Research Article

Formation of Amino Acids and Nucleotide Bases in a Titan Atmosphere Simulation Experiment

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Abstract

The discovery of large (>100 u) molecules in Titan's upper atmosphere has heightened astrobiological interest in this unique satellite. In particular, complex organic aerosols produced in atmospheres containing C, N, O, and H, like that of Titan, could be a source of prebiotic molecules. In this work, aerosols produced in a Titan atmosphere simulation experiment with enhanced CO ($N_2/CH_4/CO$ gas mixtures of 96.2%/2.0%/1.8% and 93.2%/5.0%/1.8%) were found to contain 18 molecules with molecular formulae that correspond to biological amino acids and nucleotide bases. Very high-resolution mass spectrometry of isotopically labeled samples confirmed that $C_4H_5N_3O$, $C_4H_4N_2O_2$, $C_5H_6N_2O_2$, $C_5H_5N_5$, and $C_6H_9N_3O_2$ are produced by chemistry in the simulation chamber. Gas chromatography–mass spectrometry (GC-MS) analyses of the non-isotopic samples confirmed the presence of cytosine ($C_4H_5N_3O$), uracil ($C_5H_4N_2O_2$), thymine ($C_5H_6N_2O_2$), guanine ($C_5H_5N_5O$), glycine ($C_2H_5NO_2$), and alanine ($C_3H_7NO_2$). Adenine ($C_5H_5N_5$) was detected by GC-MS in isotopically labeled samples. The remaining prebiotic molecules were detected in unlabeled samples only and may have been affected by contamination in the chamber. These results demonstrate that prebiotic molecules can be formed by the high-energy chemistry similar to that which occurs in planetary upper atmospheres and therefore identifies a new source of prebiotic material, potentially increasing the range of planets where life could begin. Key Words: Astrochemistry—Planetary atmospheres—Titan—Astrobiology. Astrobiology 12, xxx–xxx.

1. Introduction

TITAN, Saturn's largest moon, currently has the only atmosphere in our solar system that is both reducing and contains significant quantities of carbon ($\sim 2\%$ CH_4; Waite *et al.*, 2005) and nitrogen (98% N_2), and trace levels of oxygen (~ 50 ppm CO; de Kok *et al.*, 2007), thereby enabling photochemical production of complex molecules containing C, N, O, and H. This makes Titan our only planetary-scale laboratory for the atmospheric synthesis of prebiotic molecules. Infrared spectra from Voyager 1 reveal the presence of a diverse collection of organic molecules (Hanel *et al.*, 1981; Kunde *et al.*, 1981); however, it was not until the arrival of the Cassini-Huygens mission that the chemical complexity of Titan's at-

mosphere was fully appreciated. Data from the mass spectrometer carried by Cassini (Ion and Neutral Mass Spectrometer, INMS) led to the discovery of numerous species with masses up to the limit of 99 u (Vuitton *et al.*, 2007), but the instrument's resolution (1 u) limits its ability to identify prebiotic molecules unambiguously. Measurements from the Cassini Plasma Spectrometer (CAPS) indicate the presence of molecules in Titan's ionosphere with masses in excess of hundreds of u [negative ions with *m/z* up to 10,000 u/q at 950 km (Coates *et al.*, 2007), positive ions with *m/z* up to 400 u/q (Crary *et al.*, 2009)]. While the chemical pathways for synthesis of some relatively small molecules (mass less than 50 u) can be found, and the abundances of many of these molecules are now reproducible with photochemical models (Hörst

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et al., 2008; Lavvas et al., 2008a, 2008b; Vuitton et al., 2008; Krasnopolsky, 2009), the presence of very large molecules in Titan's upper atmosphere was not predicted.

Measurements from CAPS also revealed O^+ flowing into Titan's atmosphere (Hartle et al., 2006), which appears to be the source, along with micrometeorites, of the oxygen-containing molecules in Titan's atmosphere (Hörst et al., 2008). While Titan's atmosphere is relatively oxygen poor compared to terrestrial planets, CO is the fourth most abundant molecule in the atmosphere. The fact that the observed O^+ flux into Titan's atmosphere is deposited in the region now known to contain large organic molecules leads to the exciting possibility that oxygen can be incorporated into these molecules and result in the production of molecules of biological interest. Our ability to detect prebiotic molecules in Titan's atmosphere is currently limited by the mass range of the INMS to the two smallest biological amino acids, glycine (75 u) and alanine (89 u). Although INMS positive ion measurements have peaks at m/z of 76 and 90, the identification of these peaks as $HC_5NH^+/C_6H_4^+$ and $C_6H_3NH^+$ (Vuitton et al., 2007) is model-dependent due to the low resolution of the instrument, so the presence of glycine and alanine cannot be ruled out. Since our understanding of Titan's atmosphere is presently limited by the capabilities of the instruments carried by Cassini-Huygens, laboratory experiments play an important role in understanding the chemical processes occurring in Titan's atmosphere.

