

Catalytic effects of Murchison Material: Prebiotic Synthesis and Degradation of RNA Precursors

Raffaele Saladino · Claudia Crestini · Cristina Cossetti ·
Ernesto Di Mauro · David Deamer

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Abstract Mineral components of the Murchison meteorite were investigated in terms of potential catalytic effects on synthetic and hydrolytic reactions related to ribonucleic acid. We found that the mineral surfaces catalyzed condensation reactions of formamide to form carboxylic acids, amino acids, nucleobases and sugar precursors. These results suggest that formamide condensation reactions in the parent bodies of carbonaceous meteorites could give rise to multiple organic compounds thought to be required for the emergence of life. Previous studies have demonstrated similar catalytic effects for mineral assemblies likely to have been present in the early Earth environment. The minerals had little or no effect in promoting hydrolysis of RNA (24mer of polyadenylic acid) at 80°C over a pH range from 4.2 to 9.3. RNA was most stable in the neutral pH range, with a half-life ~5 h, but at higher and lower pH ranges the half-life decreased to ~1 h. These results suggest that if RNA was somehow incorporated into a primitive form of RNA-based thermophilic life, either it must be protected from random hydrolytic events, or the rate of synthesis must exceed the rate of hydrolysis.

Keywords Prebiotic chemistry · Murchison meteorite · Formamide · Catalysis

Introduction

At some point in the evolutionary process leading to the origin of life, nucleic acid like-polymers were synthesized from monomers. RNA, in particular, as recently reviewed

R. Saladino (✉)
Department of Agrobiologia and Agrochemistry, University of Tuscia, 01100 Viterbo, Italy
e-mail: saladino@unitus.it

C. Crestini
Department of Science and Chemical Technologies, University of Tor Vergata, Roma, Italy

C. Cossetti · E. Di Mauro
Department of Biology and Biotechnology Charles Darwin, University La Sapienza, 00185 Roma, Italy

D. Deamer
Department of Biomolecular Engineering, University of California, Santa Cruz, CA 95064, USA

(Cheng and Unrau 2010), may be a plausible candidate for the original biopolymer because it can act both as a catalytic ribozyme and as a carrier of genetic information. The “RNA world” scenario (Gilbert 1986; Cech 1987) assumes that RNA-like (pre)genetic polymers could arise from simpler precursors. However, it is still unclear how the phosphodiester bonds of RNA were formed, because the energy-requiring synthetic process would have been balanced by energetically downhill hydrolysis reactions. It is therefore important to understand not just synthetic pathways, but also hydrolysis rates under conditions prevailing on the prebiotic Earth (Kawamura 2001).

It is widely believed that mineral-water interfaces played a role in chemical reactions on the early Earth, and numerous previous studies have investigated such interactions (Huber and Wächtershäuser 2006; Ferris, 2006; Cody and Scott 2007; Saladino et al. 2007; Saladino et al. 2009). Because of geological processing of the Earth’s crust over the past four billion years, we can only guess what mineral-water interfaces might have been present. Presumably minerals associated with volcanism would have been abundant, including silicate ash deposits, clays and lava, as well as the sulfide minerals now found in hydrothermal marine vents. However, another site for geochemical processing would be planetesimals that preceded planet formation in the early solar system, now represented by asteroids that remain between the orbits of Mars and Jupiter. Asteroids are understood to be the parent bodies of most meteorites, and particularly the carbonaceous chondrites containing organic compounds that are products of chemical reactions occurring in the interiors of the parent bodies. This suite of organic material is at least as old as the Earth, some perhaps even predating the origin of the solar system (Ehrenfreund and Cami 2010). Their diversity, composition and isotope distribution provide clues to the history of organic carbon over the past 5 billion years, and guide thinking about the kinds of compounds available to support the origin of life.

Depending on their original size, many asteroids experienced radiation-induced heating of their interiors. In the largest, such as Vesta with a diameter of 560 km, it is known from remote sensing studies that the heat exceeded the melting point of iron so that differentiation occurred with formation of an iron-nickel core and a basaltic crust (Ghosh and McSween 1996). The earliest history of smaller carbonaceous asteroids involved microscopic crystalline particles of silicate minerals that were loosely packed by gravitational accretion into kilometer-sized objects. Over a period of many years, heat produced by radioactive decay accumulated in the interior and at some point began to melt the ice that had become incorporated along with the mineral particles. The liquid water slowly percolated toward the surface of the asteroid, perhaps speeded by vaporization of water near the center. Supporting evidence comes from the mineralogy of meteorites such as the Murchison, which contain clay particles that can only be produced by exposure to liquid water (Macdougall et al. 1984). For the purposes of our study, we assume that a variety of chemical reactions would be promoted by the heat. Furthermore, the hot aqueous phase would transport small amounts of organic compounds toward the surface of the parent body as it percolated through the mineral matrix, possibly resulting in a kind of chromatography in a column a kilometer or more in length (Krooss et al. 1991; Wing and Bada 1991). As the water approached the surface of the asteroid, it cooled, and froze again. The presence of both water ice and organic material on the surface of the asteroid Themis was recently observed by Campins et al. (2010).

