 8DRY, Scheme 2) and (b) polymeric sample that has undergone the most extended period of wet-dry cycling (cycle 8W, Scheme 2). Multiple masses consistent with glycerol-citric acid units detected as sodium adducts have been detected. The largest detected mass in the continuously dried sample corresponds to a molecule containing nine glycerols and four citrate units, whereas the largest mass detected in the cycled sample corresponds to a molecule containing eight glycerols and four citrate units. Neither of the mass spectra contains a signal corresponding to dicitrate implying that the hydroxyl group of the citric acid does not participate in the esterification reactions. The products in both spectra correspond to species enriched in glycerol consistent with the excess of glycerol in the starting material. Similar spectra have been obtained utilizing electron spray ionization (Figure S2).

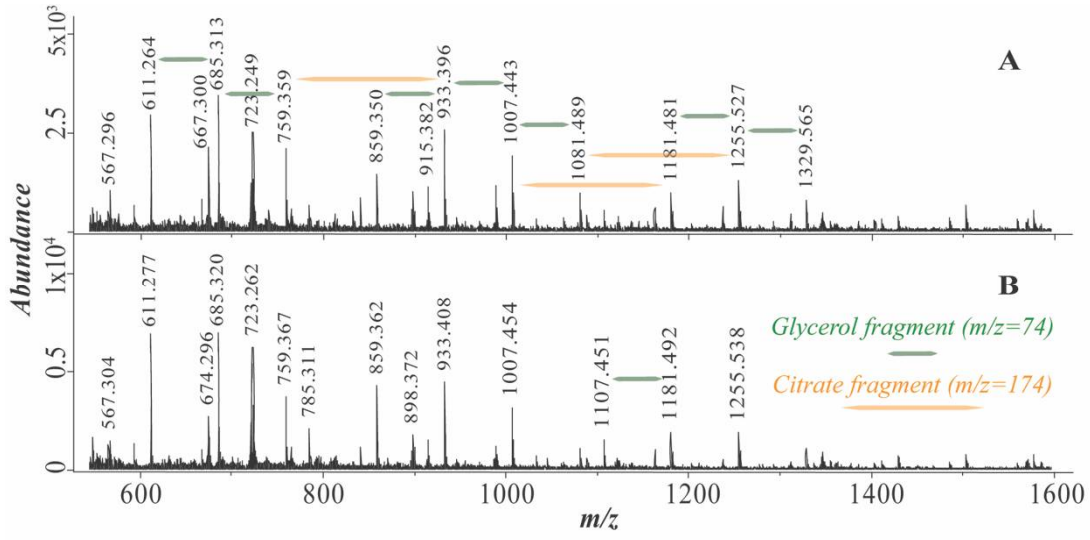

Figure 2. MALDI mass spectra of **a**) the water-soluble fraction of the continuously dried sample collected after 8 periods (8DRY) and (**b**) cycled sample collected at the end of the experiment (8W). All labeled species correspond to $(M+23)^+$ ions (sodium adducts).

3.1.Nuclear Magnetic Resonance (NMR)

Figure 3 shows representative ^1H NMR spectra in D_2O of the polymeric samples exposed to continuous drying, dry-wet cycling [Fig. 3A] as well as unreacted citric acid and glycerol [Fig. 3B] The quadruplet characterizes the spectrum of unreacted glycerol and citric acid [Fig. 1B] at ~2.8 ppm corresponding to methylene hydrogens of citric acid, multiplets at ~3.4, 3.5 and 3.6

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ppm representing methylene and methanetriyl hydrogens of glycerol. Small broad peaks associated with polymeric products are also represented in the spectrum. The unreacted sample as well as the rest of the sample has undergone a workup procedure that included a period of freeze-drying that is potentially conducive to the polyester formation. The spectrum of the sample that have undergone long wet incubation [8WET] closely resembles the spectrum of the unreacted solution [Figure S3] suggesting that polymerization did not occur during the wet incubation. The spectra of the polyesters [Fig. 1A] is described by broadened, downfield-shifted signals corresponding to citric acid unit methylene hydrogens, broadening of the glycerol unit hydrogens and the appearance of broad signals at ~4 and 5 ppm consistent with methylene and methanetriyl hydrogens at the RCOO-CH position. The exhchangeable protons of the carboxylic acid and alcohol groups were not detected. The signal assignments performed using prediction software and standards are described elsewhere [42]. The relative integrations of these signals suggest that only a small fraction of the hydroxyls [>6 %] at the 2 position of glycerol are esterified possibly due to steric hindrance. The line broadening can be explained either by the presence of multiple oligomeric isomers with overlapping signals or by slowed molecular tumbling of polymeric species.

