# Degradation of Amino Acids on Mars by UV Irradiation in the Presence of Chloride and Oxychlorine Salts

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**Abstract:** The degradation of glycine (Gly), proline (Pro), and tryptophan (Trp), were studied under simulated Mars conditions during UV-driven production of oxychlorines. The degradation of these amino acids was compared under Mars ambient and humid conditions, as films, and with addition of sodium chloride (NaCl) sodium chlorate ($NaClO_3$) and sodium perchlorate ($NaClO_4$), to understand how structure and size influences degradation and how their stabilities under Mars conditions are affected. It was shown that, in addition to the direct photochemical reaction with the salts, the degradation of the amino acids was strongly influenced by the identity of the chlorine salt and its ability to promote deliquescence. Glycine showed no significant destruction in any of the samples under Mars ambient conditions. However, its degradation increased in the presence of NaCl, $NaClO_3$ or $NaClO_4$. Proline degradation was greater than glycine with No Salt > NaCl > $NaClO_3$ > $NaClO_4$ under Mars ambient, but with the exact opposite order under Mars humid conditions. A mechanism is proposed to explain how water and silica participate in these degradation reactions. No difference was observed for tryptophan between Mars ambient and humid conditions, suggesting its degradation mechanism is different compared to glycine and proline. The results reported here will help us to better understand the survival of amino acids in the presence of oxychlorines and UV light on Mars and thus provide new insights for the detection of organic compounds on future Mars missions.

## 1. Introduction

Extraterrestrial organic compounds have been detected in comets (Kissel and Krueger, 1987), chondritic meteorites (Ehrenfreund *et al.*, 2000; Hahn *et al*., 1988; Sephton *et al.*, 1998), and interplanetary dust particles (Flynn, 1996). It has been estimated that long term bombardment of Mars by such objects has delivered up to ~ $10^4$ g/m$^2$yr of material to its surface (Flynn and McKay, 1990) including ~ $10^8$ g/yr of reduced carbon (Benner *et al.*, 2000), thus organic compounds should be abundant on its surface (Bland and Smith, 2000; Tomkins *et al.*, 2019). However, no definitive evidence of organic compounds was found by the 1976 Viking landers, which included a gas chromatograph-mass spectrometer (GC-MS) capable of detecting them (Biemann *et al.*, 1977). It was suggested that this might be due to such reasons as strong oxidants, metal oxides, oxygen radicals, degradation by UV or other radiation, or that the Viking GC-MSs failed to detect them (ten Kate, 2010; Yen *et al.*, 2000; Stoker *et al.*, 1997; Zent and McKay, 1994).

In 2008, the first wet chemical analysis of martian soil by the *Wet Chemistry Laboratory* (WCL) onboard the Phoenix Mars lander (Kounaves *et al.* 2009) revealed the presence of~ 0.6 wt% perchlorate ($ClO_4^-$) (Kounaves *et al.* 2010; Hecht *et al.* 2009; Kounaves *et al.* 2014a). The widespread occurrence of $ClO_4^-$ and chlorate ($ClO_3^-$) on Mars has subsequently been confirmed by their detection in the martian meteorites EETA79001 and Tissint (Kounaves *et al.* 2014b,

Jaramillo *et al.* 2019) and by the Sample Analysis at Mars (SAM) instrument on Curiosity at Gale crater (Glavin *et al.* 2013; Leshin *et al.*, 2013), and by detection of chlorinated hydrocarbons (Eigenbrode *et al.* 2018; Freissinet *et al.* 2015; Ming *et al.* 2014).

Degradation of organic molecules on Mars' surface is likely the result of a combination of processes. Radiation in various modes, directly or indirectly, is likely the dominant factor in destroying organic molecules. Even though much higher levels of solar energetic particles (SEP) and galactic cosmic rays (GCRs) can reach Mars' surface (Cheptsov *et al.*, 2018; Kuhn and Atreya, 1979), their total energy flux is ~ $10^4$ times less than the solar UV flux (Hassler *et al*. 2014). Although the UV at the martian surface penetrates only millimeters into the regolith (Schuerger *et al.*, 2012; Moores *et al.*, 2007), its destructive power is likely felt more widely due to such processes as cryoturbation (Levy *et al.*, 2010), aeolian transport, and impact gardening (Hartmann *et al.*, 2001), which allow regolith to be circulated and exposed to it. However, organic compounds or evidence of life could still be present where it has been sheltered from exposure to radiation (Stalport *et al.*, 2019; Tan and Sephton, 2019; Fornaro *et al*. 2018; Pavlov *et al.*, 2012).

It has been demonstrated that degradation of amino acids by UV radiation can occur rapidly at timescales of days or weeks but can extend to millions of years. It can also vary drastically depending on a variety of factors such as the

chemical environment, reaction pathways, degree of protection, molecular weight/structure, and the UV wavelength (Laurent *et al.*, 2019; Rowe *et al.*, 2019; Bertrand *et al.*, 2015; Stalport *et al.*, 2019; ten Kate *et al.*, 2005; 2006;). For example, it has been shown that a thin film of glycine has a half-life of 22±5 h when irradiated under UV between 120-180 nm at room temperature, but modelling and experiments have shown that its half-life can be extended on the order of $10^7$ years when protected by inert martian regolith (ten Kate *et al.*, 2005). When absorbed on minerals such as forsterite, antigorite, spinel, or pyrite, the half-life of glycine degradation was shown to be between 0.5 - 2 h. This relatively rapid degradation of glycine indicates that these minerals on Mars may be involved in photo-Fenton catalytic reactions (Fornaro *et al*. 2018; Poggiali *et al.* 2020). The destructive effect of UV radiation seems to involve more than just the photon energy of the different wavelengths. Using tryptophan and tryptophan analogues, it has been shown that UVB (280–315 nm) has greater destructive power than the higher energy UVC (100-280 nm) (Rowe *et al.*, 2019).