From these results, it is clear that liquid water and organic compounds co-existed in the parent bodies of meteorites. It is also likely that similar mixtures occurred early in the Earth’s history, and that polymeric compounds were continuously being produced and then degraded by hydrolytic reactions. The specific question addressed here concerns the

synthesis of monomers under these conditions in the presence of formamide, a simple one-carbon chemical precursor, and the rate at which RNA is hydrolyzed under the same conditions. The synthesis of nitrogen-containing heterocycles from formamide under homogeneous conditions has been reported by Bredereck et al. (1956, 1959), Ivanov and Vladovska (1978), Yamada and Okamoto (1972), Yamada et al. (1978) and by Miyakawa et al. (2002). The prebiotic chemistry of formamide in the presence of minerals has been also recently investigated (Saladino et al. 2007; Saladino et al. 2009). Moreover, formamide has been detected in the interstellar medium (Rubin et al. 1971; Raunier et al. 2004), in the long period comet Hale-Bopp (Bockelée-Morvan et al. 2000) and, tentatively, in the solid phase on grains around the young stellar object W33A (Schutte et al. 1999). From this, we will assume that formamide was present in the mixture of reactive precursor molecules that took part in chemical reactions that became possible when the interior of parent body asteroids warmed and liquid water was produced from trapped ices. Because the organic compounds of carbonaceous meteorites have been very instructive in guiding research on prebiotic chemistry (Ehrenfreund and Cami 2010), we chose to investigate a meteoritic mineral assemblage that may have been involved in promoting abiotic chemical reactions. We specifically address whether potential catalytic effects of mineral Murchison material affect synthesis of organic compounds from formamide. We also investigated possible effects on hydrolysis of oligonucleotides under the same conditions.

Materials and Methods

A Murchison stone (17 g) was a gift from the meteorite collection of the Field Museum of Natural History (Chicago, IL). Approximately 1.0 g of an interior sample of the stone with fusion crust removed was ground in a mortar. The extraction was carried out in two steps, the first by adding 1.0 mL 0.1 N NaOH and 3.0 mL of 2:1 chloroform-methanol. The second step was performed with 1.0 mL 0.1 N sulphuric acid and 3.0 mL of 2:1 chloroform-methanol. Between steps the powder was precipitated by a brief low-speed centrifugation (6000 rpm, 10 min) and the supernatant phase was decanted. The supernatant contained all organic compounds that were soluble in both aqueous and organic solvents at high and low pH ranges, leaving behind the mineral powder of the meteorite together with insoluble organic material.

To remove the insoluble component, half of the extracted powder was pyrolyzed at 600°C in a laboratory oven for one hour to produce a rust-red powder composed of the original meteoritic minerals and any products produced by the aerobic pyrolysis. Both the original extracted powder and the pyrolyzed powder were used in subsequent experiments.

Formamide Condensation

The synthetic reactions leading to low molecular weight compounds were performed by heating freshly distilled formamide **1** (HCONH₂, 2.5 mL), purchased from Aldrich, at 140°C for 48 h in the presence of 45 mg of the original sample of Murchison meteorite powder (2.0% by weight with respect to substrate). At the end of the reaction the mineral was recovered by centrifugation (6000 rpm, 10 min., Haereus Biofuge), the supernatant decanted, and the excess formamide removed from this by distillation under high vacuum (40°C, 4×10⁻⁴ barr). The crude product was analysed by gas-chromatography/mass-spectrometry (GC-MS) analysis after treatment with *N,N*-bis-trimethylsilyl trifluoroacetamide in pyridine (620 mL) at 60°C for 4 h in the presence of betulinic acid [3β-hydroxy-20(29)-

lupaene-oic acid] as an internal standard (3.0 mg). Mass spectrometry was performed with a Shimadzu QP5050A GC-MS, using the column WCOT fused silica (film thickness, 0.25 μm ; stationary phase, VF-5 ms; Li, 0.25 mm; length, 30 m) and the following program: injection temperature 280°C, detector temperature 280°C, gradient 100°C \times 2min, then 10°C/min for 60 min. The abundance of peaks is reported in parentheses in the relevant Tables. In order to identify the chemical structure of main reaction products, two strategies were followed. First, the spectra of identifiable peaks were compared with commercially available electron mass spectrum libraries such as NIST (Fison, Manchester, UK). Secondly, GC-MS analysis was repeated using standard compounds. In order to evaluate the possible role of the organic constituent of the Murchison powder in the condensation of formamide, the reaction was repeated under similar experimental conditions with a pyrolyzed sample of the mineral.

RNA Oligomers

The degradation of ribonucleotides by water was studied by using the 24mer 5'-AAAAAAAAAAAAAAAAAAAAAAAAA-3' (PolyA₂₄) purchased from Dharmacon Inc. (Chicago, IL, USA). PolyA₂₄ was selected because of its homogeneous sequence, allowing the verification of the regularity of the first-order kinetic degradation (Saladino et al. 2006a,b; Ciciriello et al. 2008).

RNA Preparation and 5' labelling PolyA₂₄ RNA was labeled with [γ -³²P]ATP using polynucleotide kinase (Roche Applied Science). The oligo was then purified on a 16% denaturing acrylamide gel (19:1 acrylamide/bisacrylamide). After elution, the residual polyacrylamide was removed using a NuncTrap Probe purification column (Stratagene). Then, 2 pmol (typically 30,000 cpm) RNA were processed for each sample.