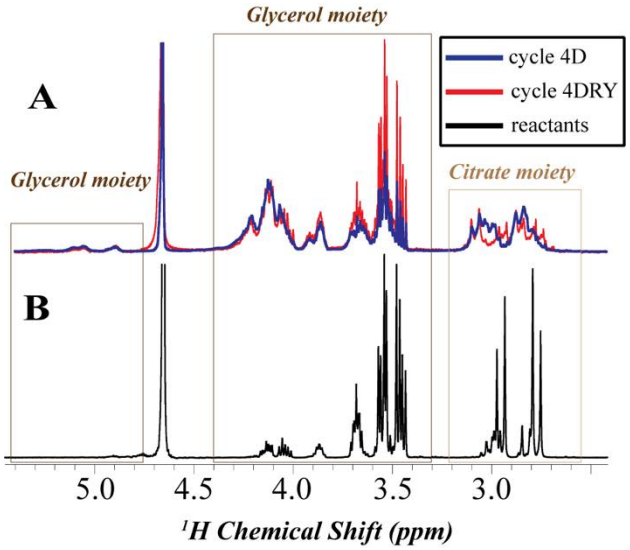

Figure 3. Representative ^{1}H NMR spectra of the glycerol-citric acid polymerization products in D2O along with general peak assignments. [**a**] The overlayed spectra of the soluble fraction of the 4DRY sample [red] and the 4D sample [blue]; [**b**] The spectrum of the unreacted sample.

Table 1 shows the relative integrations, normalized to the number of non-exchangeable protons, of the sum of the signals corresponding to citric acid and glycerol moieties. In all the cycled samples, the initial stoichiometry of two glycerols to one citrate is roughly preserved. The slight deficit of glycerol, approximately 9.9 protons instead of expected ten could be attributed to weighting and volume measurement errors during the initial solution preparation. Interestingly, the relative integrations suggest that the soluble fraction of the continuously dried samples appear to be enriched in glycerol suggesting that the insoluble fraction is enriched in citric acid. It has been shown [Halpern] that glycerol-citric acid polymer prepared at 1:1 ratio is more prone to

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crosslinking and formes an insoluble product more easily than systems with an excess of glycerol. Slightly higher glycerol excess seen in cycles 4D/W and 8D/W possibly due to some precipitation. Most considerable excess of glycerol seen in the water-soluble fraction of 4DRY [Table 1, Figure 3]. These observations suggest that during continuous drying polymer containing the similar stoichiometry of citric acid and glycerol are formed first; the excess of glycerol incorporates later. The result suggest that wet-dry cycling does not only control the molecular weight of the product but the polymer make up as well.

Table 1. Relative stoichiometries of non-exchangeable protons in citrate and glycerol moieties in the different polymeric products based on ^{1}H NMR signal integrations. In the DRY series only visibly water soluble fraction is analyzed.

Series/cycles	DRY	D	W	Initial	WET
0				4H:9.93H	
1	4H[1]:9.89H	4H:9.89H	4H:9.81H		
2	4H:10.14H	4H:9.94H	4H:9:92H		
4	4H:14.03H	4H:10.20H	4H:10.28H		
8	4H:11.99H		4H:10.37H		4H:9.74H

[1] The 4 citrate non-exchangeable protons were set as constant, the glycerol signal integrals were measures relative to citrate signal integrals.

4. Discussion

Planetary surfaces, lacking the enzymatic control of the biosphere or the synthetic chemist's skill in the lab, generically produce combinatorially complex, heterogeneous mixtures of compounds. For example, in prebiotically plausible settings self-polymerization of small molecules such as formaldehyde [58] or HCN [20] yields an intractable mixture, and has been referred to as “tar” or “asphalt” [21]. In the chemical evolution studies, the production of the tarry material is often seen as an unwanted process, or even paradox implies that the workability of elucidating life's origins might be an insuperable goal. However, recently, prebiotic systems chemistry [59–61] has begun to explore the processes of selection and organization in these heterogeneous, or "messy" systems in an attempt to understand the natural transition between the messy prebiotic chemistry and the well-controlled biochemistry, in other words, the transition between chemistry and biology. The mechanism for the organization in messy chemistry that have been explored thus far include the emergence of autocatalytic sets [62–64],molecular imprinting [65] as a mechanism for heredity in chemical systems, as well as specific selective reactions [66–68]. Herein the environmentally driven selective formation of HBPEs with a specific property is explored.