Under Mars ambient conditions both $ClO_4^-$ and $ClO_3^-$ can be photochemically produced on Cl-bearing mineral surfaces, most likely due to silicate ($SiO_2$) and/or metal-oxides acting as photocatalysts to generate radicals such as $O_2^-$, which can then react with chloride (Carrier and Kounaves, 2015; Yen *et al.*, 2000). During this process several oxychlorine intermediates such as hypochlorite ($ClO^-$), chlorite ($ClO_2^-$), chlorate ($ClO_3^-$), and chlorine dioxide ($ClO_2$) gas, as well as

radicals such as •OCl, •Cl, $O_2^-$ and •OH are also likely generated (Catling *et al.*, 2010). Recently, it was confirmed that the photochemical perchlorate formation pathway can exist without ozone ($O_3$) and that a highly oxidizing oxychlorine gaseous intermediate, most likely $ClO_2$ gas, was present in this pathway (Liu and Kounaves, 2019; Jain *et al.*, 2017).

Here we compared the degradation of three amino acids, glycine (Gly), proline (Pro) and tryptophan (Trp) **(Figure 1)**, selected in order to investigate how structure and size influences their destruction by UV photons under Mars conditions. Both glycine and proline have been found in meteorites (Kvenvolden *et al.*, 1971). Tryptophan was selected due to its aromatic ring structure. The overall aim of the reported effort was to investigate the UV degradation of glycine, proline, and tryptophan in the presence of NaCl, $NaClO_2$, $NaClO_3$, and $NaClO_4$ under Mars conditions.

Based on the data obtained in this study, we propose different degradation mechanisms for glycine, proline and tryptophan (under simulated Mars conditions) that are related to their chemical structures as well as the role of oxychlorine salts in these reactions. These results will help us to better understand the survival of amino acids in the presence of oxychlorines and UV light on Mars and provide important insights for the detection of organic compounds on future Mars missions.

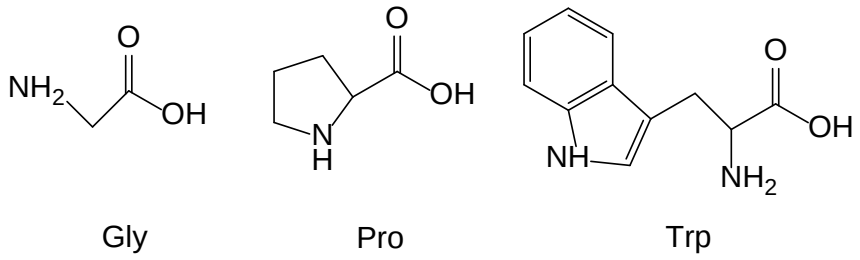


**Figure 1.** Structures of glycine (Gly), proline (Pro) and tryptophan (Trp).

## 2. Materials and Methods

### *2.1 Reagents*

The salts used were: sodium chlorite ($NaClO_2$, Sigma-Aldrich technical grade); sodium chlorate ($NaClO_3$, ACS reagent, ≥99.0%); sodium perchlorate ($NaClO_4$, ACS reagent, ≥99.0%); chlorite ion ($ClO_2^-$, 1000 ppm IC standard, >99.9%); and chlorate ion ($ClO_3^-$, 1000 ppm IC standard, >99%). Silica sand ($SiO_2$, 50-70 mesh calcined quartz, with ≤ 0.05 % HCl-soluble substances, Sigma-Aldrich 1075360250) was used as the supporting matrix. The amino acids glycine, DL-proline and L-tryptophan were purchased from Sigma-Aldrich (ReagentPlus®, ≥99% HPLC grade). A 1000 ppm amino acid stock was prepared in Nanopure 18.2 MΩ-cm deionized (DI) water and kept at ~ 4°C until use. The amino acid solutions were sterilized by filtration with a 0.2 µm syringe filter (Thermo Scientific™ 7239920). It has been shown that there is no difference between the D and L forms of amino acids with respect to UV photolysis

(Bertrand *et al.*, 2015) and thus the chirality of the amino acids is not of any consequence in these studies.

*2.2 Mars Ambient and Humid Simulation Chamber*

The Tufts *Mars Simulation Chamber* (MSC) has been previously described in detail (Liu and Kounaves, 2019). It consists of a stainless-steel cylindrical chamber (~$5\times10^4$ $cm^3$) with a cold plate at the bottom maintained at -15°C. The simulated Mars ambient atmosphere is composed of 95.3% $CO_2$, 2.8% $N_2$, 1.8% Ar, 0.10% $O_2$, <0.1% $H_2O$ (Airgas USA), and maintained at a pressure of 10 Torr. The UV light is provided by a 450W xenon lamp through a 22 cm fused silica port and monitored using a UV spectrophotometer probe (BLUE-Wave, StellarNet). Since UV radiation on Mars of wavelengths < 200 nm is attenuated by the $CO_2$ atmosphere (Kuhn and Atreya, 1979), the experiments described here used UV at wavelengths >200 nm. Mars humid conditions were roughly controlled by placing or removing a 9 cm diameter petri dish filled with 50 mL of DI water ice on the cold plate ~ 30 cm from the sample, creating water vapor with ~1 Torr partial pressure at -15°C.

