Prebiotic reactions in a Mars analog iron mineral system: Effects of nitrate, nitrite, and ammonia on amino acid formation

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Iron minerals are highly reactive drivers of abiotic/prebiotic organic chemistry, and in the presence of ammonia (NH3/NH4+) or other reduced nitrogen (N) compounds, have been shown to promote amino acid synthesis from organic precursors. On early Mars, oxidized nitrogen species (NOx/C0) such as NO3− and/or NO2− may have been present, which could be reduced by Fe(II) to form various species including N2O and/or NH3/NH4+. The production of NH3/NH4+ from Fe(II)-driven NO3− or NO2− reduction may be able to feed into prebiotic organic reactions including amino acid formation. In this study, we tested whether iron mineral-driven reduction of NOx/C0 could provide a source of NH3/NH4+ to form amino acids from two prebiotically relevant precursors (pyruvate and glyoxylate); or, whether an exogenous source of NH3/NH4+ would be required. We observed that pyruvate and glyoxylate reacted with Fe-oxyhydroxide minerals in NOx/C0-containing experiments to form reduced hydroxy acid products; and in experiments containing only NH3/NH4+, amino acids were also formed. However, significant amino acid formation was not observed in any experiments containing NO3− or NO2− unless sufficient NH4+ was also added; furthermore, colorimetric analysis did not show any generation of NH4+ from NO3− or NO2− reduction at these conditions. NO3− was observed to be highly reactive with Fe2+ and Fe(II)-bearing minerals, resulting in Fe oxidation during mineral precipitation and the formation of oxidized mineral phases (hematite). The Fe(II)/Fe(III) ratio in oxyhydroxide minerals is an important parameter for determining organic product distributions from pyruvate and glyoxylate; therefore, Fe-mediated NOx− reduction does impact organic chemistry. However, amino acid formation, at least under these conditions, would also require an exogenous source of NH3/NH4+ or other reduced N species. These results have implications for organic-N chemistry on early Mars, as well as for some early Earth origin of life scenarios regarding organic synthesis in mineral-containing systems.

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1. Introduction

The detection and characterization of organic molecules is a high priority goal in the search for habitable environments and signs of ancient life on Mars (e.g. Grotzinger et al., 2012; Williford et al., 2018). Recently, NASA’s Mars Science Laboratory’s Curiosity rover discovered chlorohydrocarbons and organo-sulfur compounds in 3-billion-year-old mudstones at Gale crater with the Sample Analysis at Mars (SAM) instrument (Freissinet et al., 2015; Eigenbrode et al., 2018; Szopa et al., 2020). In order to assess the astrobiological importance of Martian organics detected by current or future missions, it is important to understand processes by which organics can be generated and transformed abiotically in reactive mineral systems and what sorts of organic distributions these might exhibit. Additionally, the impact of organic molecules on mineral precipitation in redox-active systems could play a role in governing surface compositions.

Mars may host different geochemical redox cycles from what we observe in terrestrial environments. Nitrogen (N) cycling, in particular, is not well understood in a Martian context (Capone et al., 2006; Laneuville et al., 2018). Data from SAM show nitrates (NO3−) and other oxidized nitrogen (NOx−) species are present in aeolian materials and lacustrine mudstones at Gale crater.
etal., 2015; Stern et al., 2017). SAM data do not show the presence of reduced nitrogen species (e.g., NH$_3$/NH$_4^+$), although they would be difficult to detect with this instrument and are also easily destroyed photochemically, making it unlikely they would be preserved unless incorporated into minerals (Stern et al., 2015). A variety of reactive minerals are present in the mudstones where the nitrates and organic matter were detected, including iron oxyhydroxides, iron phyllosilicates, and iron sulfides (Vaniman et al., 2014; Rampe et al., 2017). Some of these minerals, especially those containing Fe(II), are known to be capable of driving redox reactions of organics and N species. For example, iron silicates, sulfides, and oxyhydroxides can drive nitrate (NO$_3^-$) or nitrite (NO$_2^-$) reduction (e.g. Postma, 1990; Summers, 2005; Gordon et al., 2013; Hansen et al., 1996; Zhang et al., 2021); and iron sulfides and oxyhydroxides have been shown to drive formation of amino acids from α-ketoacids and NH$_3$/NH$_4^+$ (Barge et al., 2020, 2019; Huber and Wächtershäuser, 2003; Novikov and Copley, 2013).

