State-of-the-art instruments for detecting extraterrestrial life

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"Surely one of the most marvelous feats of 20th-century science would be the firm proof that life exists on another planet. In that case, the thesis that life develops spontaneously when the conditions are favorable would be far more firmly established, and our whole view of the problem of the origin of life would be confirmed." Stanley Miller and Harold Urey wrote that in 1959 (1). Unfortunately, their dream has not been realized, and as we begin this new millennium the question of whether life exists beyond the Earth remains unanswered. However, there are reasons for optimism that in the not-too-distant future we may have an answer.

In assessing the possibility that life either exists now, or has in the past, on some other planet or moon, we must be able to evaluate whether the conditions there are, or were, compatible with life as we know it on Earth. As Alfred Russel Wallace emphasized at the beginning of the 20th century (2), the first requirement for life is liquid water; without it, as far as we know, life is impossible. The same reasoning also applies to organic compounds. Carbon-based polymers such as nucleic acids and proteins make up the core molecules required to carry out the central biological functions of replication and catalysis. Without these functions, life as we know it could not exist.

Life in Our Solar System

In 1990, a year after its launch, the Galileo spacecraft had circled the sun and flew within 960 km of Earth to give it the extra gravitational push needed to reach Jupiter. As Galileo passed by its home planet, it made a series of observations to test its onboard instruments. It detected abundant oxygen in the Earth's atmosphere, along with the chemically incapable components ozone and methane. Water in the form of vapor, ice, and oceans was detected on the Earth's surface. Galileo's camera took pictures showing extensive land areas covered with some sort of pigment. Narrow-band, pulsed radio transmissions were detected. Carl Sagan and the other scientists who conducted this remote sensing study of Earth from space concluded that taken together this evidence implied that not just life, but intelligent life, existed on Earth (3). Of course we knew that already.

Detecting the existence of life elsewhere in the solar system and beyond will not be as simple as the Galileo-based investigations of Earth. If Earth is a typical example, only photosynthetic life will enrich an atmosphere with abundant oxygen, and the oxygen build-up requires perhaps billions of years to take place. As we search for life elsewhere, we do not want to miss detecting less advanced, primitive life that has not yet developed the capacity for photosynthesis, or even oceans rich in the prebiotic ingredients considered necessary for the origin of life.

Consider what a Galileo-like spacecraft would have observed if it had swung by the Earth 3 billion years ago. Life had evolved to the point that photosynthesis had been invented, but significant amounts of oxygen had not yet accumulated in the atmosphere. Any life on the land surfaces was confined to environments protected from the intense UV radiation that penetrated to the surface and thus probably would not be detectable from space. It is uncertain whether the spacecraft would have detected the presence of life on Earth at this time. What would the spacecraft have detected if it flew by the Earth 4 billion years ago?

Spacecraft have explored all of the planets in our solar system except Pluto, and so far there are no signs of extraterrestrial life. Nevertheless, optimism persists. Whether life originated on Mars, and perhaps even still exists there today, remains an area of intense debate. Although we have sent spacecraft to Mars to search for life and analyzed Martian meteorites for possible evidence of life, these efforts have left us with no definitive answers. With increasing evidence of a large subsurface water ocean on the Jovian moon Europa, there is the possibility that this body could have a primordial soup similar to that which gave rise to the origin of life on Earth (4), or potentially even harbor living organisms (5).

It is important that we not just focus on searching for the presence of life. So far, meteorites have provided us with a wealth of information about the prebiotic chemistry that took place in the early solar system. To enhance our understanding of this chemistry, the direct analyses of the organic constituents of asteroids, comets, and moons also must be carried out. Titan, the largest moon of Saturn, may be a giant active prebiotic chemical factory, although a chilly one. The inventory of organic compounds that has been synthesized on Titan may give us a glimpse of the degree of molecular complexity that abiotic chemical reactions can generate over long periods of time. We will learn more about Titan organic chemistry when the Huygens probe onboard the Cassini spacecraft descends through the Titan atmosphere and lands on its surface in November 2004 (6).

Life on Mars?

During the coming decades, the search for evidence of extinct or extant life on Mars will be a central focus of both the National Aeronautics and Space Administration (NASA) and European Space Agency (ESA) as a flotilla of spacecraft explore the Martian surface and return Martian samples back to Earth for comprehensive state-of-the-art analyses. The challenges are daunting. Missions to Mars are costly and risky, as evidenced by the recent losses of the Mars Climate Orbiter and Mars Polar Lander spacecraft. Collecting and returning samples to Earth, while appealing because of the direct hands-on analytical advantages they provide, are constrained by the amount of material that can be returned and sample containment issues related to potential biological hazards associated with possible extant Martian organisms being transported back to Earth. Spacecraft-based robotic instruments designed to carry out direct analyses for evidence of water, prebiotic chemistry, or biologically derived molecules are constrained by mass and power limitations, as well as challenges associated with obtaining samples suitable for analysis.