*2.3 Sample Preparation*

Amino acid films were prepared by adding 100 µL of 1000 ppm amino acid stock to custom made glass plates (~25 mm dia.) cut from the bottom ~ 8 mm of 20 mL borosilicate glass scintillation vials (Fisher Scientific) and then oven dried

overnight at 60°C. All glass plates were cleaned before use with Piranha solution (2.5:1.0 v/v concentrated $H_2SO_4$:$H_2O_2$) to ensure a hydrophilic surface. The custom-made plates with the dried amino acid film were placed symmetrically under the UV light spot in the Mars chamber.

The sand+amino acid sample stocks were prepared by combining 10 g silica sand, 2.0 mL of 1000 ppm amino acid, and 5 mL water, then oven dried overnight at 60°C to avoid degrading the amino acid. After drying the stock was homogenized using a mortar and pestle and its homogeneity verified with Electrospray Ionization Mass Spectrometry (ESI-MS). For each experiment, four replicates (0.5 g each) from the stock were used as samples and four as controls. For the samples with salt, an extra 0.60 mL of $1\times10^5$ ppm NaCl, $NaClO_3$, or $NaClO_4$ was added to the sample stock before drying at 60°C overnight. The amino acid degradation in each sample with the added salt was confirmed by comparing with room temperature controls that showed no significant degradation after drying. The amino acid to salt ratio was optimized based on the ESI-MS signal and the amount of amino acid degraded while maintaining an excess amount of oxychlorine salt, in addition to ensuring sufficient amino acid ionization in the ESI-MS while still at least 10 times more than the amount of amino acid present (Constantopoulos *et al.*, 1999). The detection limit for each sample combination was determined to ensure it was < 10% of the starting amino acid concentration. A 0.5 g sample was placed in each vial during the experiment

with a resulting sample layer thickness of ~ 1 mm. The samples were configured as previously described (Liu and Kounaves, 2019).

Sodium chlorite was not used for the amino acid+salt degradation test because it would cause amino acid degradation during sample preparation. This is due to the decomposition temperature of sodium chlorite decreasing as the moisture content increases, making the chlorite more reactive towards organic compounds during drying (White *et al.*, 1942). This process was evidenced by the presence of a yellowish discoloration (See SI, Figure S2).

*2.4 Calibration and Recovery Test*

Calibration curves for each amino acid and internal standard in the presence of each salt were obtained during method optimization using ESI-MS. It was confirmed that the ratio of the amino acid to the internal standard was linear within the range of 10-200% of the starting amino acid concentration (10-200 ppm).

*2.5 Electrospray Ionization Mass Spectrometry (ESI-MS ) Analysis*

ESI-MS instead of gas chromatography (GC) was used to determine the concentration of the amino acids because oxychlorine salts will cause thermal decomposition of organic compounds at the high temperatures typically found in GC analyses. The amino acids were leached by adding 10 mL of an optimized solvent consisting of a 1:1 (v/v) of 1% acetic acid : methanol, stirred for 1 hour, and then filtered through a 0.2 µm filter. The internal standards were added prior

to leaching. The ESI-MS was cleaned prior to use by running a mixture of water/ methanol/acetonitrile (1:1:1 v/v) as cleaning solvent, and then methanol and water individually at 30 µL/min for 5 min. The ESI-MS was also rinsed with this solvent between samples to minimize interference.

Individual ESI-MS direct injection methods were developed for each type of sample (no salt, NaCl, $NaClO_3$, $NaClO_4$) by Xcalibur 2.0 built-in calibration using proline m/z=116 peak with flow rate at 10 µL/min. Internal standards were used for each amino acid to ensure signal reproducibility and to account for the adsorption of the amino acids on the silica sand surface during leaching (glycine-$2^{13}C$ for glycine, alpha-Methyl-L-proline for proline and 5-Methyl-DL-tryptophan for tryptophan). The internal standards for each amino acid were selected to be chemically similar to the target amino acid. All the amino acids and internal standards were calibrated and verified before use.

The peaks were selected based on reference data from MassBank (http://massbank.us/) and NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/). The peak at m/z=76 was monitored for glycine and at m/z=78 for glycine-2-$^{13}C$ internal standard. The peak at m/z=116 was monitored for proline and at m/z=130 for alpha-Methyl-L-proline internal standard. Peak m/z=205 was monitored for tryptophan and m/z=219 was monitored for 5-Methyl-DL-tryptophan internal standard. The presence of the salts in the sample solution led to lower target peak intensity due to lowered

ionization efficiency of the target amino acid. This was especially the case in the glycine+NaCl samples. Thus, precise peak positions instead of nominal peak positions were used in these samples for glycine (m/z=76.45) and Glycine-2-$^{13}$C internal standard (m/z=78.45).

Different solvent additives such as ammonia and ammonium acetate were also evaluated due to concerns about UV activated oxychlorine salts causing additional amino degradation in acidic solutions during storage. However, the acetic acid additive yielded the best signal stability as well as peak intensity. Tests were also conducted for all sand+salt+amino acid combinations to demonstrate that maintaining the sample leachates at 7°C for up to four days would not cause noticeable degradation of the amino acids. All the samples were analyzed within 48 hours after leaching.