It is not fully understood how the reaction conditions in Martian aqueous systems could have varied over Mars’ history; including parameters such as pH, iron redox state, chemical concentrations, temperature, and successive fluid alterations. Any or all of these conditions could have had an effect on abiotic organic chemistry driven by reactive iron minerals and N species. In planetary environments containing reactive Fe(I)/Fe(II) or Fe(II/III) minerals, it is possible that NO$_3^-$ and/or NO$_2^-$ could be reduced to NH$_3$/NH$_4^+$, thus providing a reduced N source for abiotic/prebiotic organic reactions (Summers and Lerner, 1998; Nishizawa et al., 2021; Summers, 2005). For example, glyoxylate and pyruvate – two simple carboxylic acids that are of interest for prebiotic chemistry – can react with NH$_4$Cl and Fe$^{2+}$ or Fe minerals to form the amino acids glycine and alanine (Barge et al., 2019, 2020; Huber and Wächtershäuser, 2003; Novikov and Copley, 2013). This is because Fe(II) is a strong reductant and can drive reductive amination of α-ketoacids, even using various N sources (Muchowska et al., 2019; Huber and Wächtershäuser, 2003). In a putative Mars surface or subsurface system containing reduced and oxidized Fe, NO$_3^-$ and/or NO$_2^-$, and organic precursors, one might therefore expect an abiotic N/Fe/organic redox cycle to proceed, that – under certain conditions – might be capable of generating prebiotically relevant compounds such as amino acids or other N-bearing organics. Similar conditions could also have been relevant for prebiotic chemistry on the early Earth. For example, one aspect of the alkaline hydrothermal vent emergence of life theory (Russell and Hall, 2006) is the proposal that NO$_3^-$/NO$_2^-$ could be reduced to NH$_3$/NH$_4^+$ in hydrothermal Fe-hydroxide precipitates, and thus drive alanine formation from pyruvate (Russell and Nitschke, 2017; Russell, 2018; Barge et al., 2019).

In this work, we tested 1) whether Fe-mediated NO$_3^-$/NO$_2^-$ reduction would have any effect on reactions of pyruvate and glyoxylic acid; and 2) whether NH$_3$/NH$_4^+$ for amino acid formation in this mineral reaction system could be sourced from Fe(II)-driven NO$_3^-$/NO$_2^-$ reduction or whether NH$_3$/NH$_4^+$ would need to be supplied by an exogenous source. We conducted experiments to test reactions of pyruvate and glyoxylic acid in Mars analog mineral systems with Fe(II)-containing oxyhydroxide minerals and NO$_3^-$, NO$_2^-$, and/or NH$_3$/NH$_4^+$. These specific organic molecules have not been detected on Mars, but represent simple organic precursors that can be formed abiotically in geological settings (Cody et al., 2000; Eckhardt et al., 2019; Egglins et al., 1988); they are also organic compounds of interest for biology and prebiotic chemistry since they are highly relied upon in metabolism. We hypothesized that amino acids could form from pyruvate and glyoxylic acid if NH$_3$/NH$_4^+$ were present, via reductive amination which is a well-known reaction in prebiotic chemistry (Barge et al., 2019, 2020; Hafenbradl et al., 1995; Huber and Wächtershäuser, 2003; Muchowska et al., 2017, 2019, 2020; Novikov and Copley, 2013).

Our experiments tested whether this general amino acid formation mechanism would be expected to proceed in a NO$_3^-$/NO$_2^-$-containing Fe-oxyhydroxide mineral environment in a Mars setting; the signs of which might be detected by future missions.

2. Materials and methods

The experiments carried out contained N species (NO$_3^-$, NO$_2^-$, or NH$_3$/NH$_4^+$), freshly precipitated Fe-hydroxide minerals, and organic substrates (pyruvate or glyoxylic acid). All reagents were mixed in aqueous suspension and then heated at 70 °C for time periods ranging from 3 to 21 days. We tested several variations of the reaction conditions including: varying the ratio of Fe(II)/Fe(III) in the mineral precipitate; order of addition of the NO$_3^-$ or NO$_2^-$ and precipitation of the iron; and inclusion of NH$_3$/NH$_4^+$ in addition to NO$_3^-$ or NO$_2^-$. Detailed experimental conditions are presented below.

2.1. Experimental conditions

All reactions were carried out in a N$_2$-filled glove box to recreate anoxic conditions postulated for the early Mars atmosphere and Martian subsurface (Catling and Moore, 2003; King et al., 2004; King and McSween, 2005). All solutions were prepared with Milli-Q water (18.2 MΩ-cm) that had also been sparged for ~30 min per 100 mL water with N$_2$ or Ar gas to remove dissolved oxygen. The Fe-hydroxide minerals were prepared using FeCl$_2$-4H$_2$O and FeCl$_3$·6H$_2$O (Fisher Scientific) where the total Fe concentration in each serum bottle was 50 mM. The relative amount of Fe(II) and Fe(III) was either 100% Fe(II) or 50% Fe(II). After 50 mL solutions of Fe-containing salts were made, 5 mL of a 1 M solution of NaOH was added to precipitate with the iron to synthesize an iron oxyhydroxide mineral.

NO$_3^-$, NO$_2^-$, and/or NH$_3$/NH$_4^+$ were introduced into the reaction by addition of NaNO$_3$, NaNO$_2$, or NH$_4$Cl (Fisher Scientific). NaNO$_3$ or NaNO$_2$ stock solutions were prepared and added to the mineral for a total concentration of 25 or 50 mM and NH$_4$Cl stock solution was added for varying concentrations from 5 to 375 mM. For this study, the reactant concentrations of N species were designed to keep high N:Fe ratios in the experiment, and to facilitate ease of detection of N species, iron oxidation, and organic products to observe reactions occurring; in a real early Mars environment the NO$_3^-$ concentrations would likely be much lower (Adams et al., 2021).