There is still considerable uncertainty about whether water in amounts sufficient

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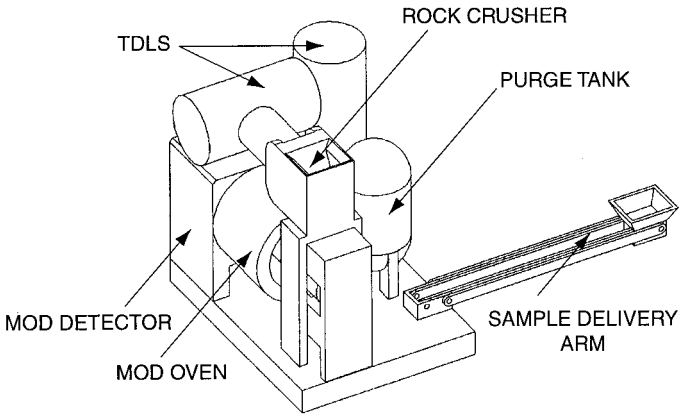

Fig. 1. The 2003 MOD instrument. The purge tank is used to flush the MOD oven and attached TDLS with an inert gas such as argon between analyses. The peak power requirement is about 28 W. The base plate dimensions are 11 cm × 14 cm; the height is about 15 cm. The entire instrument weighs ≈2 kg and fits in the palm of your hand.

to support life has ever existed on Mars. Evidence suggests that liquid water flowed, accumulated, and evaporated on Mars billions of years ago (7, 8) and perhaps even more recently (9). One of the major goals in the Mars exploration program is to provide a better assessment of the amount of water present on the planet. This appraisal is important not only for evaluating the possibility that life may have once existed, or still exists, on Mars, but also for providing information about potential *in situ* resources available for future human explorers.

The possible presence of organic compounds on Mars is also uncertain. Using a pyrolysis procedure, in combination with a gas chromatograph/mass spectrometer (GCMS), Viking did not detect any organic compounds above a level of a few parts per billion in near surface samples at two different landing sites (10). However, it is now apparent that the Viking pyrolysis GCMS instruments would not have detected the presence of millions of bacterial cells in 1 g of soil (11). In addition, oxidation reactions involving organic compounds on the Martian surface would likely produce nonvolatile products that also would not have been detected by the Viking GCMS (12).

Meteorites from Mars have been extensively investigated to assess whether they contain organic compounds possibly derived from life. Unfortunately, contamination of Martian meteorites by terrestrial organic compounds greatly compromise these investigations (for example, see ref. 13 and references therein). If there is any indigenous organic material present in Martian meteorites, it appears to be derived from the infall of carbonaceous meteorites rather than from Martian biology (14). The problems associated with terrestrial contamination underscores the importance of doing *in situ* organic compound analyses on Mars before samples are returned to Earth, where even under the best of circumstances they will be exposed to some level of terrestrial contamination.

Because amino acids are the building blocks of proteins and enzymes in terrestrial organisms, they are excellent target compounds in the search for life on Mars and elsewhere. While it is not certain that Martian biology would use the exact same set of amino acids as life on Earth, their ubiquity as constituents of organic material in the solar system (15) suggests that amino acids would have been available for incorporation into living entities on Mars just as they were on Earth. Amino acids derived from either extinct or extant life, and from the infall of meteorites and cosmic dust (16), could be present on the surface of Mars.

Another class of organic compounds of interest are polycyclic aromatic hydrocarbons (PAHs). Although PAHs have no known role in biochemistry on Earth, they can be produced from the long-term (tens of millions of years or more) degradation of biologically derived organic compounds (17). PAHs have been identified in the interstellar medium (18) and in carbonaceous meteorites (15). They may be the most abundant single class of organic compounds in the universe. Given the infall of meteorites and cosmic dust throughout the history of Mars, PAHs could be one of the organic components of the Martian surface, especially if retrieved samples contained fragments of carbonaceous chondrites.

Analyses for Organic Compounds on Mars

To evaluate whether organic compounds are present on Mars, *in situ*-based analytical techniques that have sensitivities several orders of magnitude greater than the Viking GCMS are required. In addition, given the limitations of available resources (especially money!), instruments must be compact and able to detect the targeted compounds without requiring extensive sample processing.