## 3. Results

### *3.1 Amino Acid Films*

Glycine, proline, and tryptophan in the form of dried films were directly exposed to UV irradiation (200-400 nm) for ~24 hours under both Mars ambient and humid conditions. For Mars ambient samples, ESI-MS analysis showed that 100±2% of the glycine remained in respect to the control sample. In contrast only 77±5% of the proline and 48±7% of the tryptophan remained in comparison to the controls **(Figure 2)**, with the tryptophan film also showing a yellow discoloration after irradiation similar to that when reacted with $NaClO_2$ with increased

moisture. In comparison, under Mars humid conditions no degradation of glycine or proline was observed, with 100±2% of both remaining, while the amount of tryptophan remaining increased to 70±4% (**Figure 2**).

*3.2. Amino Acids Combined with Sand and Sand+Salt*

Unlike the films, when the amino acids were mixed with silica sand and irradiated, degradation was observed at varying degrees in all the samples. As shown in **Figure 2**, for the glycine+sand sample, 95% and 99% of the glycine still remained under Mars ambient and humid conditions, respectively. Under Mars ambient the addition of NaCl, $NaClO_3$ or $NaClO_4$ to glycine+sand led to a small but significant change in glycine degradation, with ~94-88% of the glycine remaining. However, under humid conditions the addition of a chlorine-bearing salt resulted in an additional ~10% increase in degradation, with only 82-77% of glycine remaining.

The proline+sand samples under both ambient and humid conditions showed a ~ 40% decline in proline. However, the addition of NaCl, $NaClO_3$ or $NaClO_4$ under Mars ambient led to a drastic decrease in degradation from 62% to 74% to 94% of proline remaining, respectively. Yet under Mars humid conditions, the addition of NaCl, $NaClO_3$ or $NaClO_4$ had the opposite effect, accelerating its degradation process with only 54%, 50%, and 41% remaining, respectively.

Overall, tryptophan showed the greatest degradation of the three amino acids when irradiated. The tryptophan+sand samples showed a similar level of

degradation as the tryptophan film (48-57%). Addition of NaCl, $NaClO_3$ or $NaClO_4$ under either Mars ambient or humid conditions resulted in ~ 43%, ~36%, and ~41% tryptophan remaining, respectively.

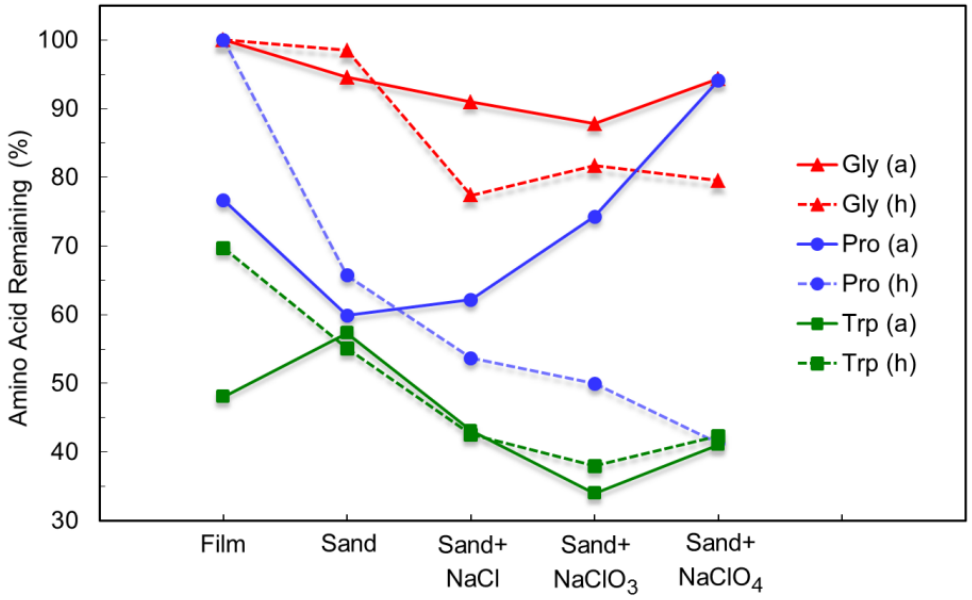


**Figure 2.** Amino acid degradation as a film and in the presence of NaCl, $NaClO_3$ or $NaClO_4$ under Mars ambient (a) or humid (h) conditions. The average shown for each sample group was normalized to 100% in respect to the control group. (For numerical data and error analyses see SI Table S1).

## 4. Discussion

### *4.1 Amino Acid Films*

Because the amino acid films were translucent and allowed some transmission of UV through the layer, their degradation did not strictly correlate with the total amount of UV absorbed. However, the molar absorptivity of each amino acid **(Figure 3)**, calculated using Beer's law, was found to be consistent

with the NIST standard spectra as well as the theoretical estimation based on functional group values (Tannenbaum *et al.*, 1953).

The tryptophan film for both ambient and humid Mars conditions showed the most degradation and developed a visible yellowish discoloration. This is likely due to its higher and broader UV molar absorptivity in the 200-300 nm range compared to proline and glycine (Figure 3). However, the discoloration of the tryptophan film also blocked the UV and thus afforded some protection from degradation. This was seen in preliminary tests where using a high-concentration tryptophan film resulted in little overall tryptophan degradation even though the film showed significant discoloration (see SI, Figure S1).

Aromatic amino acids, such as tryptophan, in the free state are rapidly destroyed when irradiated with UV in the presence of oxygen ($O_2$), but less so when $O_2$ is absent (Vladimirov *et al.*, 1970). The rapid degradation of tryptophan is due to the presence of the aromatic ring, and thus UV irradiation below 275 nm (>4.5 eV) can drive it to the first excited singlet state. One possible mechanism for its photochemical degradation is the removal of one hydrogen from the nitrogen of the indole ring to produce a tryptophan radical (Vladimirov *et al.*, 1970).