There is a long history of research into the production of prebiotic molecules in an atmosphere by naturally occurring chemical processes, particularly in the context of early Earth. Miller (1953) and Miller and Urey (1959) subjected gas mixtures thought to be representative of early Earth's atmosphere to electrical discharge (simulating lightning), in the presence of liquid water, with the aim of producing prebiotic molecules. These experiments produced amino acids, but recent research indicates that their gas mixture was more reducing than the atmosphere of early Earth (see, e.g., Delano, 2001). Sagan and Khare (1979) performed similar experiments, using gas mixtures more representative of Titan. Experiments in which energy is deposited into Titan-like gas mixtures produce dark organic material that Carl Sagan named "tholin." Titan aerosol analogues, or tholins, are currently produced in a wide variety of experimental setups that employ different gas mixtures, energy sources, temperatures, and pressures (see, e.g., Imanaka et al., 2004). Previous early Earth and Titan atmosphere simulation experiments have produced prebiotic molecules, but their formation required liquid water during (Miller, 1953; Miller and Urey, 1959) or after production (Kobayashi et al., 1995; Neish et al., 2010). We show here that inclusion of oxygen in the gas mixture in a Titan atmosphere simulation experiment results in the production of amino acids and nucleotide bases. The unique combination of CO and energetic particles and photons in a Titan-like reducing atmosphere is shown to produce molecules of astrobiological interest, that is, amino acids and nucleobases. This means that prebiotic molecules may be produced in Titan's atmosphere, despite the lack of liquid water. Additionally, similar processes may have occurred in the reducing upper atmosphere of Earth, which has implications for the origin of life on Earth and elsewhere in the Universe.

Three previous Titan atmosphere simulation experiments have included CO in the initial gas mixture. Bernard et al.

(2003) and Coll et al. (2003) analyzed the gas phase products of their experiments via IR spectroscopy and gas chromatography–mass spectrometry (GC-MS) but did not analyze the solid experimental products. From their analyses, they reported the detection of oxirane (C_2H_4O), whose mass is smaller than the lower mass limit of our mass spectrometer and therefore could not be detected by our analysis if it was also present in the solid product. Tran et al. (2008) also analyzed the gas phase products of their experiment, using GC-MS, and analyzed the solid phase products of their experiment that included CO, using UV-visible and IR spectroscopy. They identified 14 oxygen-bearing molecules in the gas phase products, mostly ketones, only one of which (C_4H_8O) is detected in the measurements described below. Their solid phase products are observed to contain ketones and carbonyls from IR spectroscopy. These analytical techniques are less sensitive to small amounts of specific molecules. The differences between analytical techniques used in the previous experiments that included CO and the work presented here make it impossible to directly compare results. Amino acids and nucleotide bases were not discussed in any of the previous works.

2. Sample Production and Analysis

Tholins were produced in the PAMPRE apparatus (Production d'Aérosols en Microgravité par Plasma Réactifs) (Szopa et al., 2006). This apparatus uses a capacitively coupled radio frequency (RF) discharge to initiate chemistry in gas mixtures composed of N_2, CH_4, and CO that results in the formation of tholins. While the primary energy source in Titan's atmosphere is solar UV, the cold plasma produced by RF discharge is a useful laboratory analogue. It produces electrons with enough energy to dissociate N_2 and CH_4, while having little effect on the temperature of the neutral gas, unlike a spark discharge. The solid particles form and grow while levitated in the plasma, which is confined within a stainless steel grid cage (Szopa et al., 2006). Their weight and the gas drag forces are balanced by electrostatic forces. The particles grow in levitation until the forces become unbalanced and eject them from the plasma into the glass vessel surrounding the cage. This unique production setup minimizes wall effects during production and more accurately reproduces the conditions under which aerosols form in a planetary atmosphere.

The tholins were produced by a 30 W, 13.6 MHz RF discharge at a pressure of 0.9 mbar (Szopa et al., 2006) and a temperature of $\sim$330 K (Alcouffe et al., 2010). The production chamber is a flow apparatus, with a gas flow rate of 55 sccm (standard cubic centimeters per minute). The N_2 and 10% CH_4/90% N_2 were both >99.999% pure (Air Liquide). The $C^{18}O$ (Cambridge Isotope Laboratories) was >95% pure. Before the tholins are produced, the chamber is baked out to remove H_2O adsorbed on the walls of the chamber and electrodes, while being pumped down to $\sim10^{-5}$ mbar. A pure N_2 plasma discharge is then used to aid in the removal of remaining contaminants. Samples were removed under atmospheric conditions, placed in plastic vials in sealed bags, and stored at room temperature.