Sodium pyruvate (J.T. Baker) or glyoxylic acid monohydrate (Alfa Aesar) stock solutions were added for a total concentration of 2.5 mM in the final solution. The order of chemical addition was varied in order to test the effect on the organic reactions: in some experiments the NaNO$_3$ or NaNO$_2$ and Fe-chloride salts were dissolved in solution together and then the NaOH was added to precipitate the Fe mineral; and in other experiments, the Fe(II) and Fe(III) was precipitated with NaOH before the NaNO$_3$/NaNO$_2$ was added. Each solution was titrated to pH = 10 using 1 and 10 M aq. NaOH and 1 and 2.5 M aq. HCl. Each experiment was placed in a water bath set to 70 °C for up to three weeks and sampled at various time points. A complete list of experimental conditions is found in Table SI-1.

2.2. Analysis

$^1$H NMR Spectroscopy: Aliquots (1 mL, triplicates) were taken at each time point (1 = 30 min, 3 or 4 days, 1 week, 3 weeks) while agitating the bottles to ensure an even distribution of liquid and solid in each sample. 0.5 mL of 1 M NaOH was added to each aliquot to precipitate the iron from solution (since paramagnetic iron...
broadens the NMR peaks. Samples were centrifuged and the supernatant was transferred to a new tube. In some cases, a fourth sample was taken and spiked with some of our expected products to verify the presence/absence of the amino acid and α-hydroxy acid products. To prepare organic samples for NMR analysis, 0.54 mL of each sample was pipetted into an NMR tube along with 60 µL of DSS/D2O standard. 1H NMR spectra were acquired on a 400 MHz Bruker spectrometer equipped with an auto sampler. The spectra were processed with MestReNova NMR analysis software.

14N NMR: Samples were prepared in an identical manner to the 1H NMR samples. 14N spectra at 28.91 MHz were acquired on a 400 MHz spectrometer. 24,000 scans were acquired with an acquisition time of 0.05 s and a delay between scans of 0.01 s. Authentic samples of the N compounds were used as positive controls.

Colorimetry analysis: Additional samples were taken for iron, nitrate, and ammonium colorimetry. Iron colorimetry analysis was conducted to determine how the iron mineral oxidized in the presence of N species. To dissolve the solid, 2.5 M HCl was added to three 1 mL samples and an aliquot from each was analyzed using a method from Aguirre et al. (2021) where the total assay volume was adjusted to 3.3 mL. Additional aliquots from the dissolved samples were used for nitrate and ammonium colorimetry. A Superior Enzymes nitrate test kit (NEC: Nitrate Elimination Company, SKU: NTK-TSSR-100) was used to determine concentrations of NO3− and NO2−: a Supelco ammonium test kit (Millipore Sigma, CN: 100683) was used to measure the concentration of NH4+. Samples were analyzed with a Thermo Fisher GENESYS 30 Visible spectrophotometer. See the Supplementary Material for a more detailed description of colorimetry analyses and results.

X-ray diffraction (XRD): For XRD analysis, the supernatant solution was first decanted from the experiment vials, and the remaining solution with the mineral precipitate was transferred to 15 mL falcon tubes and placed in a freeze dryer (SP Scientific VirTis Advantage XL-70). Dried samples were stored in a dry glove box. A Kapton film covered the samples, sealed with a sealing ring. A single crystal silicon backing was used. XRD measurements were taken using a benchtop Aeris system with copper anode (Malvern Panalytical). In order to minimize the influence of noise from the Kapton film and from iron fluorescence, a divergence slit of 0.1146° was used and pulse height distribution lower limit was set to 7.2 keV.

Liquid chromatography-mass spectroscopy: The identity of organic products was verified by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF/MS). Reaction samples were stored at room temperature for short term storage, and at ~20 °C for long term storage. The samples were mixed by vortex agitation for ~1 min to ensure proper mixing. All samples were analyzed on an Agilent 6545 Q-TOF coupled to an Agilent 1260 Infinity II HPLC fitted with an Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm column. The mobile phases for both modes were 10 mM ammonium acetate adjusted to pH 9. For analysis in ESI negative mode, 300 µL of sample was transferred to an HPLC vial and 0.2 µL was injected. All samples were run 5 times. The identified compounds are summarized in Table SI-4. For analysis in ESI positive mode, 10 µL of sample was diluted to 100 µL with high purity water in low volume HPLC vial inserts and 1 µL was injected. The samples were analyzed using MassHunter Quantitative Analysis Workflows for peak extraction and were identified by matching to retention time and accurate mass of known standards.

3. Results

Mineral precipitation and iron oxidation: NO3− and NO2− were reactive in experiments containing dissolved Fe2+ and/or Fe(II)-bearing hydroxide minerals, as evidenced by iron colorimetry results. For NO3−, we observed that the majority of Fe(II) (in either a 50% or 100% Fe(II) mineral experiment) oxidized to Fe(III) within ~30 min; NO2− experiments also showed some Fe(II) oxidation (Fig. 1). After several days, Fe minerals in NO3− experiments exhibited a dark black color and in NO2− experiments exhibited a brown/orange color (Fig. SI-1). XRD patterns of dried mineral precipitates from NO2− experiments containing pyruvate, at 100 % Fe (II) and 50 % Fe(II), correspond well to hematite, Fe2O3 (RRUFF ID: R110013.9; Lafuente et al., 2015) (Fig. SI-31). In experiments where the NO3− species was added to the solution containing dissolved Fe before the NaOH was added to precipitate the mineral, we observed effervescence upon addition of the NO3− species (see Supplementary Material).

pH variation: pH was monitored throughout the reaction. We observed that although all experiments were initially titrated to pH ~ 10, the pH sometimes changed throughout the course of the reaction (they were not further titrated to maintain a pH of 10). For experiments containing NO3−, the pH measured at t = 3 weeks was ~7.0–7.6; however, for reactions containing NO2−, the pH at t = 3 weeks was pH ~ 9.5–10.5. (This was true regardless of the starting % Fe(II).) A full time series of pH measurements for all conditions is presented in Table SI-2.

