

EFFECTS OF NITRATE, NITRITE, AND AMMONIA ON AMINO ACID FORMATION IN MARS-ANALOG IRON MINERAL SYSTEMS. L. M. Barge¹, J. M. Weber¹, E. Flores¹, A. A. Fraeman¹, E. Martinez¹, Y. L. Yung², M. M. Baum³. ¹NASA Jet Propulsion Laboratory, California Institute of Technology (laura.m.barge@jpl.nasa.gov); ²California Institute of Technology; ³Oak Crest Institute of Science.

Introduction: The Curiosity rover has detected organic matter in 3-billion year old mudstones at Gale crater perhaps containing carboxyl groups, N-bearing groups, and organic-sulfur compounds [1,2]. Nitrates / nitrites have also been detected on the Martian surface [3,4]. A variety of reactive minerals are present in the vicinity of where the organic matter was detected, including iron oxides and oxyhydroxides, phyllosilicates, and trace amounts of iron sulfides [5,6]. Some of these iron minerals are reactive and are known to be capable of driving nitrate / nitrite reduction to produce species such as ammonia ($\text{NH}_3/\text{NH}_4^+$) [7,8], which can react with simple organics (e.g. carboxylic acids such as pyruvate) to abiotically produce amino acids [9]. The Martian surface and near-subsurface might therefore provide a reactive setting to drive abiotic organic reaction networks that produce products similar to those found in biological metabolic cycles [9,10]. Here, we report experiments simulating the abiotic organic reactions that could occur in Mars analog iron oxyhydroxide mineral systems in the presence of various nitrogen species (nitrate, nitrite, and ammonia) [12].

Methods: All reactions were carried out in a nitrogen-filled glove box, and all solutions were prepared with ultrapure water that had also been purged with nitrogen or argon gas to remove any dissolved oxygen. The iron hydroxide minerals were prepared using $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ with 50 mM total iron concentration in each vial. The amount of Fe(II) and Fe(III) varied depending on the desired mole fraction of Fe(II):Fe(III). Here we tested 100% Fe(II) and Fe(II):Fe(III) = 1:1; in previous work this procedure produced magnetite [9] which is an analog for an iron phase that could be present at Gale crater [11]. Nitrate, nitrite, or ammonia were introduced into the reaction by addition of sodium nitrate, sodium nitrite, or ammonium chloride. The organic precursors were α -keto acids (glyoxylic acid and/or pyruvate); organic precursors were added at a total concentration of 2.5 mM, and sodium hydroxide (NaOH) was added to precipitate with the ($\text{Fe}^{2+} / \text{Fe}^{3+}$) as iron hydroxide minerals. Experiments were titrated to pH = 10, placed in a water bath at 70°C, and sampled at t=0, 24h, 48h, 72h. Reactions were monitored via ^1H NMR spectroscopy and iron colorimetry.

Results: Starting with these simple organic precursors (pyruvate and glyoxylate), the organic reaction pathways that occurred in our experiments depended on which nitrogen species was present - nitrate, nitrite,

or ammonia. In the presence of ammonia, both pyruvate and glyoxylate reacted with iron hydroxide minerals to form amino acids (alanine or glycine) and/or α -hydroxy acids (lactate or glycolate). In ammonia experiments, the iron mineral did not noticeably oxidize throughout the experiment. Using either nitrate or nitrite yielded identical results in glyoxylate reactions with iron hydroxide, forming only the α -hydroxy acid (glycolate), and no amino acid. However, in pyruvate

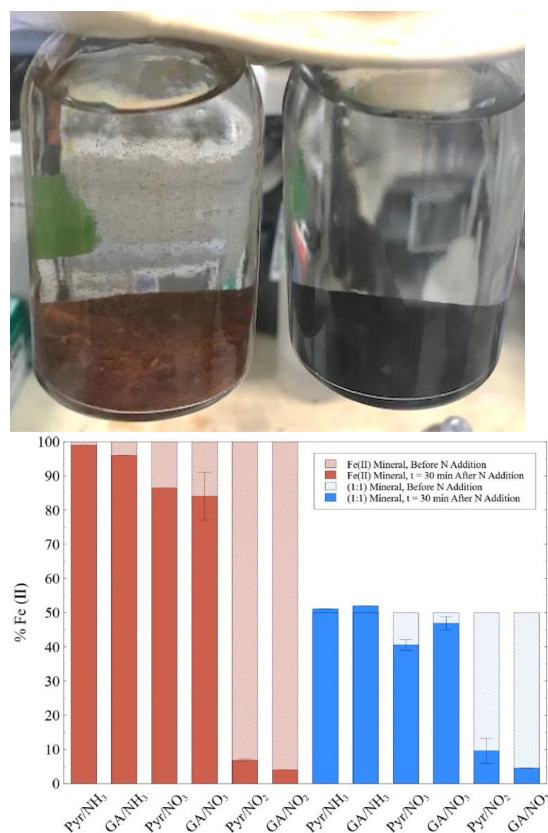


Figure 1: (Top L) Pyruvate reaction with nitrite and iron hydroxides (Fe(II):Fe(III) = 1:1) and (Top R) glyoxylate reaction with nitrate and iron hydroxides (Fe(II):Fe(III) = 1:1), after 72 hours. The color change in the nitrite-containing experiment indicates complete iron oxidation, as opposed to the nitrate-containing experiment where the mineral remains dark. %Fe(II) in the precipitates was measured via colorimetry and in all cases, nitrate experiments yielded ~60% iron oxidation and nitrite experiments yielded ~90-100% iron oxidation (data from pH 10 pyruvate experiments shown) [12].