Tholins were produced from $N_2/CH_4/CO$ gas mixtures of 96.2%/2.0%/1.8% (P2CO) and 93.2%/5.0%/1.8% (P5CO). These gas mixtures have been enhanced in CO relative to

Titan's atmosphere to produce an abundance of oxygen-containing molecules sufficiently large for our measurement techniques. Sciamma-O'Brien *et al.* (2010) showed that an initial concentration of 5% CH_4 results in a Titan-like steady-state abundance of 1–2% CH_4 in the production chamber. Since an initial concentration of 2% CH_4 is generally used in tholin experiments (*e.g.*, Neish *et al.*, 2010), both gas mixtures are presented here. For each gas mixture, a corresponding sample was produced with $C^{18}O$ instead of CO (P2COi, P5COi). The introduction of $C^{18}O$ allows for differentiation between oxygen incorporation from CO during aerosol production and ^{16}O incorporation from contamination during production, removal from the chamber, or subsequent sample preparation and analyses. A sample was also produced from only 98% N_2 and 2% CH_4 (P2), with a corresponding isotopic sample produced with $^{13}CH_4$ (P2i). The ^{13}C sample was used to rule out contamination for molecules that do not contain oxygen.

The samples were produced for 8 h with production rates from ∼0.1 mg/h (P2COi) to ∼7.9 mg/h (P5CO). Sciamma-O'Brien *et al.* (2010) investigated the variation of production rate with initial N_2/CH_4 gas mixture; however, the effect of inclusion of CO on production rates has not yet been systematically studied. Due to the extreme chemical complexity of the samples, very high-resolution mass spectrometry is necessary to determine the chemical composition of the tholins (see Fig. 1). An LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) with a resolving power ($M/\Delta m$) of 10^5 up to 400 u/q and accuracy of ±2 ppm was used to characterize the tholins. The samples were dissolved in CH_3OH (1 mg/mL) followed by sonication (30 min) and centrifugation (3 min, 9000*g*). The soluble fraction was in-

jected into the Orbitrap by electrospray ionization (ESI). ESI is a soft ionization source that produces positively charged ions (protonated) and negatively charged ions (deprotonated) but does not fragment molecules. Solubility measurements made with N_2/CH_4 PAMPRE tholins in CH_3OH reveal solubility ratios from 19% to 35%, depending on the initial gas mixture (Carrasco *et al.*, 2009). As the tholins are not fully soluble in CH_3OH, only the soluble fraction is analyzed. Both the large number of peaks and the very similar mass defects of C, N, O, and H make manual molecule identification impossible. Accordingly, custom computer software has been written to assign molecular formulae to the measured peaks quickly and accurately. The software uses a list of molecules known to exist in tholins (Somogyi *et al.*, 2005) to perform an internal mass calibration of the data. After internal calibration, the molecular formula identifications are unique up to 300 u.

The Orbitrap measurements provide the masses of the ions, from which we can infer the molecular formulae, but do not provide any information about the structure(s) of the observed molecules. Instead, GC-MS has been used to assign structure (Buch *et al.*, 2009). GC-MS measurements were made on P5CO, P5COi, and P2i. P2CO and P2COi were not analyzed due to the limited amount of sample produced. The tholins underwent chemical derivatization by using *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), which is sensitive to all compounds with acidic hydrogen, to produce volatile derivatives for measurement in the gas chromatograph column. Pyrene was used as an internal standard. The analyses were performed with a GC-MS ThermoScientific Trace gas chromatograph coupled with a DSQII mass spectrometer operated in quadrupole mode
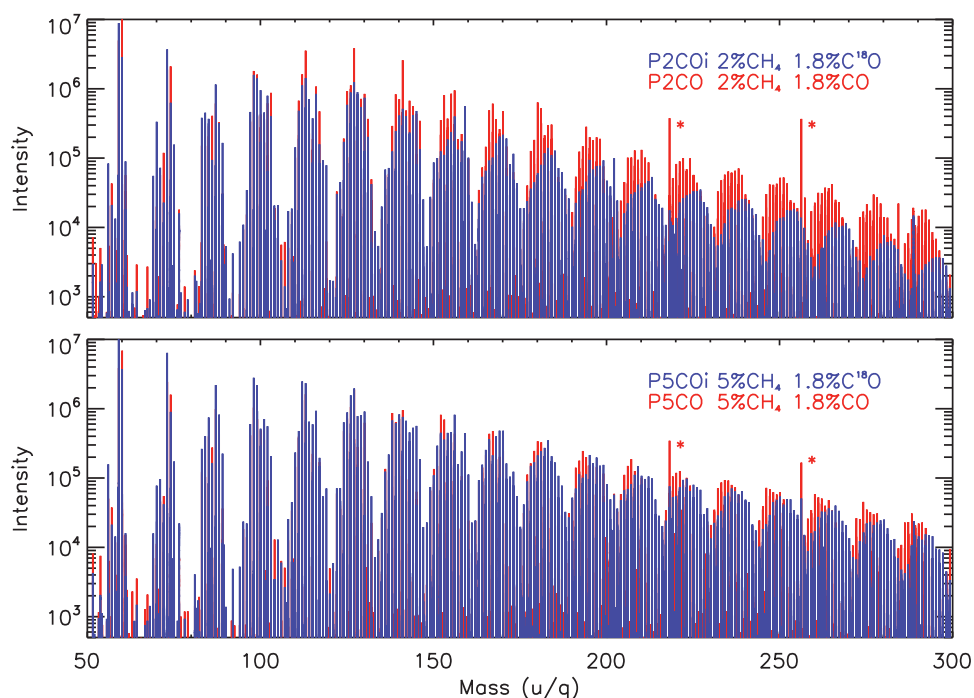