N species analysis: 14N NMR analysis of Fe-hydroxide mineral experiments containing 50% Fe(II), 2.5 mM pyruvate, and NO3− or NO2− showed only the N species that was originally added and no additional products (Fig. SI-6 – SI-8). This was confirmed by colorimetry as well at t = 0 and t = 1 week: in experiments with NO3− and 100% or 50% Fe(II), colorimetric analysis only observed NO3− and not NO2− or NH3/NH4+ (Fig. SI-26); NO3− experiments only showed NO3− (Fig. SI-27); and NH4-only experiments showed only the NH4+ that was added (no NO2− or NO3−) (Fig. SI-28). In 25 mM NO2− experiments that also contained 50 mM NH4Cl, the NH4+/NH4+, and NO2− were both detected via colorimetry (Fig. SI-25).

Analysis of organic reactivity: We conducted pyruvate and glycolate experiments with NaNO3, NaNO2, or NaH4Cl alone; as well as some mixed N source experiments in which NaH4Cl was added into an experiment containing NaNO2, in order to represent addition of exogenous NH3/NH4+. A high NH3/NH4+ concentration (375 mM) was used in NaH4Cl-only experiments to be consistent with previous experiments of this type (Barge et al., 2019); though lower, more geologically realistic NH3/NH4+ concentrations can also produce amino acids under these conditions (Barge et al., 2020). As we have observed in previous work, the major products detected by 1H NMR spectroscopy in reactions with 375 mM NaH4Cl were glycine and glycolate (in glyoxylate experiments) and alanine and lactate (in pyruvate experiments) (Figs. SI-13, SI-14). Other organic products not detectable by 1H NMR spectroscopy were likely also present in the system, and the total distribution of organics produced was previously observed to depend on the pH and mineral iron redox state (Barge et al., 2019, 2020). With LC-QTOF/MS, we looked for nitrogen-bearing organics (such as 2-nitroacetic acid) and we did not observe formation of those products (LC-QTOF/MS data are reported in the Supplementary Material).

In 50 mM NO3− or NO2− experiments with glyoxylate or pyruvate, the α-hydroxy acid products glycolate and lactate were observed, respectively, which is indicative of Fe(II)-driven organic reduction (Figs. 2 & 3). However, no amino acids (glycine or alanine) were confirmed in experiments containing only NO3− or
NO\textsubscript{3}^-. We also conducted a test where we added progressively increasing amounts of NH\textsubscript{4}Cl (5–25 mM) to 50 mM NO\textsubscript{2}-experiments with glyoxylate, inspired by similar [NH\textsubscript{4}Cl] conditions under which we previously observed amino acid formation (Barge et al., 2020). However, even with addition of NH\textsubscript{4}Cl, amino acids were not observed in NO\textsubscript{2}- containing glyoxylate experiments until 50 mM NH\textsubscript{4}Cl was added to a 25 mM NO\textsubscript{2}-experiment (at which condition, some glycine was observed) (Fig. 4).

4. Discussion

We hypothesized that the reducing power of Fe(II) in these experiments would lead to reduction of pyruvate and glyoxylate to form α-hydroxy acids, as well as reductive amination in the presence of NH\textsubscript{3}/NH\textsubscript{4}+ to form amino acids; all of which has been observed in our previous studies (Barge et al., 2019, 2020). One issue of interest here was whether amino acid formation in this Fe-oxo-hydroxide mineral system would require exogenous NH\textsubscript{3}/NH\textsubscript{4}+, or whether Fe(II)-driven reduction of NO\textsubscript{x}- in the presence of the organic substrate could produce the amino acids in a one-pot reaction. It has been shown in previous studies that NH\textsubscript{3}/NH\textsubscript{4}+ can be generated from NO\textsubscript{3}− or NO\textsubscript{2}−reduction under geologically realistic conditions involving Fe(II) or Fe minerals (Smirnov et al., 2008; Summers and Chang, 1993; Hansen et al., 1996; Nishizawa et al., 2021; Summers 2005); it has also been shown that glycine can form from Fe(II)-driven reduction of NO\textsubscript{2} to NH\textsubscript{3}/NH\textsubscript{4}+ (Summers and Lerner, 1998; though this was via Strecker synthesis rather than reductive amination). However, in our experiments we did not observe significant amino acids (alanine from pyruvate, or glycine from glyoxylate) in any of our NO\textsubscript{x}− only experiments (see Table 1); and colorimetry also did not detect any NH\textsubscript{4}+ being formed. Exogenous NH\textsubscript{3}/NH\textsubscript{4}+−, represented by the addition of NH\textsubscript{4}Cl to a NO\textsubscript{2}−-containing glyoxylate experiment, was required in order to produce significant glycine in this Fe-oxo-hydroxide mineral system.