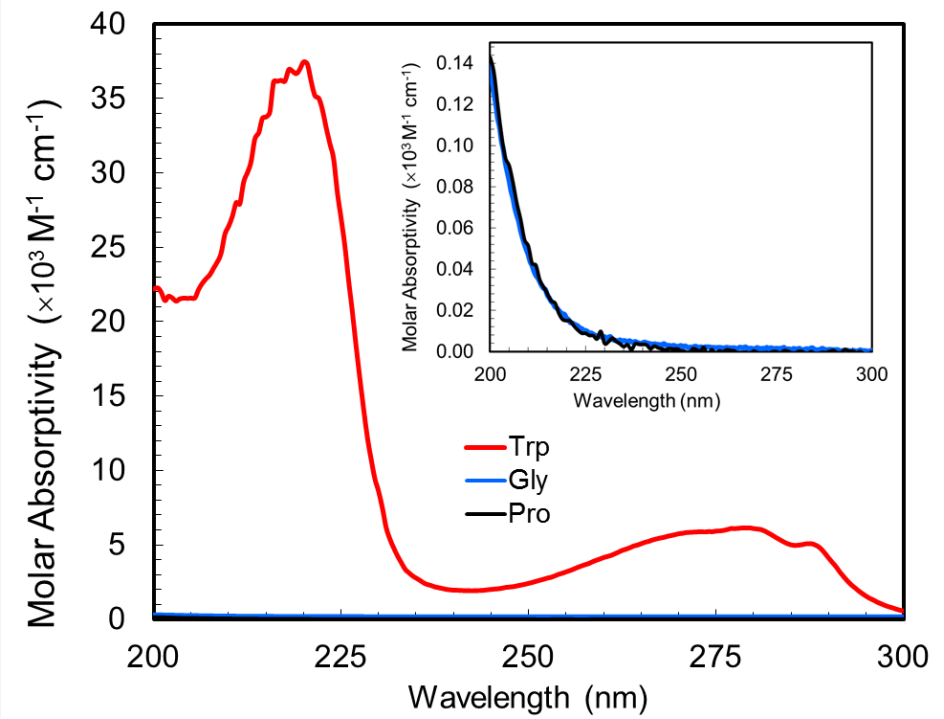


**Figure 3.** Molar absorptivity ($M^{-1}$ $cm^{-1}$) between 200-300 nm for 10 ppm tryptophan and 100 ppm of glycine and proline in water. Inset: Molar absorptivity axis ×300.

As can be seen in Figure 3 (Inset), the molar absorptivity of glycine and proline are almost identical in the 200-300 nm range. This is consistent with the absorption data for primary amines and pyrrolidine accounting for those in glycine and proline, respectively (Getoff and Schwörer, 1970; Tannenbaum *et al.*, 1953). The increased degradation of proline under Mars ambient conditions cannot be explained solely by the UV absorptivity, but is also likely caused by the presence of the pyrrolidine ring being more susceptible under UV radiation.

Aliphatic amino acid degradation can be induced by photo-excited molecules or by free radical precursors (Bonifačić *et al.*, 1998). Glycine has only a weak UV absorption below 250 nm, which increases as the wavelength decreases. Decarboxylation free radical generation is the starting point for this reaction. For glycine, under γ radiation in basic aqueous solution, the •OH radicals created from $H_2O$ radiolysis induced a degradation reaction for glycine. Thus, the increase of humidity in this case could provide more radicals for degradation. These reactions generate $CO_2$, CO, and HCN, which are some of the end products as the result of decarboxylation (Bertrand *et al.*, 2015; Ehrenfreund *et al.*, 2001). In our amino acid film tests, glycine films showed no significant degradation after 24 h under Mars ambient UV levels. This is consistent with the data from amino acid experiments onboard the international space station (ISS) (Bertrand *et al.*, 2015).

It has been shown that glycine degradation under 200-300 nm UV irradiation depends on the concentration of $O_2$. After 109 h of UV irradiation at room temperature, glycine was almost completely degraded in a pure 100 equivalents $O_2$ atmosphere (stoichiometrically 100 times the amount of $O_2$ needed to degrade all the glycine) yet showed no degradation in the absence of $O_2$ (Oro and Holzer, 1979). Considering the concentration of $O_2$ in the Mars gas (0.1% $O_2$), this is consistent with our observation. Furthermore, it has also been shown that UV radiation >300 nm is not likely to contribute to amino acid degradation (Orzechowska *et al.*, 2007).

UV-Vis absorption data for proline indicates that it also absorbs in the 200-250 nm range but with a high molar absorptivity that increases exponentially as the wavelength decreases. One key difference between proline compared to glycine and tryptophan is that proline does not have a $-NH_2$ terminal but rather a secondary amine attached to an α-carbon, and thus in this case HCN is not likely to be the product of proline degradation. It has been shown that proline is an effective quencher for singlet oxygen due to the cyclic secondary amine and has a low ionization potential (Alia *et al.*, 2001). Additionally, secondary radicals formed from proline are more stable than primary radicals formed from glycine, thus the proline degradation is much faster.

The absence of significant degradation for proline under Mars humid conditions was likely caused by the opaque frost that formed on the surface of the films. It has been observed that snow in Antarctica can reflect 96%-98% of UV and visible radiation (Grenfell *et al.* 1994). Similarly, the frost on the films reflected most of the UV radiation, shielding the amino acid and thus leading to a decrease in degradation. The frost formation was unavoidable due to the increased water vapor present under higher humidity. No frost formation was observed on any of the sand-based samples.