FIG. 1. Orbitrap positive ion mass spectra of P2CO (top, red), P2COi (top, blue), P5CO (bottom, red), and P5COi (bottom, blue) from 50 to 300 u/q. Asterisks indicate known contaminants. Plotted spectra are an average of 200 spectra with a mass resolution of 10^5. The observed mass shift in the isotopic samples results from the incorporation of ^{18}O, which has been confirmed through assignment of the peaks.

(resolution 1 u). The molecules were ionized with 70 eV electron impact. The temperatures of the split/splitless injector (Optic 3, AtasGL) and the detector were 270°C and 200°C, respectively. A fused Rxi-5SilMS (Restek) capillary column was used. The tholins were dissolved in CH_3OH (5 mg/mL) and centrifuged (10,000 rpm, 10 min). The supernate was separated from the solid phase and evaporated at 40°C under nitrogen flow. Then, a mixture of 30 μL of MTBSTFA and 10 μL of *N,N*-dimethylformamide was added to the supernate solutes. The derivatization reaction occurred for 20 min at 75°C. Then 4 μL of the solution was injected directly into the GC-MS operated in split mode (1:4). For comparison, the retention times and cracking patterns (from dissociative ionization) of amino acid and nucleotide base standards were measured.

3. Results

For the purposes of this work, the molecules of interest are the 5 nucleotide bases (adenine, cytosine, guanine, thymine, uracil) and the 19 amino acids utilized by life on Earth (biological amino acids) composed only of C, N, O, and H. Orbitrap mass spectra of P2CO, P2COi, P5CO, and P5COi are shown in Fig. 1. Detailed analysis of these spectra resulted in the identification of over 8,000 different molecular formulae, which corresponds to a much greater number of molecules if structural isomers are taken into account. Here,

we focus on the definitive detection of a handful of species that are interesting for prebiotic synthesis.

Eighteen peaks with masses that correspond to the molecular formulae of biological amino acids (14—isoleucine/leucine are isomers) or nucleotide bases (5) were detected in the spectra (see Table 1). Formulae that correspond to nonbiological amino acids and purine bases were also identified and will be discussed in a future publication. To confirm that these molecules were created in the chamber and that the oxygen comes from the CO in the gas mixture and not contamination, the spectra of P2CO and P5CO were compared to the spectra of P2COi and P5COi, respectively. Detailed examination of the tholin spectra, shown in Fig. 2, reveals the presence of isotopic molecules with the formulae of cytosine, uracil, thymine, and histidine (P2COi and P5COi) and adenine (P2i). The peaks that correspond to these isotopic molecules are above the level of naturally occurring ^{18}O and ^{13}C on Earth. Isotopic versions of the other 13 molecules identified in P2CO and P5CO were not detected above the noise level, nor were any amino acids or nucleotide bases containing two ^{18}O atoms. It is possible that they were present in abundances too low to be detected with the techniques used here as a result of an ^{16}O contamination source in the chamber (see Section 4).

Molecular structure was confirmed with the GC-MS measurements, which indicate that all five nucleotide bases and two biological amino acids (glycine and alanine) were

TABLE 1. SUMMARY OF OBSERVED PREBIOTIC MOLECULES

Name	Mass	Formula	Fig.	P2CO %N$_2$ 96.2 %CH$_4$ 2 %CO 1.8	P2COi 96.2 2 1.8 C^{18}O	P5CO 93.2 5 1.8	P5COi 93.2 5 1.8 C^{18}O
Nucleotide base							
cytosine	111	$C_4H_5N_3O$	2	OT	OT	OT/GC-MS	OT
uracil	112	$C_4H_4N_2O_2$		OT	OT	OT/GC-MS	OT
thymine	126	$C_5H_6N_2O_2$		OT	OT	OT/GC-MS	OT
adenine[a]	135	$C_5H_5N_5$	2	OT		OT/GC-MS	
guanine	151	$C_5H_5N_5O$		OT		OT/GC-MS	
Biological amino acid							
glycine	75	$C_2H_5NO_2$		OT		OT/GC-MS	
alanine	89	$C_3H_7NO_2$		OT		OT/GC-MS	
serine	105	$C_3H_7NO_3$		OT		OT	
proline	115	$C_5H_9NO_2$		OT		OT	
valine	117	$C_5H_{11}NO_2$		OT		OT	
threonine	119	$C_4H_9NO_3$		OT			
isoleucine/ leucine[b]	131	$C_6H_{13}NO_2$		OT		OT	
asparagine	132	$C_4H_8N_2O_3$		OT		OT	
glutamine	146	$C_5H_{10}N_2O_3$		OT		OT	
lysine	146	$C_6H_{14}N_2O_2$		OT		OT	
histidine	155	$C_6H_9N_3O_2$	2	OT		OT	OT
phenylalanine	165	$C_9H_{11}NO_2$		OT		OT	
arginine	174	$C_6H_{14}N_4O_2$		OT		OT	