This discrepancy with previous work may partially be explained by the varying redox potentials of Fe(II)/Fe(III)-minerals and N species as a function of pH and experimental conditions. The redox potential of Fe\textsuperscript{2+}/Fe\textsuperscript{3+} is +0.77 V in acidic solution, but in alkaline solution (where our Fe-mineral precipitation occurs) the redox potential of Fe-bearing minerals can span a wide range down to <−0.5 V (Table SI-5), depending on mineral structure, stoichiometry, and/or Fe(II):Fe(III) ratio (Bird et al., 2011; Génin et al., 2001, 2006; Ilbert and Bonnefoy, 2013; Ruby et al., 2006). The minerals in our experiments could have been a combination of various Fe-oxo-hydroxides (including hematite, magnetite, lepidocrocite, ferrihydrite, and/or green rust) depending on Fe(II):Fe(total). As seen in Table SI-5, many of these minerals at pH 10 would have negative redox potentials sufficient to reduce pyruvate to lactate (E\textsuperscript{0} = −0.190 V) or glyoxylate to glycolate (E\textsuperscript{0} = −0.092 V) (Génin et al. 2001; Ruby et al. 2006; Bird et al. 2011; Thauer et al. 1977; Dellero et al. 2015). From Figs. 2 and 3 we can see that lactate and glycolate formed under all conditions tested, but more lactate and glycolate were formed at higher Fe(II)/Fe(total). This makes sense given the relative reactant concentrations in these experiments: [Fe]:[NO\textsubscript{x}/C\textsubscript{0}] was either 2:1 or 1:1, whereas [Fe]:[organics] was 20:1; thus, even though much of the Fe(II) present had been oxidized by the NO\textsubscript{x}− species, there was still enough Fe(II) to reduce the relatively small amount of organics that were present. NO\textsubscript{3}- or NO\textsubscript{2}− reduction potential is pH dependent (Génin et al. 2001; Ruby et al. 2006; Fanning 2000) but our observed Fe(II) → Fe(III) oxidation in the presence of NO\textsubscript{3}− or NO\textsubscript{2}− is consistent with expectations based on redox potentials. Even though there are differing reports in the literature of the potential for specific N redox reactions (NO\textsubscript{3}−/NO\textsubscript{2}−; NO\textsubscript{3}−/NH\textsubscript{4}+; NO\textsubscript{2}−/NO), they are more positive than many of the Fe(II)/Fe(III) minerals in Table SI-5. Even though various Fe minerals should have sufficient potentials for NO\textsubscript{3}−/NO\textsubscript{2}− reduction to NH\textsubscript{4}+, the selective formation of NH\textsubscript{4}+ over other products (such as N\textsubscript{2} or NO) is also highly dependent on the properties of the reductant/catalyst as well as the experimental conditions (e.g. Lu et al. 2021; Wu et al. 2021; Raikshit et al. 2021).
et al. 2005; Hirakawa et al. 2017). It is possible that the specific mineral phases formed in this study favored reduction to other N products instead of accumulation of NH$_3$/NH$_4^+$, but that other types of minerals might give a different result.

Even despite the lack of mineral-driven NH$_3$/NH$_4^+$ synthesis, we observed that the presence of NO$_x^-$ species affected the organic chemical reactions in this mineral system due to the impacts of NO$_x^-$ (particularly NO$_2^-$) on the redox state of Fe. In all NO$_2^-$ experiments, most of the Fe(II) initially added was oxidized even by the t = 30 min iron colorimetry time point (Fig. 1), resulting in an oxidized mineral phase. We have previously shown that the Fe(II)/Fe(III) ratio in iron hydroxide minerals is an important parameter for determining their reactivity with pyruvate or glyoxylate; in particular, our previous work did not show any alanine formation from pyruvate with a mostly oxidized Fe mineral; though some glycine could still form from glyoxylate at oxidized Fe-hydroxide conditions (Barge et al., 2019, 2020). Depending on the order of addition of reactants in these experiments (N source added before vs. after the Fe-hydroxide was precipitated), the mineral-bound Fe(II) available for reduction of pyruvate (or glyoxylate) to form lactate (or glycolate) could vary, depending on how much NO$_2^-$ driven Fe(II) oxidation had already occurred. Our observed formation of hematite in NO$_2^-$ experiments (Fig. SI-31) is consistent with expected hematite precipitation directly from solution at similar experimental conditions (low temperature and controlled addition of a base to ferric chloride solution; Dutrizac and Riveros, 1999).

We measured significant Fe(II) oxidation in experiments containing NO$_2^-$, and somewhat in experiments containing NO$_3^-$ and NO$_2^-$ reduction before the mineral was formed (Fanning, 2000; Nguyen et al., 2003), depleting the NH$_3$/NH$_4^+$ concentration. Reductive amination is thought to proceed through an imine intermediate (Mayer et al., 2021), and so, if N species reacted even before organics were added, that could prevent imine formation and thus amino acid formation.

