

Mineral Interactions with Ester-Mediated Amide Bond Forming Systems: Prebiotic Dipeptides. Aaron D. McKee*¹, Christopher J. Bennett¹, Andrew Saydjari¹, Nicholas V. Hud¹, Ramanarayanan Krishnamurthy² and Thomas M. Orlando¹, ¹NSF/NASA Center for Chemical Evolution, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, ²NSF/NASA Center for Chemical Evolution, Department of Chemistry, The Scripps Research Institute, La Jolla, CA

Introduction: As a component of extant biology, peptide polymers have been the focus of many model prebiotic reactions and the presence of amino acids on extraterrestrial bodies and in simulation experiments suggests the plausibility of their existence on early Earth. As condensation of these monomers in aqueous solution is nonspontaneous, the use of drying cycles to drive off water and favor the formation of, in the case of amino acids, peptide linkages has been employed. For a prebiotic system, heterogeneity would likely come in the form of some mineral interacting with solvated monomers or the near-evaporated state of a puddle rich in those monomers. However, formation of functional length, linear peptides has been challenging in part due to the propensity of amino acid dimers to cyclize and become less reactive, rendering many mineral-including peptide formation experiments largely inconclusive. Recent work [1] has introduced α -hydroxy acids alongside α -amino acids, expanding the molecular inventory involved in the peptide problem to other molecules likely present on early Earth. By the exchange of an ester-linked hydroxy acid (HA) unit by an amino acid (AA), long mixed acid oligomers, or depsipeptides, have been produced via an ester-mediated peptide formation mechanism under simple wet/dry cycling conditions. Now, investigation of some potential effects on oligopeptide formation due to the presence of a mineral substrate have been investigated by employing this mixed acid system.

Here we demonstrate that mineral addition to an AA/HA system alters the compositional distribution of oligomers for all chain lengths, in that it affects the ratio of amino acid and hydroxy acid residues in the depsipeptides, relative to a mineral-absent control. While depsipeptides might be a crucial intermediate step in a plausible prebiotic route towards poly-amino acids, ester linkages are not present in proteins, so depsipeptides with decreased HA content and increased AA content are more desirable. Several minerals have been examined, with varying effects on oligomer component distribution, but the abundant mineral SiO₂ produces oligomers enriched with amino acids.

Experimental: The majority of experiments employed a single step dry-down with open glass vials incubated in an oven at 125°C for durations ranging 0.25–48 hours. SiO₂ (14 nm, 200 m²/g) was added at 100 mg/mL to 0.5 mL, 100 mM solutions of the 1:1 HA/AA mixtures for reaction, along with mineral-absent control reacted under otherwise identical condi-

tions. Samples were then rehydrated at room temperature and minerals were removed by centrifugation. Analysis by ESI-LC-MS and ¹H-NMR gave qualitative and quantitative information regarding the composition of the oligomers.

Results: From ESI mass spectra, the distribution of oligomer compositions has been derived. The composition distributions show that, compared to mineral absent experiments, silica containing samples produce AA enriched oligomers for all present chain lengths (<10 units). Furthermore, for the silica containing samples, the dominant species within a given chain length consistently contains only one HA, which is likely amide linked, so contains no ester linkages. When examining the composition as a function of time, it appears that the mineral absent samples produce HA and ester linkage rich oligomers, while silica containing samples do not ever contain higher levels of HA units. Additionally, trials without AA, producing only polyesters, revealed that esterification in the presence of silica did not proceed extensively while control trials saw the formation of many long chain poly lactic acid species.

From ¹H-NMR analysis of samples after reaction and after an aqueous ester hydrolysis procedure, corroborations of compositions and some sequence motifs were achieved. From the methyl region associated with poly-HA, mineral-absent samples had a large peak that reduced significantly with hydrolysis, yet silica samples did not show signal here. Additionally, signal from the AA methylene region associated with specifically the C-terminal unit was indifferent to hydrolysis for the silica containing sample, while it significantly grew for mineral-absent samples post-hydrolysis. Both these features point to the mineral-absent samples possessing larger quantities of HA that are located on the oligomer terminals, while silica containing samples allow esters to form to a much lesser degree.

Therefore, it is likely due to a reduced effective forward esterification rate, imparted to the system by the presence of SiO₂ via a general acid catalytic mechanism, that leads to a depsipeptide oligomer composition distribution that is more AA enriched. Monitoring composition of depsipeptides produced in the presence of other prebiotically relevant minerals and correlating those results with readily available mineralogical data, such as point of zero charge, might provide a method for predicting protopeptide behavior given a particular geological environment.

[1] J. G. Forsythe, et. al. (2015) *Angew. Chem. Int. Ed.*, 54, 9871–9875