OT indicates that the molecular formula was present in the Orbitrap spectrum. GC-MS indicates confirmation of structure from GC-MS measurements. In all cases, presence in the isotopic samples (P2COi, P5COi) refers only to the isotopic molecule. GC-MS measurements were not performed on P2CO and P2COi.

[a]Due to the lack of oxygen, adenine is a special case, and additional measurements were made using P2 and P2i. Orbitrap measurements show clear incorporation of ^{13}C into $^{13}C_5N_5H_5$ (as shown in Fig. 2), and GC-MS measurements provide structural confirmation that the ^{13}C-containing molecule is adenine (as shown in Fig. 3).

[b]Note that isoleucine and leucine are isomers (mass 131) and cannot be differentiated based on Orbitrap measurements.
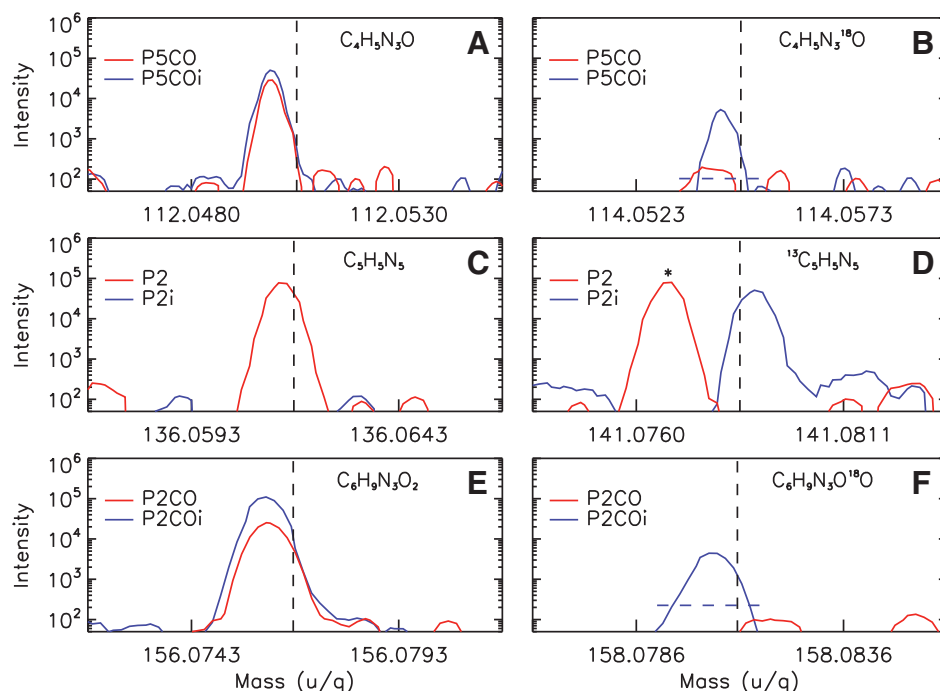
FIG. 2. Orbitrap mass spectra of the samples produced with isotopic gas (blue lines) and non-isotopic gas (red lines). The peaks shown are ions with the molecular formulae of cytosine (top), adenine (middle), and histidine (bottom). Vertical dashed black lines indicate the exact masses of the ions. The left panels (**A**, **C**, **E**) show the peaks for the non-isotopic molecules, and the right panels (**B**, **D**, **F**) show the corresponding peaks for isotopic molecules. The blue peaks in (A) and (E) are indicative of ^{16}O contamination in the samples, which is discussed at length in Section 4. Horizontal dashed blue lines in the right panels (B, F) indicate the predicted intensity of the ^{18}O-containing peak if it contains ^{18}O only from Earth's atmosphere, based on the intensity of the ^{16}O-containing peak. The ^{12}C molecule (C) is not observed in the isotopic sample; therefore (D) has no horizontal dashed blue line. The observed peaks are significantly more intense than can be explained by contamination. For $C_4H_5N_3O$ and $C_6H_9N_3O_2$, the non-isotopic molecules are observed in both samples. There is no ^{18}O or ^{13}C contamination in the unlabeled samples. The peak marked by an asterisk (D) is $C_5H_8N_4O$.

present in P5CO (see Figs. 3 and 4). ^{13}C-containing adenine was present in P2i. Although there is some evidence in the analysis of P5COi for the corresponding isotopic (^{18}O) molecules (particularly for cytosine), the fact that the cracking patterns for the isotopic and non-isotopic molecules overlap strongly makes it difficult to separate the isotopic signal for molecules other than adenine. The lack of chromatographic identification for the other 11 molecules does not necessarily indicate that these molecules do not have the structure of prebiotic interest, but rather could result from abundances below the detection limit.

