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Amino acids generated from hydrated Titan tholins: Comparison with Miller–Urey electric discharge products

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ABSTRACT

Various analogues of Titan haze particles (termed 'tholins') have been made in the laboratory. In certain geologic environments on Titan, these haze particles may come into contact with aqueous ammonia (NH_3) solutions, hydrolyzing them into molecules of astrobiological interest. A Titan tholin analogue hydrolyzed in aqueous NH_3 at room temperature for 2.5 years was analyzed for amino acids using highly sensitive ultra-high performance liquid chromatography coupled with fluorescence detection and time-of-flight mass spectrometry (UHPLC-FD/ToF-MS) analysis after derivatization with a fluorescent tag. We compare here the amino acids produced from this reaction sequence with those generated from room temperature Miller–Urey (MU) type electric discharge reactions. We find that most of the amino acids detected in low temperature MU $CH_4/N_2/H_2O$ electric discharge reactions are generated in Titan simulation reactions, as well as in previous simulations of Triton chemistry. This argues that many processes provide very similar mixtures of amino acids, and possibly other types of organic compounds, in disparate environments, regardless of the order of hydration. Although it is unknown how life began, it is likely that given reducing conditions, similar materials were available throughout the early Solar System and throughout the universe to facilitate chemical evolution.

1. Introduction

It is generally believed that life on Earth originated from environmentally supplied organic precursors (Oparin, 1924; Haldane, 1929; Miller and Orgel, 1974; Cleaves, 2008). A variety of possible sources have been invoked for such an "Oparin–Haldane" type origin of life, including atmospheric synthesis (Miller, 1953; Schlesinger, 1983a, 1983b), extraterrestrial delivery from meteorites and comets (Chyba and Sagan, 1992), and geothermal synthesis (Wächtershäuser, 1988; Steele et al., 2012).

Although the composition of the atmosphere during the time life originated on Earth is debated (Tian et al., 2005; Zahnle et al., 2010; Trail et al., 2011), other bodies in the Solar System have atmospheres which may be more conducive to atmospheric organic synthesis. One example is Saturn's moon Titan, which has an upper atmosphere consisting of ~98% N_2 and 2% CH_4, along with minor amounts of other species including H_2, HCN, CO and organics such as ethane, ethylene, acetylene and cyanoacetylene (Niemann et al., 2005). However, due to Titan's low temperature (~95 K), most of its water is present as ice in the crust and mantle, acting similar to bedrock on the Earth. In contrast, the early Earth was likely above the freezing point of water for long periods of time, with possible early excursions above and below the conditions at which water can exist as a liquid (Kasting and Pollack, 1984; Bada et al., 1994).

Titan's atmosphere is exposed to various types of energetic processing, producing higher molecular weight organic polymers. When produced under laboratory conditions, these polymers are collectively known as tholins (Sagan and Khare, 1979). Similar materials have also been suggested to be formed in the atmospheres of Triton and Pluto (McDonald et al., 1994). On Titan, it has been proposed that as the organic haze particles precipitate from the

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atmosphere they may come into contact with eutectic brines highly enriched in NH_3, in short-lived 'oases' of liquid water formed through impact melting or cryovolcanism (Thompson and Sagan, 1992). These interactions could lead to reactions producing molecules of biological interest, for example amino acids and nucleobases (Neish et al., 2008, 2009, 2010).

The energetic processing of Titan's present atmosphere is likely somewhat different than that which acted upon the primitive Earth's (Chyba and Sagan, 1992), or that which contributed to interstellar ice grain organic formation (Meierhenrich et al., 2005; Öberg et al., 2009). For example, the Earth has always received a significantly higher flux of solar radiation than Titan, and the flux in interstellar environments is attenuated distinctly due to the differences in path lengths, composition and density of the medium. However, it was noted almost 30 years ago that electric discharge reaction products and organic molecules found in carbonaceous chondrites are very similar with respect to their amino acid contents (Wolman et al., 1972). The inference is that there is some similarity in the mechanism of formation in both cases (Peltzer et al., 1984), for example *via* Strecker-like synthesis from small reactive organic precursors such as ketones or aldehydes (Miller, 1957). Analytical studies since the 1970s have revealed a much richer molecular complexity in many carbonaceous chondrites (Schmitt-Kopplin et al., 2010; Burton et al., 2012). It is worth bearing in mind the various degrees of post-accretional processing the natural samples have undergone over ~4.5 Ga compared to the laboratory samples, and that, with few exceptions (Johnson et al., 2008; Vuitton et al., 2010), laboratory samples have not received the same degree of scrutiny.

A number of laboratories have modeled the synthesis of Titan haze particles using CH_4/N_2 gas mixtures at low temperature using various energy sources, such as electric discharge and UV light (Ferris, 1981; Khare et al., 1986; Thompson et al., 1991; McDonald et al., 1994; Ehrenfreund et al., 1995; Kobayashi et al., 1997; Neish et al., 2008; Cable et al., 2012). A subset of these laboratories also investigated the products of tholin hydrolysis, which is the subject of this paper.