The RNA Stability Assay

The 5'-labeled oligonucleotide was treated under the time, temperature, pH and solution conditions indicated in the figure and table legends. A typical assay consisted of 5.0 μL of 400 nM RNA, 10 μL of mineral (suspension of 1 mg/ml of ground material) in water, brought to a final volume of 75 μL with water. To stop the reaction a solution of 5×10^{-4} M (final concentration) of tetrasodium pyrophosphate (Sigma) dissolved in water, pH 7.5, was added. This addition desorbed the oligomers. The reaction was stopped by the following steps: the samples were vortexed for 1 min, then centrifuged at 13000 rpm for 20 min (Haereus Biofuge).

This latter procedure was performed twice. The supernatant was ethanol precipitated, resuspended in 5.0 μL of loading solution (90% formamide, 10% H₂O containing xylen cyanol and bronophenol blue), heated for 2 min at 65°C, and loaded on a 16% denaturing polyacrylamide gel (19:1 acrylamide/bisacrylamide). For further details, see (Saladino et al. 2008). No labelled RNA remained associated with the Murchison mineral powder, as tested by Cerenkof measurement.

The half-life of the oligonucleotide was determined with standard graphical procedure from plots of the percent disappearance of the intact 24mer molecules. Cerenkof measurement allowed to verify the full recovery by desorption of the 2 pmol of the RNA used in each assay. Given that the disappearance of one full-length molecule is caused by one cleavage, the half-life of the single 3'-phosphoester bonds in the ribooligonucleotide

can be obtained from the half-life of the oligonucleotide $\times 23$ (that is: the number of 3'-5' phosphodiester bonds in the 24-mer). This procedure is described in detail in Ciciriello et al. 2008.

The Murchison Sample and RNA Stability Assay

The ground meteorite powder was suspended in water at a concentration of 1 mg/ml. A 10 mL aliquot of the vortexed solution was immediately taken and added to the RNA sample and the assay was run as indicated. The amount of Murchison powder to be added was selected on the basis of previous experience with other minerals (Saladino et al. 2008). In the previous studies 1 mg/mL of the minerals under analysis (iron sulphur minerals) quickly degraded RNA. Similar results were obtained in the analysis of the effect of RNA stability in the presence of a large panel of borate minerals (Cossetti et al. 2010). Thus, at this concentration the mineral is in excess, as also shown by the fact that a 10-fold increase or decrease did not change the results (data not shown).

Results

Synthesis of Heterocycles

The condensation of formamide alone yielded purine **2** as the only recovered product (ca. 34.0 mg per gram of formamide). In the presence of the Murchison mineral, a number of additional compounds were produced (Fig. 1), including nucleic acid bases and other heterocycles **3–7** and **9** (Scheme 1, Table 1), isocyanate **8**, carboxylic acids **10–13**, *N*-formyl glycine **14** and one of the chemical precursor of sugars during the formose condensation, dihydroxy acetone (DHA) **15** (Scheme 2, Table 2, Table 3).

Among the nucleic acid bases, adenine **3**, uracil **4** and isocytosine [2-aminopyrimidin-4(3*H*)-one, iC] **5** were synthesized in comparable yield (Table 1, entries 2–4), while 4(3*H*)-pyrimidinone **6** was the most abundant heterocycle derivative (Table 1, entry 5). It is noteworthy that the reaction performed in the presence of the pyrolyzed Murchison sample (Fig. 2) produced the same set of nucleic acid bases and heterocycles (Table 1, entries 9–13).

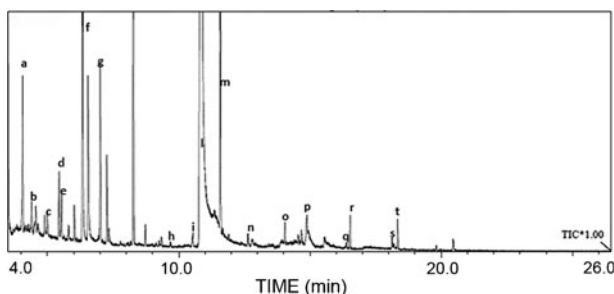
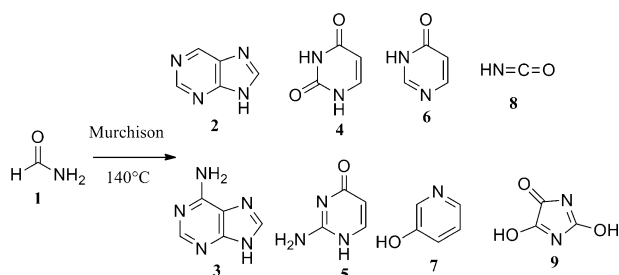