The yields of these molecules were not calculated for a number of reasons. First, quantitative information is difficult to extract from ESI-Orbitrap measurements. A number of molecular properties, including solubility in methanol and proton affinity, affect the relative intensity of peaks in the Orbitrap measurements. Additionally, based on investigations of ^{13}C and ^{15}N isotope patterns, not discussed in this work, it appears that the low intensity peaks in the Orbitrap data have a lower intensity than expected. As the molecules discussed here have peak intensities between 0.1% and 1% of the most intense peak in the spectrum, the intensities likely suffer from this problem; therefore quantification, even in a relative sense, is not currently possible. Second, for a number of reasons, Titan atmosphere simulation experiments do not precisely reproduce Titan's atmosphere. For this reason, extrapolation of the yields from these types of experiments,

including our own, to Titan is likely to result in large uncertainties.

Given the large number of molecular species present in the tholins and the lack of information on ions and radicals in the plasma, we can form no conclusions about likely reaction mechanisms responsible for the formation of the observed prebiotic molecules. Some reaction pathways have been investigated in the context of amino acid synthesis in the interstellar medium. Experiments by Blagojevic *et al.* (2003) showed that reaction of ionized or protonated hydroxylamine with acetic acid or propanoic acid produces glycine or alanine, while theoretical calculations by Largo *et al.* (2010) showed that reaction of NH_3^+ with CH_3COOH and CH_2COOH leads to ionized or protonated glycine. Additional experimental and theoretical calculations are needed to determine whether these or similar reaction pathways operate in the PAMPRE chamber.

4. Contamination

Possible contamination of our samples has been carefully considered in the analysis of our measurements. In Fig. 2, panels A and E show that the ^{16}O-containing peaks do not disappear when $C^{18}O$ is substituted for CO in the initial gas mixture. The ^{16}O observed in our samples has several possible sources: Earth's atmosphere during production, removal or sample preparation/analysis, a biological source
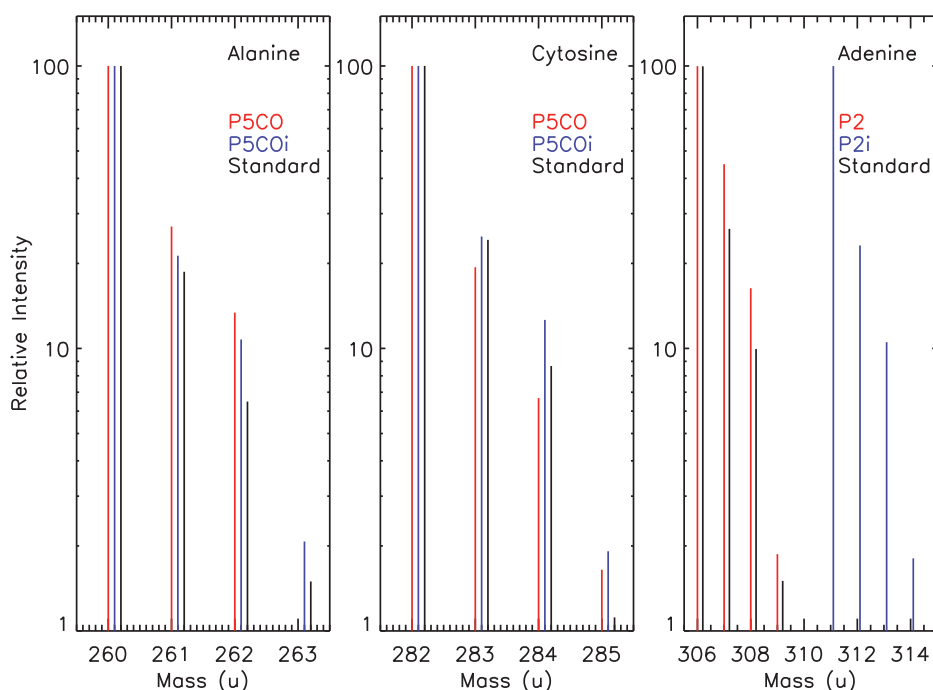
FIG. 3. GC-MS mass spectra (resolution 1 u) of alanine (left), cytosine (middle), and adenine (right) after derivatization of 2 H and dissociative ionization resulting in the loss of a *tert*-butyl fragment. Peaks have been normalized to the base peak for each molecule [260 (alanine), 282 (cytosine), 306/311 (adenine)]. Strong agreement between the non-isotopic samples (red lines), isotopic samples (blue lines), and the standards (black lines) verifies the identities of alanine, cytosine, and adenine. For adenine, the shift of 5 u demonstrates the presence of ^{13}C adenine in P2i. Retention times (minutes) for the non-isotopic, isotopic, and standard samples are 15.41, 15.39, and 15.43 (alanine); 26.09, 26.10, and 26.16 (cytosine); and 34.44, 35.62, and 34.50 (adenine). For clarity, the masses of the isotopic samples and the standards have been offset by +0.1 and +0.2 u, respectively.
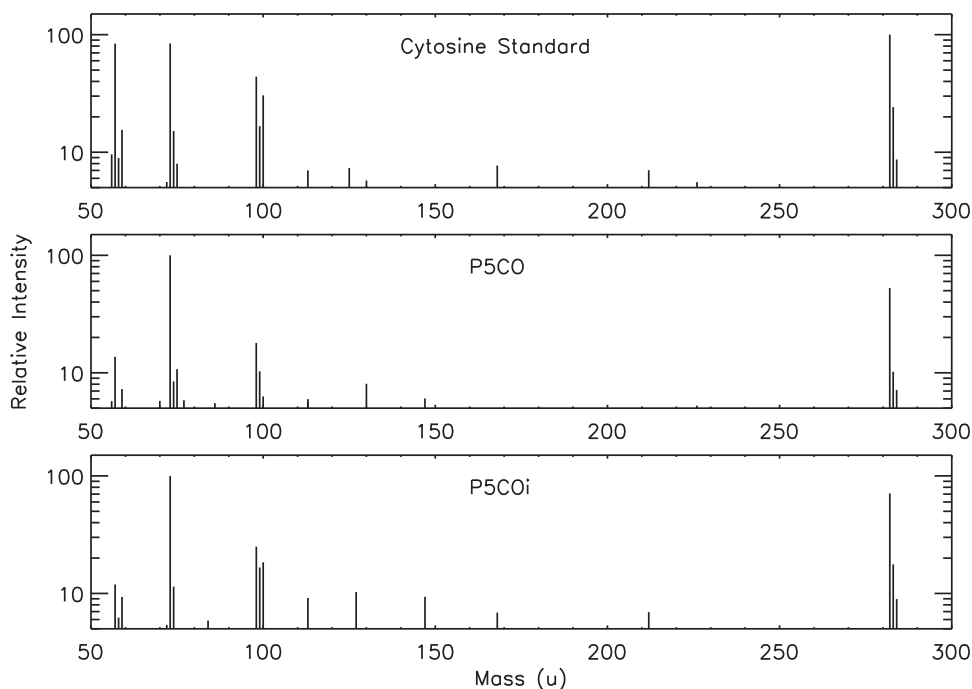