There are numerous variables involved in these simulations which could render comparison difficult, for example the conditions under which the tholins are synthesized, and their products processed and analyzed (Scheme 1); nevertheless, as we will show, the detected products remain remarkably similar despite these variables.

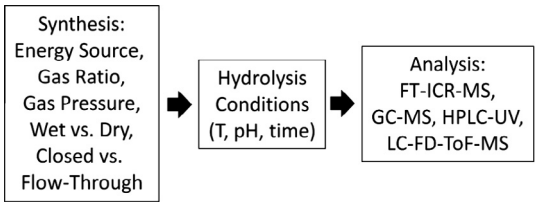
For example, during tholin synthesis, a wide-range of ratios of N_2 to CH_4 (from ~1000:1 to 1:1), total gas pressures (from ~400 mmHg to 1 mmHg) and reaction temperature (from 296 K to 195 K) have been used. Some use flow reactors while others use static ones. Workup has ranged from high temperature (>100 °C) aqueous acid to extremely low temperature (−20 °C) aqueous base over widely varying time ranges. Lastly, a variety of analytical techniques have been used to measure the amino acid products of these reactions.

A short historical note regarding the terminology used here is useful for clarifying this discussion. While there was some experimental work on the generation of discrete low molecular weight organic compounds, such as amino acids, using electric discharges acting on various gas mixtures prior to Miller's pioneering 1953 publication (Miller, 1953) (see for example, Löb, 1913), no formal name was applied by Miller or his predecessors to the complex organic mixtures which result from such experiments. It should be stressed that Miller's experiments were produced in the context of a reducing early Earth atmosphere, which adds a level of intentional contextualization to the materials produced. Furthermore, Miller recognized that discrete organic compounds were isolated from hydrolysis of the products of the electric discharge reaction, which could include a mixture of those synthesized directly in the discharge, those made in aqueous phase reactions from intermediates produced in the gas phase, and those derived from hydrolysis of larger more complex organic polymers produced in either phase. Collectively these were termed "electric discharge products".[1]

Thus the principle practical distinctions between MU electric discharge products and tholins, are that MU products can be considered as alternatively (1) organic materials derived from the action of electric discharges acting on reduced gases, (2) organic materials derived from energetic (be it *via* electric discharge, UV, or high energy particle) processing of gas mixtures (reducing or otherwise) simulating planetary atmospheric chemistry, or (3) chemistry as described in (2) in the presence of a liquid phase. Tholins, in contrast are generally considered to be the resulting non-volatile organic products derived from reduced gases *via* any type of energetic processing simulating Solar System environments at temperatures below the freezing point of water. In both cases, the materials are considered to be precursors which require some work-up for the release of biomolecules, and in neither case is the potential importance of the molecular weight of the precursors considered significant, *i.e.* either or both might liberate small molecules in water, such as HCN, NH_3 and aldehydes and ketones, which may undergo other types of secondary reactions, such as the Strecker reaction, to give the final observed small molecule products such as amino acids.

The salient points we wish to make are that there is considerable similarity between tholins and MU discharge products when derived from methane and molecular nitrogen, with respect to the amino acids, and potentially other products, produced regardless of the temperature of reaction or the presence of liquid water during energetic processing. This suggests that the gas-phase chemistry of non-oxygen-containing species dominate the amino acid synthesis pathways, and that as these are highly transient species the temperature may be of little importance (Khare et al., 1984). This point may have considerable consequence for the types of organic materials which can be expected to be delivered to almost any reducing planetary environment throughout the universe.

Among the various other studies of these materials (Pernot et al., 2010; Vuitton et al., 2010), it has often been of interest to measure the amino acids produced from these simulations. As mentioned above, the paucity of water or other O-containing



Scheme 1. Some variables involved in the preparation of amino-acid yielding tholins reported in the literature.

[1] It was not until Sagan and co-workers recognized the potential parallels between MU type reactions and the chemistry of the atmospheres of the jovian planets and their satellites that the term "tholin" was coined, which Sagan derived from the Greek root "tholos" ("θολοσ"). It appears Sagan intentionally chose a Greek root which could be alternatively interpreted as being derived from the word meaning "muddy" or "unclear", referencing both the color and molecular complexity and heterogeneity of these materials, as well as from the root meaning "dome", referencing their astronomical significance. In a personal communication from Miller to Sagan discussing Sagan's suggested name, Miller, tongue-in-cheek, suggested "tholin" was a poor choice of terminology, partly because the English voiceless dental fricative "Θ" ("th-") would be difficult to pronounce in German (where it would be pronounced as "/d/") (Miller, personal communication to HJC).