Fig. 1 GC-MS chromatogram of the condensation of formamide in the presence of unpyrolyzed Murchison. Column WCOT fused silica (film thickness, 0.25 μm ; stationary phase, VF-5 ms; Li, 0.25 mm; length, 30 m). GC program: injection temperature 280°C, detector temperature 280°C, gradient 100°C \times 2min, then 10°C/min for 60 min. Selected peaks: **a**: isocyanate; **b**: 3-hydroxypyridine; **c**: lactic acid; **d**: oxalic acid; **e**: *N*-formylglycine; **f**: 4(3*H*)-pyrimidinone; **g**: dihydroxyacetone; **h**: parabanic acid; **i**: uracil; **j**: purine; **k**: malic acid; **l**: oxaloacetic acid; **m**: adenine; **n**: tetradecanoic acid; **o**: hexadecanoic acid; **p**: oleic acid; **q**: octadecanoic acid

Scheme 1 Synthesis of heterocycles from formamide

Among carboxylic acids, oxalic acid **13** was synthesized in the highest yield, followed by oxaloacetic acid **12**, malic acid **11** and lactic acid **10** (Table 2, entries 1–4). *N*-formylglycine **14** and DHA **15** were obtained in yield comparable to that of carboxylic acids (Table 2, entries 5–6). Again, the selectivity and efficiency of the reaction performed with the pyrolyzed Murchison sample was similar to that previously obtained with the parent mineral (Table 2, entries 2–6 versus entries 8–12).

We note that when the condensation products of formamide in the presence of unpyrolyzed Murchison powder were analyzed, traces of tetradecanoic acid, hexadecanoic acid, oleic acid and octadecanoic acid were also detected in the mixture (Fig. 1). Since these compounds were not observed in the presence of the pyrolyzed sample, they were probably in the parent mineral sample, most likely as contaminants since these fatty acids are abundant components of biological oils such as triglycerides.

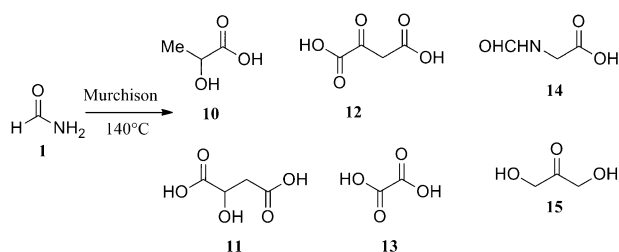
RNA Stability in the Presence of Murchison

The major problem in the origin of informational polymers is the instability of their precursors, and the stability of the polymers themselves once formed. Therefore we also investigated the effect of Murchison minerals on RNA stability. Figure 3 Panel A shows the kinetics of hydrolysis of PolyA₂₄ ribonucleotides in water pH 7.3 at 80°C.

Table 1 Synthesis of nucleobases and heterocyclic derivatives

Entry	Product	catalyst	Yield ^a
1	Purine (2)	Murchison	27.0±0.1
2	Adenine (3)	Murchison	0.019±0.002
3	Uracil (4)	Murchison	0.014±0.002
4	Isocytosine (5)	Murchison	0.034±0.003
5	4(3 H)-pyrimidinone (6)	Murchison	0.115±0.003
6	3-hydroxypyridine (7)	Murchison	0.014±0.002
7	Isocyanate (8)	Murchison	0.003±0.001
8	Parabanic acid (9)	Murchison	0.009±0.001
9	Purine (2)	Pyrolyzed Murchison	28.0±0.1
10	Adenine (3)	Pyrolyzed Murchison	0.016±0.002
11	Uracil (4)	Pyrolyzed Murchison	0.009±0.001
12	Isocytosine (5)	Pyrolyzed Murchison	0.025±0.002
13	4(3 H)-pyrimidinone (6)	Pyrolyzed Murchison	0.089±0.002

^a The yield is defined as mg of product per gram of starting formamide.

Scheme 2 Synthesis of carboxylic acid and sugar precursor from formamide

The pattern of degradation indicates that the 24mer was randomly broken by hydrolytic events along its entire length, as shown by the appearance of ladder-like degradation profiles. After about 10 h of residence time in water, faster degradation of the RNA started. This behaviour was reported and discussed in detail (Ciciriello et al. 2008). The stability of PolyA₂₄ was then analyzed in the presence of both untreated and pyrolyzed Murchison material, as a function of pH in the range 4.5–9.2. The results are reported in Figs. 3 and 4. Figure 3 panel B shows, as an example, the kinetics of PolyA₂₄ degradation at pH 9.2. Figure 4 plots all of the data collected in the set of analyses over the pH range of 4.2 to 9.3.

Discussion

Reaction Mechanism

Murchison minerals are a complex mixture of microscopic silicate particles ranging from sub-micron single crystals to aggregates tens of microns in size. Very little metallic iron is present (0.03 % by weight). The mineral components are primarily classified as pyroxene and olivine (Fuchs et al. 1973). The olivine has a Mg:Fe ratio ranging from 40 to 85 (Fa to Fo in mineralogical terminology) and the red color of the powder following aerobic pyrolysis is presumably due to iron oxides produced by reaction of the iron content with oxygen. The catalytic effect reported here is most likely due to trace amounts of iron and

