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Surface-active substances in a laboratory simulated Titan's organic haze: Prebiotic microstructures

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ABSTRACT

Titan, the largest satellite of Saturn, is a key planetary body for astrobiological studies due to its active organic chemistry, hydrocarbon lakes and possible subsurface water-ammonia liquids. We have investigated the physicochemical properties of organic compounds synthesized in a simulated Titan atmosphere. A laboratory analog of Titan's aerosols, called tholin, was produced by irradiation of a nitrogen/methane gas mixture. The primary aim was to determine whether tholin represent possible sources of surface-active substances that could have been involved in the formation of prebiotic structures. A tholin sample was extracted with chloroform-methanol and the chloroform soluble material was separated by two-dimensional thin layer chromatography. Fluorescence excited by UV light was used to identify the major components on the plates. After being scraped from the TLC plate, the components were eluted as specific fractions and investigated by surface chemical methods, FTIR, scanning electron microscopy and cyclic voltammetry. Fractions 1 and 2 were strongly fluorescent and surface active, producing films at air-water interfaces. When exposed to aqueous phases, components in fraction 1 form spherical microstructures resembling prebionts. The prebionts are precursor structures that might have evolved into the first living cells.

1. Introduction

Surface-active compounds tend to concentrate at interfaces and form aggregates in solution. Such compounds have both polar and nonpolar groups in their chemical structure and are often referred to as amphiphilic molecules. The Surface-active molecules are of interest because they are present in aerosols and influence the surface tension of haze and cloud droplets (Shulman et al., 1996; Kiss et al., 2005). When exposed to aqueous phases, they often self-assemble into micelles, vesicles, and a variety of other supramolecular structures. Surfactant assemblies can provide local conditions that are very different from the bulk medium, which can markedly affect the rate of chemical reactions and change product distributions (Yoshida et al., 2000; Vane and Giroux, 2000). Because of their central role in forming biological membranes, amphiphilic compounds have been proposed to provide compartments that would be essential for the origin of cellular life. For instance, the Murchison meteorite contains amphiphilic compounds that assemble into boundary membranes (Deamer, 1985; Deamer and Pashley, 1989).

Titan, Saturn's the largest moon, has a nitrogen-methane atmosphere that may be analogous to the Earth's atmosphere shortly after primary accretion. Previous investigations have demonstrated that a complex organic chemistry is occurring in Titan's atmosphere, and Cassini-radar observations hint at the existence of an internal water-ocean (Stofan et al., 2007; Griffith et al., 2003; Raulin et al., 2012). Laboratory simulations of Titan's atmosphere have shown that a dark reddish-brown high-molecular-weight polymer named “tholin” is produced when the gas mixture is exposed to ultraviolet irradiation or electrical discharge (Sagan and Khare, 1979; Khare, 1986; Cable et al., 2012). The primary aim of this research is to investigate the physical and chemical properties of laboratory produced tholin, particularly whether surface-active substances are present and assemble into microstructures.

2. Materials and methods

A liposome kit composed of L-α-phosphatidycholine/stearylamine/cholesterol was purchased from Sigma-Aldrich, as was stearylamine.

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Stearonitrile was purchased from Santa Cruz Biotechnology, Inc. The solvents chloroform and ethanol were of spectroscopic grade from Sigma-Aldrich. Stock solutions of stearyl amine, lipid mixtures and stearyl nitrile were prepared in chloroform.

2.1. Tholins production

Tholin was produced according to the procedure described earlier (McKay, 1996). Pre-mixed $CH_4/N_2=10/90$ gas mixture (purity $>99.99\%$, Matheson Tri-Gas) was introduced into a custom made reaction vessel equipped with three sets of tungsten electrodes, each with a 0.4 in. arc gap. The gas mixture was exposed to electrical discharge from a tesla coil (model BD-10). The flow rate is set such that the pressure of the chamber is maintained slightly above ambient ($\sim$1150 mb). The apparatus was operated at room temperature continuously for three weeks and accumulated a film of brown material (tholin) on the walls. Several hundred milligrams of tholin were scraped off the walls of the reaction vessel under atmospheric conditions, placed in glass vials and stored in vacuum dessicator for use in experiments.

2.2. Two-dimensional thin layer chromatography

Tholin samples were extracted in chloroform-methanol 8:2 by volume, and the extracted sample was further separated by two-dimensional thin layer chromatography. First, 21 mg of tholins was dissolved in 2 mL of 8:2 chloroform/methanol and stirred vigorously by vortexing for 10 mins at room temperature. An aliquot of the chloroform-methanol dissolved tholin was spotted in the lower left-hand corner of a (20×20 cm Silica gel 60 F_{254}) TLC plate. The first chromatographic run was performed with chloroform. The second run was performed with 4:1 heptane-butanol at 90° to the original direction. After viewing under UV-light and labeling, the fluorescent spots on the TLC plate were recovered by scraping them from the plate along with the silica, followed by extraction in chloroform. Atmospheric oxidation might have altered some of the components of the tholin. The chromatographic patterns of the chloroform soluble tholin sample were not altered even after repeating the experiment number of times. This suggests that the chloroform preparation of tholin did not further oxidize the sample.