FIG. 4. GC-MS mass spectra of cytosine from Fig. 3 expanded to show the full mass range of the fragments produced by dissociative ionization of the derivatized molecules. Peaks have been normalized to the most intense peak in the spectrum. Strong agreement between the standard (top), the non-isotopic sample (middle), and the isotopic sample (bottom) verifies the identity of cytosine.

such as fingerprints, reactions with solvents during measurements, or contaminants in the solvent, Orbitrap, GC-MS, and so on.

To ensure that the observed oxygen-containing molecules did not result from reactions between the tholins and the CH_3OH or the formation of solvent clusters, identical measurements were acquired by using CH_3CN. The same molecules were observed with both solvents; thus we concentrate on the CH_3OH results because of the higher ESI ionization efficiency of CH_3OH. Isotopic gases were used to evaluate the possibility of biological contamination. While the measurements of the ^{18}O-containing samples have not fully eliminated this possibility, the complete lack of ^{12}C-containing adenine in the ^{13}C sample (P2i) in both the Orbitrap and GC-MS measurements indicates that a biological source of the other molecules is highly unlikely. We have run blanks (only CH_3OH) before each set of GC-MS and Orbitrap measurements. The molecules of interest observed in both instruments are not present in the blanks; therefore it is unlikely that the molecules are a contaminant in the solvent, gas chromatograph column, Orbitrap, or storage vials.

The tholins were generally analyzed within days of production to minimize any aging processes, except P2i, which was produced in 2006 and analyzed in 2010. Accordingly, this sample was only searched for adenine, which does not contain oxygen.

The large abundance of ^{16}O molecules in the ^{18}O samples indicates the presence of terrestrial, but not biological, contamination. It is possible that the oxygen contamination occurred during sample production because of incomplete removal of water from the walls of the chamber and electrodes; small leaks resulting in the introduction of H_2O, O_2, and CO_2 from Earth's atmosphere; or impurities in the gas cylinders. If the oxygen contamination occurred during sample production, the oxygen would not have come from the intended source, but the amino acids and nucleotide bases would still have been formed in the production chamber and would still be of interest for prebiotic synthesis, though further investigation of the causes and consequences of the contamination is required. The parallel production of biomolecules in the simulation from both CO and impurity $H_2O/O_2/CO_2$ implies that both sources either produce radicals or ions that react to form these biomolecules or react with organic radicals or ions to produce them. The parallel chemistry in no way detracts from the fact that CO is shown to produce biomolecules in the gas phase under these conditions. The relative intensities of the molecules that originated from CO and the oxygen-containing background gases simply speak to relative rates. Since the background gases in the experiment are not present in Titan's atmosphere to any appreciable extent relative to CO, the production of biomolecules from CO is significant. Detailed production mechanisms and reaction rate coefficients are required before the quantitative nature of this chemistry can be determined.