Table 2 Biological carboxylic acids, amino acids and sugars

Entry	Product	Catalyst	Yield ^a
1	Lactic acid (10)	Murchison	0.003±0.001
2	Malic acid (11)	Murchison	0.004±0.001
3	Oxaloacetic acid (12)	Murchison	0.009±0.001
4	Oxalic acid (13)	Murchison	0.049±0.002
5	<i>N</i> -Formylglycine (14)	Murchison	0.006±0.001
6	Dihydroxy acetone (15)	Murchison	0.003±0.001
7	Lactic acid (10)	Pyrolyzed Murchison	0.002±0.001
8	Malic acid (11)	Pyrolyzed Murchison	0.003±0.001
9	Oxaloacetic acid (12)	Pyrolyzed Murchison	0.006±0.001
10	Oxalic acid (13)	Pyrolyzed Murchison	0.033±0.002
11	<i>N</i> -Formylglycine (14)	Pyrolyzed Murchison	0.003±0.001
12	Dihydroxy acetone (15)	Pyrolyzed Murchison	0.003±0.001

^a The yield is defined as mg of product per gram of starting formamide.

Table 3 Selected mass Gas-Chromatography Mass-Spectroscopy data (GC-MS) of condensation products **2-15**^[a]

Products	<i>m/z</i> (%)
Purine (2) ^[b]	192 (79) [M], 177 (100) [M-CH ₃], 120 (10) [M-Si(CH ₃) ₃]
Adenine (3) ^[c]	279 (27) [M], 264 (100) [M-CH ₃], 249 (1) [M-(CH ₃) ₂], 192 (17) [M-Si(CH ₃) ₃]
Uracil (4) ^[c]	256 (35) [M], 241 (100) [M-CH ₃], 225 (15) [M-(CH ₃) ₂], 182 (7) [M-Si(CH ₃) ₃], 142 (70), 113 (55)
Isocytosine (5) ^[d]	327 (18) [M], 312 (100) [M-CH ₃], 282 (9) [M-(CH ₃) ₃], 255 (6) [M-H-Si(CH ₃) ₃], 240 (7) [M-H-Si(CH ₃) ₃ -CH ₃], 182 (2) [M-2Si(CH ₃) ₃]
4(3 H)pyrimidinone (6) ^[b]	168 (25) [M], 153 (100) [M-CH ₃], 123 (3) [M-(CH ₃) ₃]
3-Hydroxy pyridine (7)	167 (10) [M], 152 (100) [M-CH ₃], 137 (6) [M-(CH ₃) ₂], 122 [M-(CH ₃) ₃]
Isocyanate (8) ^[b]	115 (79) [M], 110 (100) [M-CH ₃], 87 (10) [M-CO]
Parabanic acid (9) ^[c]	258 (15) [M], 243 (35) [M-CH ₃], 215 [M-2xCH ₃]
Lactic acid (10) ^[c]	219 (6) [M-CH ₃], 190 (14) [M-CO ₂], 147 (71) [M-Si(CH ₃) ₃ -CH ₃], 133 (7), 117 (76) [M-Si(CH ₃) ₃ -(CH ₃) ₃].
Malic acid (11) ^[c]	278 (65) [M], 206 (100) [M- Si(CH ₃) ₃], 191 (23) [M-Si(CH ₃) ₃ -CH ₃], 162 (35) [M- Si(CH ₃) ₃ -CO ₂]
Oxaloacetic acid (12) ^[c]	276 (54) [M], 204 (100) [M- Si(CH ₃) ₃], 189 (31) [M-Si(CH ₃) ₃ -CH ₃], 160 (33) [M- Si(CH ₃) ₃ -CO ₂]
Oxalic acid (13) ^[c]	219 (3) [M-CH ₃], 189 (5) [M-(CH ₃) ₃], 147 (78) [M-Si(CH ₃) ₃ -CH ₃], 117 (10) [M-Si(CH ₃) ₃ -3CH ₃], 73 (100) [M-2Si(CH ₃) ₃ -O]
<i>N</i> -formylglycine (14) ^[c]	246 (5) [M-H], 231 (7) [M-CH ₄], 174 (12) [M-H-Si(CH ₃) ₃], 129 (11) [M-H-Si(CH ₃) ₃ -(CH ₃) ₃], 101 (11) [M-H-2xSi(CH ₃) ₃], 73 (100) [M-H-2xSi(CH ₃) ₃ -CO]
Dihydroxy acetone (15)	68 (100) [M], 53 (45) [M-CH ₃]

^a Mass spectroscopy was performed by using a GC-MS QP5050A. Samples were analyzed after treatment with *N,N*-bis-trimethylsilyltrifluoroacetamide and pyridine. The abundance of peak is reported in parenthesis.