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Even accounting for the observation that Fe oxidation by NO$_2^-$ can impact reactions of pyruvate and glyoxylate in this system, it is still curious that glycine did not form from glyoxylate with the addition of 5 mM or 25 mM NH$_4$Cl, both of which are [NH$_4$Cl] conditions where we have previously observed glycine formation (Barge et al., 2020). NH$_3$/NH$_4^+$/NO$_3^-$/NO$_2^-$ are all highly reactive and can undergo interconversions to form gas-phase products e.g. N$_2$, N$_2$O, NO (Doane, 2017 and references therein). It is also possible that NH$_3$/NH$_4^+$ could have acted as a reductant and driven NO$_2^-$ reduction before the mineral was formed (Fanning, 2000; Nguyen et al., 2003), depleting the NH$_3$/NH$_4^+$ concentration. Reductive amination is thought to proceed through an imine intermediate (Mayer et al., 2021), and so, if N species reacted even before organics were added, that could prevent imine formation and thus amino acid formation.

We measured significant Fe(II) oxidation in experiments containing NO$_2^-$, and somewhat in experiments containing NO$_3^-$. As our goal was to investigate the organic reactions, not the N redox chemistry, we did not focus on identifying all of the reduced N-bearing products of NO$_3^-$ or NO$_2^-$ or the reaction stoichiometry, beyond the absence/presence of NH$_4^+$. However, we did observe
evidence of gas-phase N product formation. In both NO₃⁻ and NO₂⁻ experiments where the NOₓ⁻ species was added to the solution containing dissolved Fe²⁺ (before the NaOH was added to precipitate the mineral), we observed effervescence upon addition of the NOₓ⁻ species (see Research Data); therefore, these gaseous products are likely an effect of N redox reactions with Fe²⁺ which do not necessarily require a solid mineral precipitate in order to proceed (Jones et al. 2015). Various gas-phase products of NO₃⁻ or NO₂⁻ reduction with Fe²⁺ or Fe minerals can be abiotically produced in geological systems, including N₂, NO, and N₂O (Summers and Chang, 1993; Hansen et al., 1994, 1996; Buchwald et al., 2016; Samarkin et al., 2010; Postma, 1990; Doane, 2017; Zhu-Barker et al. 2015; Stanton et al., 2018). The Fe²⁺- or Fe-mineral-driven reduction of NO₃⁻ or NO₂⁻ can produce various iron minerals and reduced N products (Table SI-5), and can have varying Fe²⁺/NOₓ⁻/H⁺ stoichiometries, depending on the reaction conditions. Some possible reactions for Fe²⁺-driven NO₂⁻ reduction in our experiments include (Buchwald et al., 2016; Ranjan et al., 2019):

\[ 2\text{NO}_2^- + 4\text{Fe}^{2+} + 5\text{H}_2\text{O} \rightarrow \text{N}_2\text{O} (\text{g}) + 4\text{Fe}^{3+} + 6\text{H}^+ \]

\[ \text{NO}_3^- + 3\text{Fe}^{2+} + 4\text{H}_2\text{O} \rightarrow \text{N}_2\text{(g)} + 3\text{FeOOH} + 5\text{H}^+ \]

And some possible reactions for NO₃⁻ reduction include (Postma, 1990; Samarkin et al., 2010; Ranjan et al., 2019; Ottley et al., 1997):

\[ 2\text{NO}_3^- + 12\text{Fe}^{2+} + 11\text{H}_2\text{O} \rightarrow \text{N}_2\text{O} (\text{g}) + 4\text{Fe}_3\text{O}_4 + 22\text{H}^+ \]

\[ 2\text{NO}_3^- + 10\text{Fe}^{2+} + 14\text{H}_2\text{O} \rightarrow \text{N}_2\text{(g)} + 10\text{FeOOH} + 18\text{H}^+ \]

\[ \text{NO}_3^- + 12\text{Fe}^{2+} + 13\text{H}_2\text{O} \rightarrow \text{NH}_4^+ + 4\text{Fe}_3\text{O}_4 + 22\text{H}^+ \]

\[ 2\text{NO}_3^- + 15\text{Fe}^{2+} + 13\text{H}_2\text{O} \rightarrow \text{N}_2\text{(g)} + 5\text{Fe}_3\text{O}_4 + 28\text{H}^+ \]

\[ \text{NO}_3^- + 12\text{Fe(OH)}^+ + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + 4\text{Fe}_3\text{O}_4 + 10\text{H}^+ \]

\[ \text{NO}_3^- + \text{Fe(OH)}^+ + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + 8\text{FeOOH} + 6\text{H}^+ \]

A previous study found that NO₂⁻ reduction rates with Fe(II) (at pH 7) were similar when solid Fe-hydroxide mineral was added at the beginning compared to an initially homogenous reaction, because Fe-hydroxides precipitate rapidly either way under these conditions (Jones et al. 2015). Therefore, our “NOₓ⁻ added pre-precipitation” or “NOₓ⁻ added post-precipitation” conditions (Table SI-1) may not have been that different, since minerals would have precipitated in the first minutes of all experiments. However, NO₂⁻ reduction by Fe²⁺ has been observed to depend on relative concentrations of Fe and NO₂⁻, with faster NO₂⁻ reduction when [Fe²⁺] > [NO₂⁻] (Ranjan et al. 2019; Jones et al. 2015); Fe(II)-dependent NO₂⁻ reduction also increases with pH (Sørensen and Thorling, 1991). The rates of oxidation of Fe²⁺ by NO₃⁻ vs NO₂⁻...
in our experiments remain an open question. However, the Fe(II)/Fe(III) ratios we observed shortly after all reactants had been added and the Fe-hydroxide mineral had formed (Fig. 1) suggest that: 1) NO$_3^-$ reduction may have occurred at least to some extent at room temperature (as evidenced by the formation of bubbles, and the oxidation of Fe(II)); 2) NO$_2^-$ reduction occurred at a faster rate than NO$_3^-$ reduction under these conditions, as evidenced by the much greater oxidation of Fe(II) in the same time frame. Our observation that the NO$_3^-$ experiments ended up at a lower pH (6.47–7.56) than the NO$_2^-$ experiments (9.14–10.48) after 3 days (even though both were titrated to the same initial pH of ~10; Table SI-2) would also be consistent with the redox reactions above where NO$_3^-$ reduction by Fe$^{2+}$ releases more H$^+$ than NO$_2^-$ reduction.

