A SEARCH FOR EXTRATERRESTRIAL PEPTIDES IN METEORITES. E. T. Parker[1], H. L. McLain, L. Chou[1], X. Li[1], H. Lin[2], R. Krishnamurthy[2], J. C. Aponte[1], J. E. Elsila[1], J. P. Dworkin[1], and D. P. Glavin[1], [1]Solar System Exploration Division, NASA Goddard Space Flight Center, Greenbelt, MD 20771, [2]Department of Chemistry, Scripps Research, La Jolla, CA 92037

Introduction: The exogenous delivery of organic compounds from comets, asteroids, and their fragments [1,2] to the primitive Earth may have been an important source of prebiotic molecules that led to the emergence of life. While the plausible inventory of amino acid monomers from extraterrestrial material has been widely investigated [3,4], much less is understood about the likelihood that amino acid chemical evolution products may have been delivered to the early Earth.

In 2002, Shimoyama and Ogasawara [5] reported the use of gas chromatography and mass spectrometry (GC-MS) to search for dipeptides and diketopiperazines in both the CM2 Murchison and CM2 Yamato 791198 meteorites. The dipeptide glycylglycine (gly-gly) and diketopiperazine cyclo(gly-gly) were identified at pmol/g levels by GC-MS in solvent extracts of both meteorites [5]. No other dipeptide or diketopiperazine were detected above the 1 pmol/g level [5]. These peptide detections have never been duplicated, making it unclear if such oligomers can be detected in meteorites using newer, more sensitive analytical equipment. In 2015, McGeoch and McGeoch [6] used a Folch extraction protocol, followed by matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF/MS) to analyze the Murchison and CV3 Allende meteorites for polymers, and reported the detection of polymer amide species (~1000-1200 Da) composed of glycine, alanine, and α-hydroxyglycine. However, upon analysis of the meteorite extract hydrolysates, the presence of the polymer amide monomers was not definitively confirmed [6], which offers an internal challenge to the veracity of the polymer amide detection. More recently, in 2022, McGeoch and McGeoch [7] stated that a 1494 Da hemoglycin polymer, an oligomer partially comprised of glycine and hydroxyglycine, was detected in four different CV3 meteorites, including Allende, following Folch extraction, and UV-Vis absorption and X-ray diffraction analyses. However, this reporting did not demonstrate that this polymer was absent from appropriate experimental blanks and controls, thus making it difficult to rule out terrestrial contamination as a potential source of the polymer.

As a result, questions remain regarding whether amino acid polymers may exist in extraterrestrial material, and if such species could have consequently been delivered to the primitive Earth at or near the time of the origin of life. In an effort to investigate these uncertainties, we have set out to verify the peptide detections reported by Shimoyama and Ogasawara [5] and McGeoch and McGeoch [6,7]. Here, we summarize the current status of these explorations.

Experimental: A 0.5 g sample of the Murchison meteorite (USNM 5453) was extracted in 2 mL of ultrapure water via ultrasonication for 1 hour at room temperature (23 °C). This extraction method was first optimized with standards to minimize peptide hydrolysis. A portion of the resultant supernatant underwent acid vapor hydrolysis as described elsewhere [8], to allow for comparisons between hydrolyzed and unhydrolyzed fractions of the Murchison water extract.

An aliquot of the unhydrolyzed Murchison water extract was derivatized by 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, a salt-insensitive fluorophore with specificity for primary amines and select secondary amines [9]. Glycylglycine and 17 additional analytes composed of amino acids and their respective homopeptides were searched for in the Murchison water extract using liquid chromatography with fluorescence detection and high-resolution mass spectrometry (LC-FD/HR-MS). The analytical technique used here was based off that reported elsewhere [10]. The analytes targeted were glycine monomer through hexamer, alanine monomer through pentamer, aspartic acid monomer through tetramer, and glutamic acid monomer through trimer.

To reproduce results presented by McGeoch and McGeoch [6,7], a 10 mg sample of Allende underwent Folch extraction as executed by McGeoch and McGeoch [6,7]. The resultant extracts were analyzed by tandem mass spectrometry (MS-MS), via MALDI-TOF/MS-MS, while utilizing a cyano-4-hydroxycinnamic acid (CHCA) matrix. Portions of each of the aforementioned hydrolyzed and unhydrolyzed Murchison water extracts were analyzed in parallel by MALDI-Ion Trap (IT)/MS-MS for the presence of peptides. Details pertaining to the MALDI-based analytical techniques implemented in the current work are based on those described elsewhere [11-13].