^b Product detected as the monosilyl derivative; ^c Product detected as the bis-silyl derivative; ^d Product detected as the tris-silyl derivative

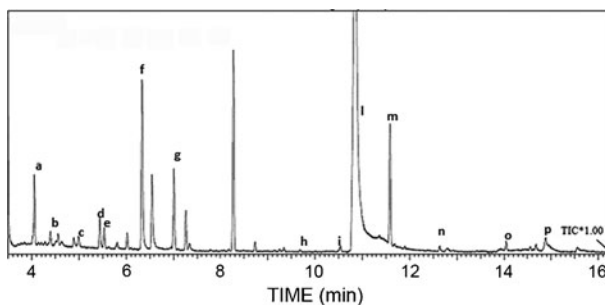


Fig. 2 GC-MS chromatogram of the condensation of formamide in the presence of pyrolyzed Murchison. Column WCOT fused silica (film thickness, 0.25 μ m; stationary phase, VF-5 ms; Li, 0.25 mm; length, 30 m). GC program: injection temperature 280°C, detector temperature 280°C, gradient 100°C \times 2 min, then 10°C/min for 60 min. Selected peaks: **a**. isocyanate; **b**. 3-hydroxypyridine; **c**. lactic acid; **d**. oxalic acid; **e**. *N*-formylglycine; **f**. 4(3 H)-pyrimidinone; **g**. dihydroxyacetone; **h**. parabanic acid; **i**. uracil; **l**. purine; **m**. isocytosine; **n**. malic acid; **o**. oxaloacetic acid; **p**. adenine

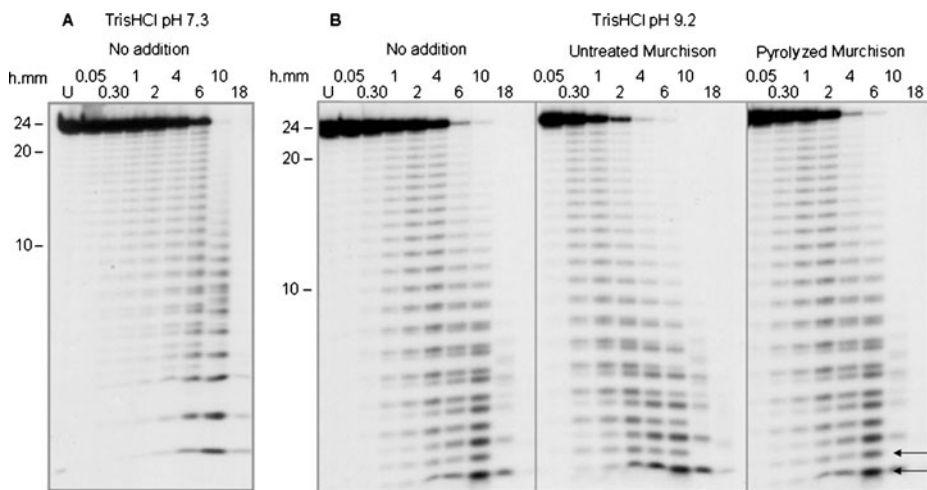


Fig. 3 The stability of RNA in the presence of ground Murchison material. **a.** degradation kinetics of polyA₂₄ as a function of time (hours, minutes: h:m) at 80°C in water at the indicated pH with no addition of Murchison material. 5'-labeled oligonucleotide and fragments were treated for the time indicated on the top of each lane and analyzed in 16% polyacrylamide gel electrophoresis. Numbers on the left side indicated the fragment length of the polyA (nucleotides). U: untreated. **b:** degradation kinetics of polyA₂₄ as a function of time at 80°C in water at pH 9.2, as shown on top of the gel, in the presence of untreated or pyrolyzed Murchison material

nickel oxides and sulfides, even though the effect of silicate itself cannot be completely ruled out (Saladino et al. 2001). Establishing which components of the mineral mixture are catalysts is beyond the scope of this study.

On the basis of the Eschenmoser hypothesis (Eschenmoser 2007) concerning the relationship between HCN and the constituents of the reductive citric acid cycle, we suggest that compounds **10–13** were produced by a common reaction pathway involving the hydrolysis of the nitrile group to form reactive intermediates, such as the HCN-dimer and the HCN-tetramer. *N*-formylglycine **14** was previously synthesized from formamide and mineral phosphates (Saladino et al. 2006a,b) probably through a Strecker condensation (Miller 1953) between HCN, formaldehyde and ammonia, possibly derived from formamide (Saladino et al. 2007), followed by formylation of the amino moiety by formamide (Aizpurua and Palomo 1983). Note that this latter reactive step is a simple model for the formation of the peptide bond.

The yields of the formamide reaction products are very small. The initial volume of formamide was 2.5 mL, and the highest yield was 27 mg of purine per gram of formamide. Apparently, the synthesis of purine was slightly inhibited in the presence of Murchison. Yields of other nucleobases and heterocyclic derivative products ranged from 9.0 to 115 micrograms, and carboxylic acid and amino acid yields were lower still, ranging from 3.0 to 49 micrograms. Since the yield of products was comparable in both Murchison samples, the catalytic activity in the condensation of formamide was mainly due to the inorganic constituent of the meteorite. However, a possible role of the organic component (mainly the kerogen-like polymer) cannot be completely ruled out because of the slightly lower yield of nucleic acid bases in the presence of the pyrolyzed sample (see for example, Table 1, entries 2–4 versus entries 10–12). The main point is that similar reactions and products were likely to proceed in the parent body of the Murchison meteorite during heating of the interior. The hot water would then transport the products to the surface of the parent body where they