experiments, nitrate vs. nitrite yielded distinct results. Nitrate led to lactate formation as expected, but nitrite led to two new ^1H NMR peaks that do not correspond to any previously observed product in these reactions [9]. Nitrate and nitrite also had a significant effect on the iron mineral oxidation state: in nitrate experiments, about 60% of the Fe(II) in the mineral was oxidized by the end of the experiment, and in nitrite experiments, almost all of the Fe(II) was oxidized at the end of the experiment (Figure 1). Amino acid formation was not observed in experiments containing only nitrate or nitrite; instead, glycine formation was only observed upon addition of 50 mM ammonia to a nitrite experiment (and not with lower ammonia concentrations) (Figure 2) [12].

Implications / Conclusions: Nitrate and nitrite can theoretically reduce to ammonia driven by iron minerals (or Fe(II)), and ammonia if present should react with pyruvate or glyoxylate to produce amino acids [9]. Though nitrate was observed to undergo redox reactions with the mineral-bound Fe(II) in our experiments, it did not create any nitrogen species that reacted with the organic precursors. Therefore we conclude that, at least for this geochemical system containing Fe(II,III)-oxyhydroxides and nitrate / nitrite at neutral to alkaline pH, amino acid formation from these organic precursors would either require a source of exogenous ammonia or a different geological reactant/catalyst to re-

duce NO_x^- to ammonia. However, the presence of nitrate / nitrite 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 [9]. More study is needed to understand the abiotic redox cycling of nitrogen and its reactions with organics in reactive iron mineral systems, and to better constrain the likely abundances of different nitrogen species in planetary environments.

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References: [1] Eigenbrode et al. (2018) *Science* 360, 1096–1101. [2] Freissinet et al., (2015) *JGR Planets*, 120, 3. [3] Stern et al (2015) *PNAS* 112: 4245–4250. [4] Navarro-González, et al. (2019) *JGR Planets*, 124, 94–113. [5] Vaniman et al. (2014) *Science* 343, 6169, 1243480. [6] Rampe et al., (2017) *EPSL*, 471. [7] Hansen et al. (1994) *GCA* 58:2599–2608. [8] Summers et al. (2012) *Astrobiology*, 12, 2. [9] Barge et al. (2019) *PNAS*, 116 (11) 4828–4833. [10] Muchowska et al. (2019) *Nature* 569, 104–107. [11] Tosca et al. (2018) *Nature Geoscience* 11, 635–639. [12] Barge et al. (2022) *GCA* 6, 469–479.

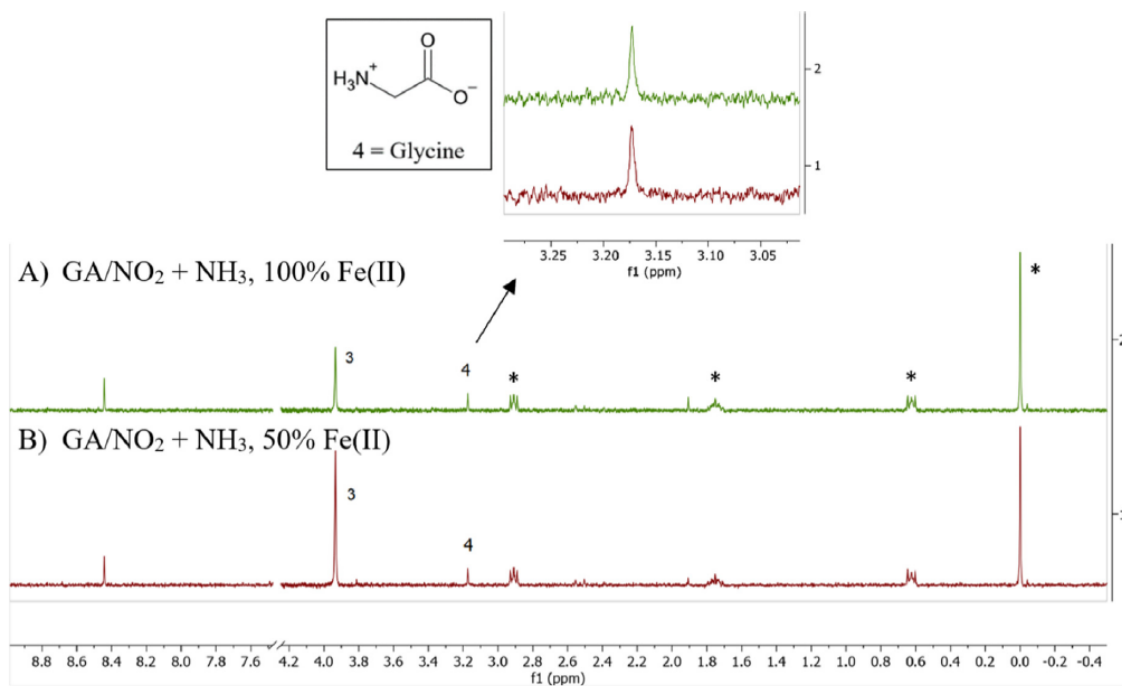


Figure 2: Glyoxylate reactions with 25 mM nitrite and 50 mM ammonia. ^1H NMR spectra of liquid supernatant indicating organic content from experiments containing Fe-hydroxide minerals (at indicated % Fe(II)). Inset shows glycine peak (4) at higher magnification. From [12].