4.1. Implications for Mars

Together, these experiments demonstrate how Fe and N redox reactions can produce a variety of conditions that can affect abiotic organic chemistry in a Mars analog iron mineral system (Table 1). This could be relevant to conditions on Mars today that might be accessible to in situ missions, as well as to interpreting processes in Mars’ geologic past.

Ancient Mars would have had iron-rich fluids of Fe$^{2+}$ and/or Fe$^{3+}$ with a variety of oxidation states depending on conditions. Groundwater and surface water under anoxic atmospheric conditions would have contained Fe$^{2+}$ dissolved from primary mafic minerals (Burns, 1993; Walker and Brimblecombe, 1985). In some very low pH environments, such as acid-saline conditions interpreted from orbital data to have been present in Terra Sirenum (Wray et al., 2011; Ehlmann et al., 2016), Fe$^{3+}$ may have also been available in solution. Little is known about nitrogen cycling on Mars, but various N species could have existed throughout its history; including NO$_x$ species produced from energetic events in a N$_2$-containing atmosphere that could dissolve in water to form NO$_3^-$/NO$_2^-$ (Adams et al., 2021; Hu and Díaz, 2019; Mancinelli and McKay, 1988; Navarro-González et al., 1998, 2019; Summers and Khare, 2007; Wong et al., 2017; Yung and McElroy, 1979); or, N$_2$O or NH$_3$/NH$_4^+$ produced from N$_2$/NO$_3^-$/NO$_2^-$ reduction by Fe$^{2+}$ and/or Fe minerals (Hansen et al., 1996; Sørensen and...
Organics on Mars could be derived from a variety of non-biological sources such as meteorites (e.g., Cobb and Pavlovitz, 2014; Callahan et al., 2011), hydrothermal/volcanic synthesis (e.g., Huber and Waechtershauer, 1997, 2006) and/or CO/CO₂ reduction on reactive minerals (Steele et al., 2018; Roldan et al., 2015; Kitadai et al., 2018; Yamaguchi et al., 2014), and could undergo subsequent mineral-catalyzed reaction networks even possibly leading toward prebiotic chemistry (e.g., Muchowska et al., 2017, 2019, 2020). NO₂⁻ reacts quickly with Fe²⁺ or Fe(II) minerals, so its residence time in Martian iron-bearing fluids may be limited (Ranjan et al., 2019). However, even if unstable, NO₂⁻ could still be relevant for organic chemistry in these systems. NO₂⁻ could indirectly affect organic chemistry via 1) oxidation of reactive Fe(II), which would affect the potential for Fe-bearing minerals to drive organic reduction; 2) production or consumption of NH₃/NH₄⁺ that can react with organics. Even if NO₂⁻ is quickly consumed and undetectable in an aqueous reservoir, it could still have impacted a system like the one we simulated here.

The pH and other geochemical conditions that determine relative solubility of Fe²⁺ and mineral-bound Fe(II) would likely affect the N redox chemistry since iron minerals are likely to be a stronger reductant than Fe²⁺ in solution (Sorensen and Tholring, 1991; Grabb et al., 2017). NO₃⁻ would be more stable than NO₂⁻ and able to persist in solution for a longer period of time (Dhakal et al., 2013; Ranjan et al., 2019). The Fe-bearing minerals eventually deposited from solutions containing Fe²⁺, Fe³⁺, NO₂⁻, and/or NO₃⁻ would be a function of the Fe²⁺/Fe³⁺ ratio, temperature, and pH. Several abiotic mechanisms have been proposed to form oxidized secondary minerals on Mars, including changes in pH or the oxidation of Fe²⁺ by small amounts of free oxygen, photooxidation, chlorate, or hydrogen peroxide (Burns, 1993; Schaefer, 1996; Hurowitz et al., 2010; Mitra and Catalano, 2019; Mitra et al., 2020).

In this study we observed that the presence of NO₂⁻ in particular affects Fe oxidation and precipitation, and so NO₂⁻ could be considered as another potential mechanism for abiotic Fe oxidation on Mars. Notably, at Gale crater, nitrates were detected in lacustrine mudstones that also contained a variety of ferric and mixed valence secondary phases which included magnetite, hematite, and ferric phyllosilicates (Stern et al., 2015; Stern et al., 2017; Rampe et al., 2017). However, additional work is needed to understand if NO₃⁻ or NO₂⁻ driven oxidation would be dominant or comparable to previously proposed processes given comparative oxidation rates and abundances of NO₃⁻ species generated over time.