The results presented here indicate that wet-dry cycles associated with day-night, tidal or seasonal environmental changes promote the formation of soluble lower molecular weight HBPE whereas continuous drying is conducive to the formation of gelated polymers. Periods of intermittent wetting trigger the partial hydrolysis controlling the chain growth of the HBPEs and therefore retarding the gelation process. Soluble HBPEs oligomers have been shown to possess

 doi:10.20944/preprints201904.0297.v1

enzyme-like catalytic activities. Therefore a process of their synthesis and maintenance in the water solution can significantly affect abiotic systems. Furthermore, the NMR studies suggest that that during the continuous drying HBPE enriched in citric acid, more prone to crosslinking, are produced preferentially. We have previously demonstrated that HBPEs can be synthesized by subjecting multifunctional organic acids and alcohol mixtures to mild heating under dry conditions [42]. This method, however, produces a multitude of polymeric products varied in size and shape. One possible way of selecting for the desired HBP shape and function is to investigate further the concept of far-from-equilibrium polymerization by subjecting the polyesterification system to intermittent drying and wetting. Period of heating the open vessel stimulates esterification. Even though periodic sample rehydration promotes hydrolysis, successive iterations of wet-dry cycles result in polymer yields, and molecular weight distributions above that observed after heating alone. Products less prone to hydrolysis would tend to persist in the system at the expense of the rest. The hydrolysis patterns of HBPEs differs from linear polymer ones. The globular nature of HBPEs prevents or delays the water intrusion into the core, slowing down the hydrolysis process and resulting in macromolecular surface erosion rather than breakdown. When wet-dry cycling applied to HPBE condensation, the first drying phase will result in a mixture of linear, branched and mixed polymers. During the hydrated periods, linear portions of the polymer would be more susceptible to hydrolysis than their branched counterparts. Therefore it is reasonable to assume that after some cycles the makeup of the polymer would consist predominantly of branched structures.

Analytical challenges have discouraged many other researchers from studying the inherent complexity of the prebiotic systems chemistry [61].Experimental studies of the complex prebiotic systems are challenging to identify all the components unequivocally. The system described here is somewhat simplified; it starts with only two reactants being connected through one chemical bond with minimal side reactions, such as water eliminations. Nevertheless, due to the production of a multitude of branched architectures, the spectroscopic and chromatographic methods produce broad unresolved signals preventing the clear identification of every component in the system. Comparative analysis between different experimental conditions in addition to the bulk properties of the products permits to derive some structural information. While it is not necessarily feasible to produce precise, complex biological structures abiotically, a functional measurements to establish whether the messy products have a particular bulk property, i.e., solubility, color, swelling in a solvent or capable of a particular function, i.e., catalytic ability, capability to absorb specific molecules, is a reasonable approach to hypothesis of the origin of life.

5. Conclusions

The results described above describe the polymerization ion citric acid-glycerol polyester formation to form a branched polyester. The polymerization was conducted under continuous drying and subjected to wet-drying cycles. The continuous drying yielded an insoluble glass-like swellable in water product after 48 hours. In contrast, the system subjected to wet-dry cycling yielded a water-soluble product after 786-hour experiment. The NMR studies indicated that the insoluble product was enriched in citric acid, a stoichiometry that favors crosslinking. While the

347 analysis has proven challenging as expected in abiotic mixtures with significant heterogeneous
348 content, the two experimental setups have produced polymeric products with different properties.
349 In branched systems, the intermittent wetting has provided means to control the chain growth and
350 delayed the gel transition. The described system is a model for further exploration of the
351 formation of functional polymers under prebiotically plausible conditions.

352 **Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure
353 S1: Normal phase SEC analysis of the citric acid glycerol polymerization products, Figure S2:
354 ESI mass spectra Figure S3: ^{1}H NMR spectra of citric acid glycerol polymerization controls

355 **Funding:** This research was supported by Kakenhi Kiban C grant # 17K01943, MEXT, Japan
356 and World Premier International Research Center Initiative[WPI], MEXT, Japan.

357 **Acknowledgments:** I thank Yuki Suna and Kugako Sugimoto for the technical support, Dr.
358 Nicholas Guttenberg and Dr. Nathaniel Virgo for helpful discussions, and Prof. Naohiro Yoshida
359 for the use of his NMR spectrometer.

360 **Conflicts of Interest:** The author declares no conflict of interest.

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