5. Discussion

In summary, isotopic tests confirmed that $C_4H_5N_3O$, $C_4H_4N_2O_2$, $C_5H_6N_2O_2$, $C_5H_5N_5$, and $C_6H_9N_3O_2$ were made in the chamber. These formulae correspond to cytosine, uracil, thymine, adenine, and histidine; and GC-MS analysis of the non-isotopic samples confirmed the presence of cyto-

sine, uracil, thymine, adenine, guanine, glycine, and alanine. Our GC-MS analysis had insufficient sensitivity to determine the structure of the isotopically labeled molecules detected in the high-resolution mass spectra, but the fact that structures were confirmed with the more abundant ^{16}O isotopes suggests that the same molecular structures existed in the isotopic samples and that cytosine, uracil, and thymine were synthesized by chemistry in the reaction chamber and incorporated into the aerosols. For adenine, we have both detection of the ^{13}C isotopologue and confirmation of its structure, which leaves no doubt that it was synthesized in the reaction chamber. The source of O for the remaining 13 prebiotic molecules listed in Table 1 could either be the CO in the simulated atmosphere or contaminants in the chamber such as O_2 or H_2O.

It is impossible to perfectly replicate a planetary atmosphere in a laboratory; instead, we look to the laboratory to further our understanding of the physical and chemical processes that occur in an atmosphere. This work indicates that prebiotic molecules can be produced in the gas phase or through gas phase/aerosol interactions under conditions that can exist in planetary atmospheres. Accordingly, these molecules may be present in Titan's atmosphere. Although reaction mechanisms responsible for the formation of some of these molecules have been suggested (see, *e.g.*, Blagojevic *et al.*, 2003; Maeda and Ohno, 2006; Largo *et al.*, 2010), the mechanisms are not known; further work is necessary to identify the specific conditions required for formation.

While prebiotic molecules have been previously observed in organic aerosols produced in the presence of water (Miller, 1953; Miller and Urey, 1959) or subjected to further processing postproduction, such as high-temperature acid hydrolysis (Sagan and Khare, 1971), and low-temperature hydrolysis (Neish *et al.*, 2010), this work represents the first detection of the formation of prebiotic molecules in conditions representative of the upper atmosphere.

Life as we know it requires C-, N-, O-, and H-containing molecules. In many atmospheres, these atoms are trapped in very stable molecules (*e.g.*, N_2 and CO). The energy required to break these triple bonds limits possible energy sources to lightning, energetic particles, or extreme UV radiation. The lightning simulated in the Miller-Urey experiments may not be present on every planet that could potentially harbor life. The simulation experiments described here are analogous to processes that occur in Titan's upper atmosphere and ionosphere. Similar to other heavy molecules in Titan's upper atmosphere, prebiotic molecules produced in these regions will diffuse downward and may be subject to further chemistry and incorporation into aerosols (*c.f.* Vuitton *et al.*, 2008). Photochemistry in an upper atmosphere or ionosphere has two attributes that may be important for the synthesis of organic molecules. First, the reducing conditions that may be more favorable for prebiotic molecule formation (see, *e.g.*, Schlesinger and Miller, 1983) are more likely to be found in a planet's upper atmosphere because diffusive separation enhances abundances of hydrogen-rich species (see *e.g.*, Chamberlain and Hunten, 1987). The difficulties with synthesis of prebiotic molecules in an oxidizing environment may be lessened or avoided in an upper atmosphere. Second, solar photons and associated photoelectrons deposited in an upper atmosphere or ionosphere have energies sufficient to break triple bonds (10–20 eV), which releases active nitrogen

and creates high-energy radicals that may facilitate production of prebiotic molecules. The combination of a reducing environment and high-energy electrons existed in our simulation chamber; therefore, the amino acids and nucleotide bases discovered in our simulations suggest that the building blocks of life may form in planetary upper atmospheres in addition to the lower atmosphere (Miller-Urey synthesis), interstellar space, or through aqueous alteration of organic material on planetary surfaces or interiors.

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Author Disclosure Statement

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Abbreviations

CAPS, Cassini Plasma Spectrometer; ESI, electrospray ionization; GC-MS, gas chromatography-mass spectrometry, gas chromatograph–mass spectrometer; INMS, Ion and Neutral Mass Spectrometer; MTBSTFA, N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide; PAMPRE, Production d'Aérosols en Microgravité par Plasma Réactifs; RF, radio frequency.

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