Results and Discussion:

LC-FD/HR-MS Analyses: The amino acids glycine and aspartic acid were quantitated in the unhydrolyzed Murchison water extract at abundances comparable to those reported previously [14], while alanine and glutamic acid were comparatively elevated. More specifically, glycine and alanine were the most

abundant and were present at 11.9 ± 2.4 nmol/g and 122.8 ± 4.4 nmol/g, respectively. Conversely, aspartic acid and glutamic acid were present at 0.4 ± 0.3 nmol/g and 5.2 ± 0.1 nmol/g, respectively.

Although several amino acids were detected in the Murchison water extract, their corresponding homopeptides were not identified in the same extract above the 0.4 – 7.9 fmol level. However, one unidentified analyte was detected both by the fluorescence detector and the mass spectrometer in the *m/z* trace of derivatized alanine homodipeptide. It is worth noting that there are isobaric species of alanine homodipeptide that are worthy of further exploration in an attempt to identify the unknown analyte. For example, considering that alanine can be present in both α- and β-isomeric forms, and that α-alanine features a chiral center, there are nine isobaric alanine dimer species that should be investigated before a firm assignment to this possible dipeptide analyte can be made. Alternatively, the dimer of glycine and α-aminoisobutyric acid, which are relatively abundant amino acids in Murchison [14], has a derivative that is also an isobaric species of alanine dimer.

Further exploration is needed to determine if the unknown compound is an isobar of alanine dipeptide. Additional studies in progress include spiking experiments to verify analyte identity, hydrolysis experiments to evaluate the presence of an amide bond, and tandem mass spectrometry experiments to confirm molecular fragmentation patterns.

MALDI/MS-MS Analyses: Upon completion of the MALDI-TOF/MS-MS analyses of the Allende Folch extract, it was confirmed that large masses (>1000 Da) were observed. However, the high mass peaks observed here were not consistent with those reported by McGeoch and McGeoch [6,7]. Furthermore, the MS/MS analyses verified that none of these high mass peaks could be assigned to peptides, indicating that amide linkages were not present in the large mass analytes observed in the Allende Folch extract.

The MALDI-IT/MS-MS analysis of the unhydrolyzed Murchison water extract, on the other hand, revealed a parent mass analyte at the *m/z* 133 trace affiliated with gly-gly. It is worth noting that this mass was not also detected in the hydrolyzed Murchison water extract, suggesting that perhaps the *m/z* 133 analyte detected in the unhydrolyzed Murchison water extract may have contained an amide bond that was cleaved during hydrolysis. Upon MS/MS analysis of the *m/z* 133 analyte in the unhydrolyzed Murchison water extract, the product ions were observed to not be of sufficiently large signal intensities necessary to confirm the detection of the glycine dimer. Therefore, this detection of gly-gly in the unhydrolyzed water extract of Murchison is considered tentative. Additionally, the MALDI-IT/MS-MS analyses of the Murchison water extract did not reveal larger (>1000 Da) peaks that could be representative of polymers. Additional work is needed to confirm the putative identification of gly-gly and to identify mid-range mass analytes (~300-1000 Da) in the Allende and Murchison meteorite extracts.

Conclusions: In this work, we have attempted to reproduce the findings of peptides in Murchison that were reported by Shimoyama and Ogasawara [5], as well as duplicate the reports of large (>1000 Da) polymer amide species in Allende by McGeoch and McGeoch [6,7]. Peptides were analyzed for in Murchison using LC-FD/HR-MS, but none of the target peptides were identified using this method. However, an intriguing, unknown fluorescent analyte that is an isobar of alanylalanine was observed, and work is currently underway to identify this compound. Furthermore, a target mass possibly representative of gly-gly was tentatively detected by MALDI-IT/MS-MS analysis of Murchison water extracts. Large oligopeptide species were searched for in Murchison and Allende via MALDI/MS-MS. Some high mass compounds were observed in Allende Folch extracts, but none matched those reported by McGeoch and McGeoch [6,7]. Furthermore, of those high mass species observed in the current work, none were identified to be peptides.

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