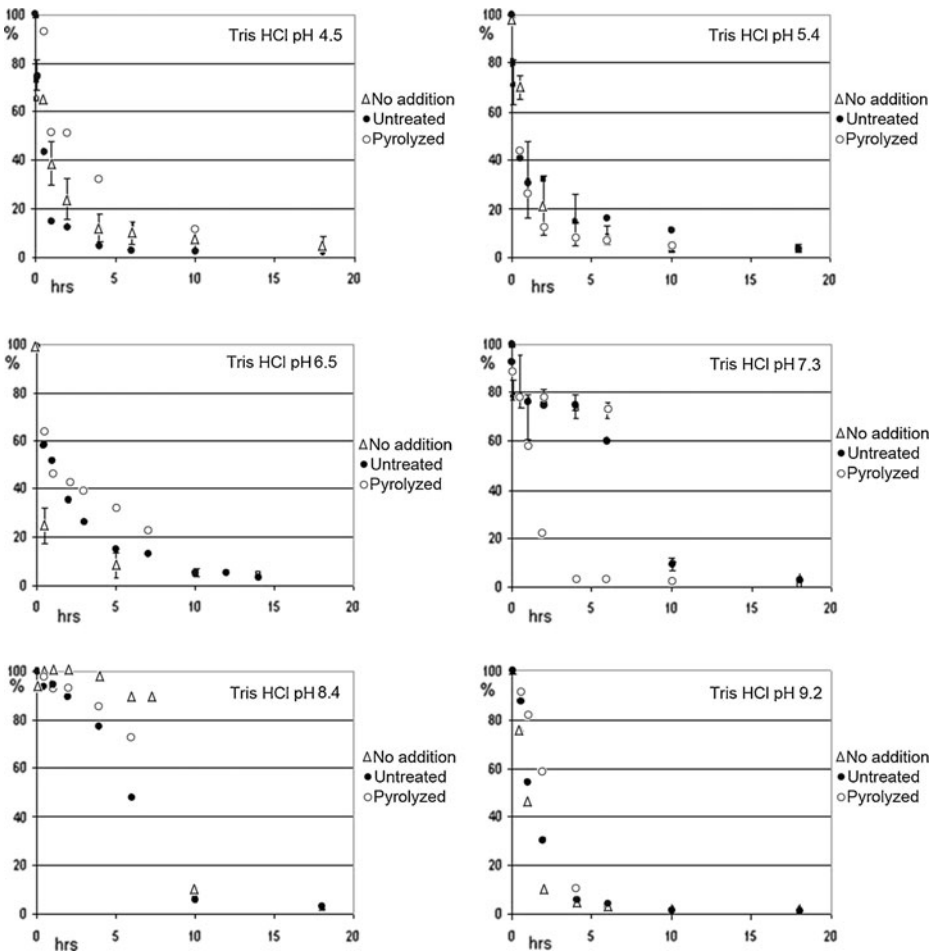


Fig. 4 Degradation of polyA₂₄ at different pH values, in the absence or presence of untreated or pyrolyzed Murchison, as indicated. The RNA molecules remaining intact at each time point (abscissa) are given as a percentage of the initial full-sized molecules. Some of the experimental points were performed in triplicate and relative *error bars* are reported

would accumulate, perhaps with some selective enrichments resulting from geochromatography (Wing and Bada, 1991; Larter et al. 2000).

Prebiological Significance of Formamide Products

Adenine and uracil are nucleic acid bases, while isocytosine is a structural isomer of cytosine that is able to recognize cytosine through a Watson-Crick hydrogen bonding pattern similar to that of the natural guanine/cytosine pair, thus possibly mimicking guanine in an alternative primordial genetic alphabet (Zhanpeisov and Leszczynski 1999). Moreover, compound **5** also recognizes guanine by a reversed Watson-Crick interaction, further enlarging the possibility of hydrogen bond interactions in alternative pre-RNA molecules (Gupta et al. 2004). In accordance with the reaction mechanism reported by Yamada et al. (1978) the synthesis of **3** and **5** from formamide probably requires the *in situ*

generation of hydrogen cyanide (HCN). The synthesis of 4(3*H*)-pyrimidinone **6** from formamide was previously reported in the presence of calcium carbonate, silica, alumina and clays (Saladino et al. 2005). Schmitt-Kopplin et al. (2010) using advanced mass spectral technique, were able to resolve tens of thousands of different organic compounds in the Murchison meteorite, including a significant fraction of nitrogen heterocyclics.

Although compound **6** was not specifically noted, its eventual presence as a minor heterocyclic component of the pyrimidine family can probably be explained as an artifact of the preparation procedure, as reported by Stoks and Schwartz (1981). The 3-hydroxy pyridine **7** and isocyanate **8** were not previously detected in formamide-based syntheses, while parabanic acid **9**, was previously obtained from formamide in the presence of different minerals (Saladino et al. 2004).

The biological carboxylic acids lactic acid **10**, malic acid **11** and oxaloacetic acid **12**, are intermediates in the reductive version of the citric acid cycle (Morowitz et al. 2000). The reductive citric acid cycle has been proposed as an autocatalytic pathway for the reductive carbon assimilation from carbon oxides in complex organic structures in primordial metabolism (Morowitz et al., 2000; Smith and Morowitz, 2004). This variant of the conventional (oxidative) citric acid cycle, a key metabolic process in modern biology, is known to function in certain bacteria (Holms 1987).