2.3. Fourier transform infrared spectroscopy analysis (FT-IR)

Measurements by infrared spectroscopy were performed on the chloroform dissolved nonpolar fractions of tholin. The faint yellow fraction was deposited onto a ZnSe window by repeated drop-wise deposition and chloroform evaporation. The final thin film samples were dried at room temperature under vacuum for at least 2 h to evaporate the chloroform from the samples. To minimize oxygen exposure the samples was analyzed immediately. Measurements were carried out 3–4 times to check the reproducibility of the results, and the blanks which included entire procedure except sample were also run. Measurements were performed with a Thermo-Nicolet, Nexus 670 FT–IR spectrometer over the region of 4000–400 cm^{-1} with 4 cm^{-1} spectral resolution. The FT–IR system was continuously purged with dry air.

2.4. Surface pressure measurements

A computer-controlled Langmuir-type film balance (Micro-Through XS, Kibron Inc.) was used to record pressure–area (π–A) isotherms of non-polar components of tholin adsorbed at the air/water interface. To eliminate organic contaminants the glassware were heated in high temperature oven at 500 °C. Prior to experiments the trough was cleaned with pure water and ethanol. After filling the trough with MiliQ water, impurities were removed by vacuum suction of the top layer with a pasteur pipet after compression of the barriers to a minimal area. Monolayers were obtained by spreading a certain amount of chloroform solution of fraction 1 and 2 on the trough subphase. After the solvent evaporated, the film was compressed at a rate of 60 A^2/chain/min at room temperature. For comparison, we also studied the surface–area per molecule (π–A) isotherms of the lipid films such as stearyl amine and stearyl nitrile by spreading chloroform solutions on the surface of an aqueous subphase. The measurement was carried out 3–4 times to check reproducibility of the results. The monolayer of tholin fractions was also transferred onto silicon substrates and CaF_2 for Scanning electron microscopy (SEM) and Infrared (IR) characterization, respectively, by a vertical dipping method. CaF_2 and silicon substrates were extensively cleaned before use. The Langmuir–Blodgett films were dried in a desiccator at room temperature for one day.

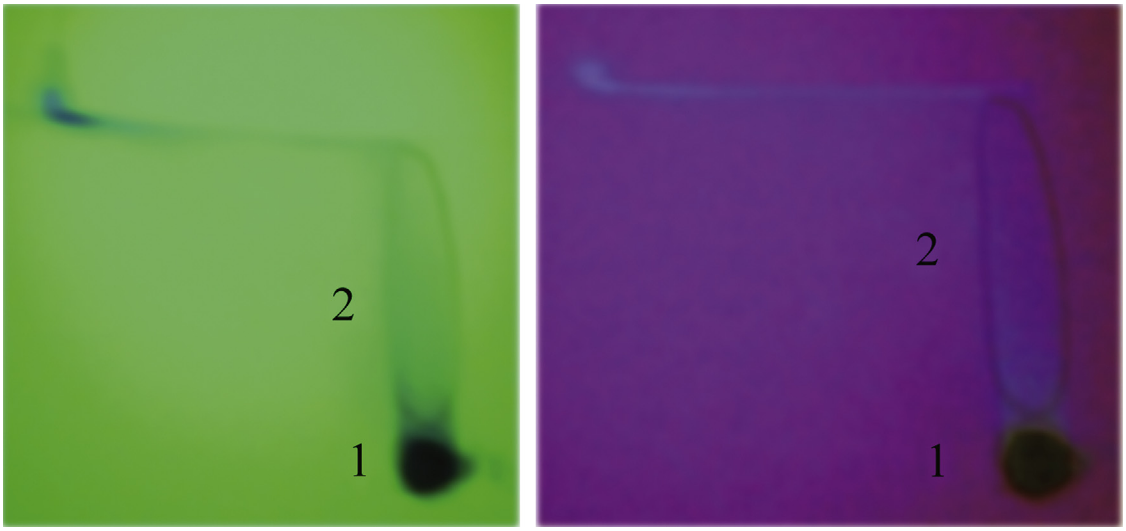

Fig. 1. 2D –TLC of tholin chloroform-methanol extracts. Left panel shows illumination with 254 nm UV light. Compounds that absorb UV of this wavelength produce dark areas against the green fluorophore embedded in the silicic acid of the TLC medium. Right panel shows fluorescence excited by near UV illumination.