*4.2 Amino Acids Combined with Sand and Sand+Salt*

Through confirmatory tests, it was found that generally >80% recovery of amino acids is possible when making amino acid+sand with/without salt stock

compared to a direct amino acid addition. Thus, the amount of amino acid present in the amino acid+sand samples was slightly less than the amount of amino acid in the film form. This does not present a problem since comparisons are made in terms of % differences.

*4.2.1 Glycine with Sand and Sand+Salt*

Glycine+sand displayed increased degradation in all the samples under both Mars ambient and humid conditions compared to the glycine film. This is likely the result of silica sand particles providing a larger surface area and therefore more reactive sites (Carrier and Kounaves, 2015). It has been shown that the presence of water is necessary for amino acid degradation under Mars ambient (usually 10-1000 ppm in the atmosphere) while the absolute level of water does not seem to affect the reaction rate significantly (ten Kate *et al.*, 2006). The absorption of UV by water is negligible, although UV radiation at 242 nm wavelength has been shown to cause $H_2O$ dissociation to produce $^{\bullet}H$ and $^{\bullet}OH$ radicals (Okabe, 1978).

There was only a slight increase in degradation observed for glycine+sand under both Mars ambient and humid conditions. However, in the presence of NaCl, $NaClO_3$ or $NaClO_4$, the degradation increased for Mars humid compared to Mars ambient conditions, although no significant difference between NaCl, $NaClO_3$ or $NaClO_4$ was observed. This indicates that at Mars ambient with the salt concentrations used here (6000 ppm in each 0.5 g amino acid+sand+salt

sample), the amount of oxidizing intermediates generated after 24 h of UV irradiation were not sufficient to cause significant degradation of glycine.

*4.2.2 Proline with Sand and Sand+Salt*

For proline+sand without any salts, there was increased degradation under both Mars ambient and humid conditions compared to the proline film sample. This trend is similar to what was noted in the previous section with glycine +sand. For proline+sand with NaCl, $NaClO_3$ or $NaClO_4$, it appears that the salts were protecting proline from degradation under Mars ambient and accelerating it under Mars humid conditions following the trend: no salt < NaCl < $NaClO_3$ < $NaClO_4$. This difference was especially obvious for the degradation of proline mixed with sand+$NaClO_4$ where it was only ~ 5% under Mars ambient yet ~ 60% under Mars humid conditions. This difference cannot be explained solely by either oxidizing intermediates or absorption of UV by the salts because $NaClO_3$ has a higher and broader UV absorption and is expected to be more reactive than $NaClO_4$ in both cases. This again confirms that the degradation of proline was not driven by the oxidizing power of the oxychlorine salts or the intermediates produced under UV irradiation.

It has been suggested that in an $O_2$ saturated aqueous solution irradiated with γ-rays, proline would be converted to hydroxyproline, initiated by intermediary $^{\bullet}OH$ radicals and peroxide (Kopoldová and Voráček-Hübsch, 1975). On the other hand, both decarboxylation and addition of $CO_2^-$ to proline radicals to form

dicarboxylic acid were observed in a nitrogen atmosphere. However, as discussed above, •OH generation in this case was not likely to be significant due to the UV wavelength used.

Another possible reaction pathway would be initiated by reacting singlet oxygen with proline, an effective singlet oxygen quencher in aqueous solution, though the exact mechanism for this reaction is unclear (Alia *et al.*, 2001). It has also been shown that UV radiation can induce photodissociation of molecular oxygen and generate an oxygen radical, ozone, as well as singlet oxygen (Brasseur and Solomon, 2006), as shown in the reactions below:

$$O_2 + h\nu \rightarrow O + O \quad (1)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (2)$$

$$O_3 + h\nu\ (\leq 320\ \text{nm}) \rightarrow O_2(^1\Delta g) + O(^1D) \quad (3)$$

Due to the low oxygen content of the Mars simulation gas (<0.1%) and the significant difference between glycine and proline absorption, it is not likely that any of the above mechanisms occurred with proline under Mars ambient. If oxygen radicals or singlet oxygen are key for proline degradation, its degradation under Mars humid conditions should decrease compared to Mars ambient, as an increased $H_2O$ level would reduce the amount of reactive oxygen species available to proline.

Given the above, it is proposed that the absorption of a UV photon by proline under Mars ambient conditions generates unstable radicals **(Figure 4)**. For

photolysis of secondary cyclic amines, it has been shown that breaking the N-H bond is likely the dominant dissociation process in non-polar solvents and that breaking the C-N bond is more likely in polar solvents (Cohen *et al.*, 1973). The adsorbed $H_2O$ and -OH on the silica surface could then react with the proline radicals and convert them to the two products shown in **Figure 4**. It has been shown that different forms of amorphous silica adsorb the same amount of $H_2O$ under the same water partial pressure (Iler, 1979). The reacted $H_2O$ and -OH groups can then be regenerated with the $H_2O$ vapor present in the Mars gas ($H_2O$ <0.1%). Under Mars ambient, when NaCl, $NaClO_3$ and $NaClO_4$ are present they will compete for the $H_2O$ and -OH groups and thus the degradation of proline would be hindered. Under Mars humid conditions, the $H_2O$ is in excess and the salts will then aid in increasing the adsorbed $H_2O$ on the silica sand and thus accelerate the degradation. The presence of water could also leach the adsorbed amino acid from the sand making it more susceptible to degradation (Fornaro *et al*. 2018). The ability of the salt to promote degradation depends on its solubility in $H_2O$. For NaCl, $NaClO_3$ and $NaClO_4$ their solubility at 25°C is 36, 100, and 209 g/100 g of $H_2O$, respectively (Lide, 2004) and their deliquescence power follows with $NaClO_4$>$NaClO_3$>NaCl. These salts are likely to behave similarly with the glycine+sand samples. However, the effects were not as obvious as with the proline+sand samples due to the structural stability of glycine as discussed above.