Hydrolytic Degradation of RNA in the Prebiotic Environment

It is uncertain whether RNA as we know it today was synthesized abiotically and then incorporated into RNA-based life. On the face of it, this scenario seems unlikely, because biological RNA incorporates D-ribose and uses 3'-5' phosphodiester bonds exclusively. It is more plausible that an earlier version of RNA-like polymers did not require homochirality and specific 3'-5' linkages. Although a plausible prebiotic RNA-like polymer has not yet been discovered, it does seem reasonable that ester bonds were the primary link that formed between monomers, so using synthetic RNA as a model system to study stability is justified.

The hydrolysis of RNA has been extensively investigated (Perreault and Anslyn 1997; Soukup and Breaker 1999a and b; Saladino et al. 2006a,b; Ciciriello et al. 2008; Saladino et al. 2008). RNA degradation in formamide has also been described (Saladino et al. 2008, 2009 and references therein). The cleavage of the phosphoester chain in water normally requires participation of the 2'-OH group as an internal nucleophile (Morrow et al. 1995) in two "nucleophilic cleavage" events: the transesterification and hydrolysis reactions. During transesterification, the 2'-OH nucleophile attacks the tetrahedral phosphorus to afford a 2',3'-cyclic monophosphate. This species is then hydrolyzed into a mixture of 2'- and 3'-phosphate monoesters. Both steps are generally catalyzed by protons and hydroxide (depending on the pH), nitrogen groups and metal ions (Saladino et al. 2006a,b). The two molecular species (2',3'-cyclic phosphate and 2'- or 3'-monophosphate) can be separated in analytical electrophoretic gels (i.e., see the lower part the gel runs in Fig. 1), as indicated in Fig. 1 by the two arrows, upper and lower, respectively. The information provided by this assay pertains to: (i) the stability of the 3' phosphoester bond, the weakest bond in RNA both in water (Saladino et al. 2008); (ii) the rate of 2',3'-cyclic phosphate ring opening.

The relatively high stability of the PolyA sequence in aqueous solution is well established (Smith and Allen 1953; Lane and Butler 1959; Kaukinen et al. 2002). The stability of RNA phosphodiester bonds has repeatedly been associated with the stacking interactions between adjacent bases (Kierzek 1992; Li and Breaker 1999; Bibillo et al. 1999). The possibility that the initial stability of the PolyA oligonucleotide towards

hydrolysis is a base stacking-related effect was discussed in Saladino et al. (2007). Kinetic details are in Ciciriello et al. 2008.

RNA synthesis in simulated prebiotic conditions has been described in reports from several laboratories. For instance, Ferris and co-workers have shown that RNA up to 50mers is synthesized when activated ribonucleotides adsorb to Montmorillonite clay mineral surfaces (see Ferris, 2006 for review). Rajamani et al. (2008) demonstrated that non-activated mononucleotides polymerize into oligonucleotides in the range of 20–100mers when exposed to cycles of hydrated and anhydrous conditions in the presence of organizing lipid matrices. Costanzo et al. (2009) found that cyclic nucleotides could undergo a polymerization reaction at neutral pH ranges and elevated temperatures.

Conclusions

The results of this study support two conclusions related to prebiotic synthesis and degradation reactions:

1. The surfaces of the minerals that compose the Murchison meteorite can catalyze the synthesis of a number of compounds from formamide that may be relevant to the origin of life. The catalytic activity resides in the actual minerals, and not in the kerogen-like polymer that is the most abundant organic material present. The yields are very small, micrograms per gram of formamide. On the other hand, the soluble organic compounds in carbonaceous meteorites are also detected in the parts per million range. Assuming that significant amounts of formamide were present in the parent body of the Murchison, the catalyzed reaction we described here represents a plausible pathway to account for the synthesis of biomolecules, including nucleic acid components.
2. The synthesis of nucleobases from formamide would not be significant unless the bases could be incorporated into polymers that had a useful lifetime in prebiotic conditions. For instance, we reported earlier that both iron sulfur and borate minerals actively promote RNA degradation (Saladino et al. 2008, Cossetti et al. 2010) in physical-chemical conditions comparable to those that promoted synthesis of RNA precursors. However, we observed little or no catalytic effect of Murchison minerals in promoting hydrolysis of phosphodiester bonds of RNA. At low and high pH ranges, the half-time of hydrolysis is an hour or less under the conditions used in this study, but significantly slower at intermediate pH ranges, with half-times of 5–10 h.

These results are a useful guide for possible synthesis of RNA-like polymers in an RNA World scenario. It is clear that RNA must be generated at a rate sufficient to overcome the back reaction of hydrolysis, otherwise RNA would be unable to accumulate in quantities sufficient to support the origin of RNA-based life. From the results reported here and in earlier studies, if ester-linked polymers of mononucleotides can be synthesized on time scales of minutes to hours, they could accumulate at a rate sufficient to overcome hydrolysis reactions, even at elevated temperatures between 80°C and 90°C. Another possibility is that the RNA of primitive microorganisms may have been protected from hydrolysis in some way, as in thermophilic microorganisms today, which maintain functional RNA for many hours at elevated temperatures. Future research efforts should be directed toward discovering mechanisms by which RNA-like polymers can not only be synthesized, but also protected from hydrolytic degradation in simulated prebiotic conditions.

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