Finally, Fe-driven N redox chemistry can drive abiotic organic reactions in mineral systems, and these conditions should be further studied in order to interpret future organic or N detections on Mars. Here we observed that the organic products produced from pyruvate and glyoxylate are affected by the presence of NO₃⁻ /NO₂⁻ /NH₃/NH₄⁺: it is also possible that in more complicated systems, more complex and/or additional N-bearing organic molecules could be synthesized. N-bearing organics were recently detected in a 4-billion-year-old Martian meteorite (Roik et al., 2020); these may have been delivered meteorically on ancient Mars, or they could have formed in-situ from various precursors (including perhaps amines, N-heterocycles, and/or nitriles) originating from abiotic N redox chemistry. In a Mars surface environment, radiation would also be a factor. We did not include the effects of radiation in these experiments, but both pyruvate and glyoxylate are known to be photochemically reactive and this could affect the resulting organic reaction networks. For example, UV irradiation of pyruvate in aqueous solution or on mineral surfaces can produce acetate and lactate as well as various longer oligomers (Griffith et al., 2013a,b; Alves et al., 2019) and UV irradiation of glyoxylate can produce lactate or oxalate depending on experimental conditions (Guzman and Martin, 2010; Ekstrom and McQuillan, 1999). It remains for future experimental studies to investigate how NO₃⁻ species in redox-active iron mineral systems drive formation of specific organic molecules, and how the resulting Fe-bearing precipitates preserve or further alter these organic molecules under early Mars surface conditions.

4.2. Implications for prebiotic chemistry on early Earth

Our results are also relevant to prebiotic early Earth environments, particularly to Fe-mineral rich systems where organics may be synthesized. Alkaline hydrothermal vents are one such example; these environments have been proposed as `hatcheries` for life due to the combined pH/redox/ion gradients that would have existed across Fe-hydroxide-rich precipitates in hydrothermal chimneys and mounds on the early Earth (Russell and Hall 2006; Russell et al., 2014). The interface between mildly acidic early Earth oceans containing Fe²⁺/Fe³⁺ (from hydrothermal systems) and NO₃⁻/NO₂⁻ (produced from atmospheric photochemistry) (Wong et al., 2017; Shibuya et al., 2016; MacLeod et al., 2019, 2020); these may have been delivered meteoritically on ancient Mars, or they could have formed in-situ from various precursors (including perhaps amines, N-heterocycles, and/or nitriles) originating from abiotic N redox chemistry. In a Mars surface environment, radiation would also be a factor. We did not include the effects of radiation in these experiments, but both pyruvate and glyoxylate are known to be photochemically reactive and this could affect the resulting organic reaction networks. For example, UV irradiation of pyruvate in aqueous solution or on mineral surfaces can produce acetate and lactate as well as various longer oligomers (Griffith et al., 2013a,b; Alves et al., 2019) and UV irradiation of glyoxylate can produce lactate or oxalate depending on experimental conditions (Guzman and Martin, 2010; Ekstrom and McQuillan, 1999). It remains for future experimental studies to investigate how NO₃⁻ species in redox-active iron mineral systems drive formation of specific organic molecules, and how the resulting Fe-bearing precipitates preserve or further alter these organic molecules under early Mars surface conditions.
5. Conclusions

We set out to explore the reactivity of glyoxylate and pyruvate with ferrous/ferric iron hydroxides and oxidized N species NO$_3^-$ and NO$_2^-$, and in particular, to investigate under what conditions amino acids can be formed. We observed that pyruvate and glyoxylate reacted in NO$_3^-$-containing systems to form reduced α-hydroxy acid products (from the Fe(II) as reductant); and, in experiments containing only NH$_4$Cl, also formed amino acids. Amino acid formation was not observed in experiments containing only NO$_3^-$ or NO$_2^-$; instead, glycine formation was only observed upon addition of 50 mM NH$_4$Cl to a NO$_3^-$ experiment (and not with lower [NH$_4$Cl] values). However, NO$_2^-$ was observed to be highly reactive with Fe$^{2+}$ in solution and with Fe(II)-bearing minerals, resulting in Fe oxidation during mineral precipitation and eventually forming oxidized Fe mineral phases. Despite various indicators of NO$_2^-$/NO$_3^-$ reduction in these experiments, we did not observe any evidence for NH$_3$/NH$_4^+$ formation. We conclude that, at least in this geochemical system containing Fe(II,III)-oxyhydroxides and NO$_3^-$ or NO$_2^-$ at neutral to alkaline pH, amino acid formation from these α-keto acid precursors would either require a source of exogenous NH$_3$/NH$_4^+$ or a different geological reactant/catalyst to reduce NO$_3^-$ to NH$_3$/NH$_4^+$. However, the presence of NO$_3^-$ or NO$_2^-$ with Fe(II)-bearing oxyhydroxide minerals can still affect organic chemistry in these systems through Fe(II) oxidation, which affects the reactivity of Fe-oxyhydroxide minerals with these organic precursors and the distribution pattern of organic products that are produced (Barge et al., 2020). Further study is needed to identify the specific N redox reactions under these conditions, and to determine whether other organic products (including N-bearing organics) could be formed under Mars-relevant conditions.

Research data

The data from this study are available in the supporting information (XRD, colorimetry, representative NMR spectra) and in the Open Science Framework. Research Data associated with this article can be accessed at https://osf.io/hxcc7/.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The research data is published in the linked repository.

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