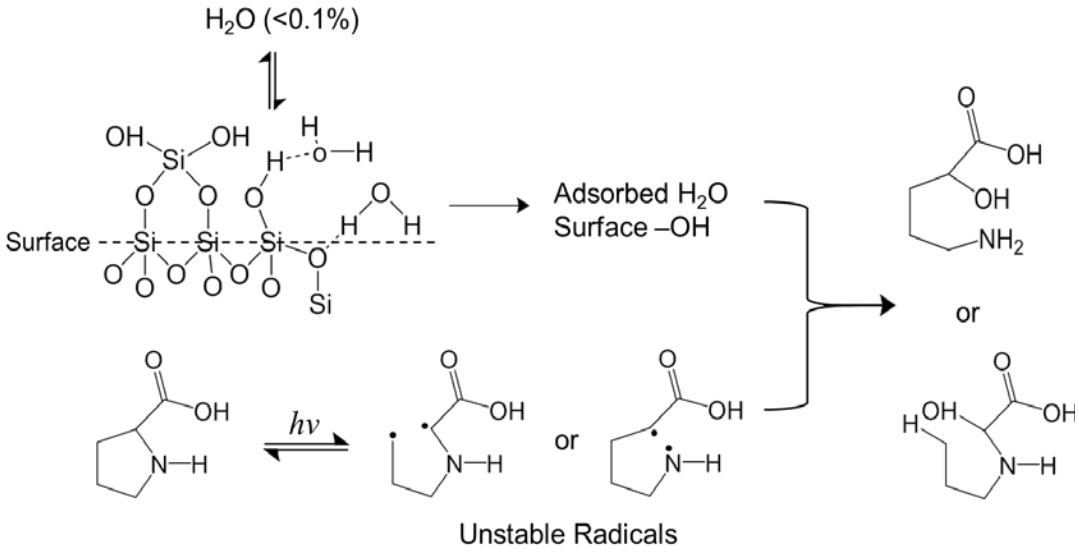


**Figure 4**. Proposed mechanism for the degradation of proline by UV under Mars ambient conditions. The $H_2O$ and -OH on the $SiO_2$ are regenerated with the <0.1% $H_2O$ present in the simulated Mars gas.

*4.2.3 Tryptophan with Sand and Sand+Salt*

Irradiation of tryptophan as a film or with any sand or salt resulted in a yellowish product, in stark contrast to glycine and proline. To explain this discoloration, it is proposed that the aromatic indole ring in tryptophan enables it to absorb much more UV energy than glycine or proline. Thus, the bond breakage in tryptophan occurs mainly on the indole ring under Mars ambient and is more significant but independent of the humidity level (Creed, 1984).

Although identification of this yellow product was beyond the scope of this research, one possible candidate is β-carboline formed via UV driven

decarboxylation. This has previously been shown to be the product of thermally decomposed tryptophan (Cuq and Cheftel, 1983). Another possible candidate is 1H-Indole-3-carboxaldehyde, which was determined to likely be the dominant fragment of tryptophan photodissociation under 265 nm UV (Talbot *et al.*, 2005). Whatever this degradation product is, the salient accompanying effect under Mars ambient is that it provides some protection from UV, and thus further degradation of the tryptophan.

## 5. Conclusions

The direct UV degradation of glycine, proline, and tryptophan, was studied under Mars ambient and humid conditions, as a film or with addition of NaCl, $NaClO_3$ or $NaClO_4$. These amino acids demonstrated different degradation behaviors. Glycine being the most stable amino acid tested; showed no significant destruction in any of the samples under Mars ambient conditions. However, the degradation of glycine increased in the presence of NaCl, $NaClO_3$ and $NaClO_4$ but with no significant difference between the three salts. The degradation of proline was significantly greater than that observed for glycine, even though the molar absorptivity of glycine and proline are similar for UV between 200-400 nm. In addition, the degradation order for proline was No salt > NaCl > $NaClO_3$ > $NaClO_4$ under Mars ambient conditions, but under Mars humid conditions, the order of degradation for proline was reversed to $NaClO_4$ > $NaClO_3$ > NaCl > No

salt. This difference is likely due to the difference in the affinity of each salt towards water.

For tryptophan, no difference was observed between Mars ambient and humid conditions. This suggests that tryptophan possesses a different degradation mechanism compared to glycine or proline. This is likely the result of tryptophan's strong broad UV absorption, which allows significantly more UV energy for degradation compared to glycine and proline. The opaque products resulting from the tryptophan degradation can also act as a UV screen for the amino acids immediately below and protect them from further photochemical degradation.

Addition of NaCl, $NaClO_2$, $NaClO_3$ or $NaClO_4$ to the amino acid+sand samples resulted in different behaviors for glycine, proline, and tryptophan. Under Mars ambient, degradation generally increased for glycine but not for proline. The salts in proline+sand appear to be protecting proline from UV degradation, with the proline+sand+perchlorate sample showing only 6% degradation.

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The authors have no competing or personal financial interests.

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# Supplemental Information

### Optimization of Amino Acid and Salt Concentrations

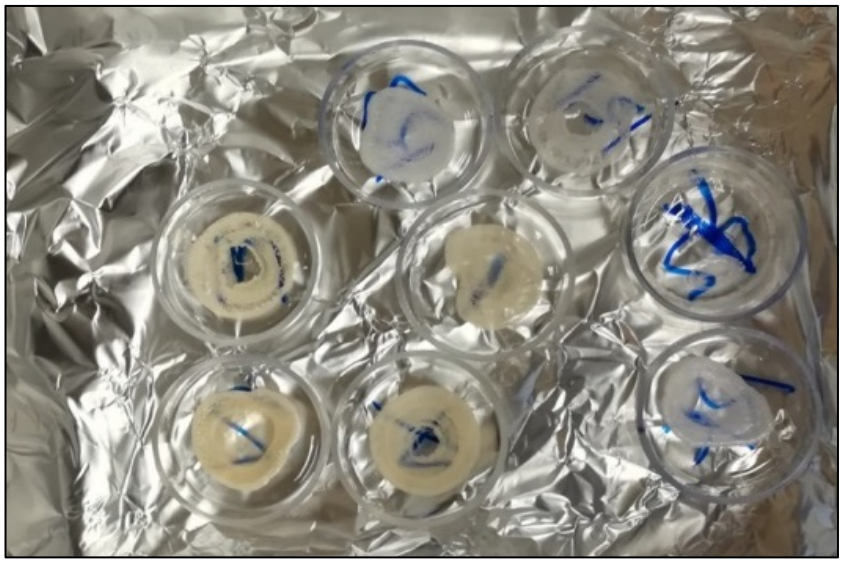

**Figure S1.** Tryptophan films made with 0.2 mL of $5\times10^3$ ppm tryptophan. Left four vials were exposed to UV at Mars ambient for 24 h. Right four vials were controls kept at 4°C for 24 h.

The amino acid to salt ratio was optimized based on the ESI-MS signal and the amount of amino acid degraded while maintaining an excess amount of oxychlorine salt. For example, if too much tryptophan was used, even though the surface turned visibly yellow indicating tryptophan degradation (**Figure S1**), the amount of tryptophan actually degraded was statistically insignificant compared to the total amount of tryptophan (less than 5% for 4 vials of samples and 4 vials of controls). This is likely due to the yellowish compound produced during the experiment which blocks UV from further reaching the amino acid below. Thus, the amount of amino acid in the sample needed to be optimized and the amino acid film needed to be thin. For amino acid+chlorate and perchlorate salt+sand samples, several pre-analysis methods, such as utilizing different organic solvents, were tested to extract only the amino acid but without any significant success.

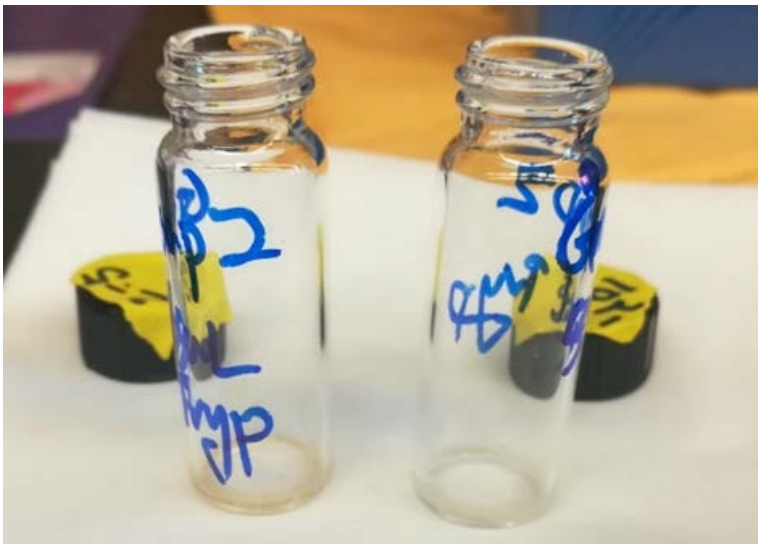

**Figure S2.** Tryptophan+sodium chlorite sample preparation after vacuum drying at room temperature. Left vial: Trp+$NaClO_2$ The color change was due to the amino acid degradation as confirmed by ESI-MS analysis; Right vial: Trp alone as control.

**Table S1.** Numerical data for percent amino acid remaining after being irradiated as a film and in the presence of NaCl, $NaClO_3$ or $NaClO_4$ under Mars ambient or humid conditions. The average shown for each sample group was normalized to 100% in respect to the control group (n=3).

| Amino Acid + | % Gly Remaining | | % Pro Remaining | | % Trp Remaining | |
|---|---|---|---|---|---|---|
| | Ambient | Humid | Ambient | Humid | Ambient | Humid |
| Film | 100±2 | 100±2 | 77±5 | 100±4 | 48±7 | 70±4 |
| Sand | 95±3 | 99±1 | 60±21 | 66±11 | 57±7 | 55±2 |
| Sand+ NaCl | 91±6 | 77±2 | 62±17 | 54±23 | 43±6 | 43±8 |
| Sand+ $NaClO_3$ | 88±5 | 82±1 | 74±10 | 50±7 | 34±12 | 38±5 |
| Sand+ $NaClO_4$ | 94±2 | 80±10 | 94±4 | 41±9 | 41±2 | 42±1 |