The Mars Organic Detector (MOD) is an instrument that has been developed to search for traces of the key organic compounds, amino acids/amines, and PAHs, directly on the Martian surface (19). MOD is based on the following concepts: (*i*) amino acids and PAHs can be directly sublimed from natural samples by heating to 450°C under partial vacuum, thus eliminating the use of the aqueous reagents and organic solvents used in laboratory analyses; (*ii*) sublimed amino acids condensed on a cold finger coated with a reagent specific for amino acids can be detected at very high sensitivities by using UV fluorescence; and (*iii*) sublimed PAHs can be directly detected on the cold finger because they are naturally fluorescent when exposed to UV light.

Laboratory experiments have demonstrated the feasibility of these concepts. A mixture of dry pure amino acids, or a crushed sample of a fossil or ocean sediment, was placed into a sublimation apparatus, which then was evacuated to 5–6 torr to approximate Martian atmospheric pressure (20). A cold finger was cooled to −195°C with liquid nitrogen, and the apparatus was heated to 450°C for various time periods ranging from 30 sec to several minutes. The material that sublimed onto the cold finger was then analyzed. No decomposition into amines, which are produced by amino acid decarboxylation, was observed with the pure amino acid mixtures. The behavior of amino acids in fossils and sediments during sublimation was found to be more complex than pure amino acid mixtures. Nearly all of the amino acids originally present in the samples decomposed into amines during the heating step. However, the amines readily sublimed onto the cold finger and could be detected by using the same methodologies used for amino acid detection. Thus, even when amino acid decomposition to amines is significant, the presence of amino acids in the original sample can still be inferred.

Primary amines were found to react with the reagent fluorescamine coated on a cold-finger surface to yield intensely fluorescent derivatives. Experiments indicate that the fluorescamine reaction proceeds in the dry state; no solvent is necessary. Amino acid/amine detection limits with this method are in the 10^{-13} mole range. Thus, even if amino acids were present in a Martian sample at a level of a few parts per trillion, they would be detectable by the fluorescamine-based method.

PAHs also readily sublime under the same conditions used for amino acids. The detection of sublimed PAHs can be carried out directly on the cold finger without the need for derivatization reagents because these compounds are extremely fluorescent when irradiated with near UV light. Detection limits are in the femtomole (10^{-15} mole) range.

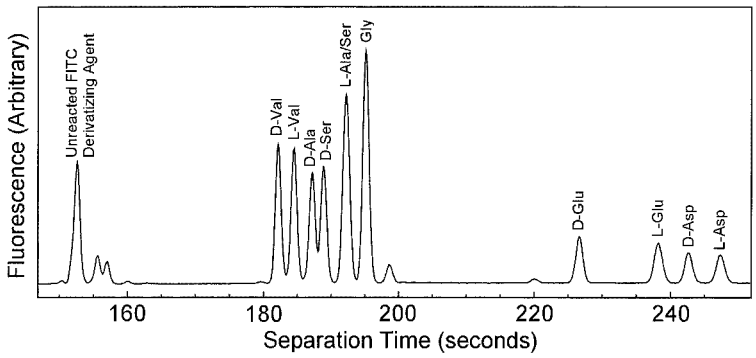

Fig. 2. Baseline resolution of several amino acid enantiomers using the μCE chip system (based on results presented in ref. 24).

A MOD design recently was selected for the definition phase of the 2003 NASA HEDS (Human Exploration and Development of Space) lander instrument package for fundamental biology and *in situ* resource utilization (unfortunately, this mission is now cancelled). The various components of the 2003 MOD instrument (see Fig. 1) consist of a delivery arm and a rock crusher; an organic detector, consisting of a sublimation cell, a chemical detector, and a fluorescence analyzer; two timable diode laser spectrometers (TDLSs), which each have a Herriott cell, a dual laser system; and a miniature capacitance manometer and Pirani gauge to measure absolute pressure. During the operational sequence, the sample delivery arm first receives a sample that then is dropped into the rock crusher where it then is pulverized before being transferred to the MOD oven for analyses. After closing the oven at Mars ambient pressure, the crushed sample will be stepwise-heated to 950°C. Amino acids and PAHs in the sample will be sublimed and collected on a cold finger (located at the rear end of the oven), which is cooled to Mars nighttime temperatures (around −100°C). The sublimed target compounds are detected by using laser-based fluorescence sensors with sensitivities in the 10 parts per trillion range, which is 100 times more sensitive than the Viking GCMS.

During the same heating sequence used to sublime the target organic compounds, bound water, along with carbon dioxide produced from the decomposition of various carbonate minerals, will be released from the samples. The quantities of water and carbon dioxide, and their isotopic compositions, evolved are determined by a TDLS directly connected to the MOD oven.

The second TDLS uses an open-path sample cell to measure the water and carbon dioxide content and carbon dioxide isotopic composition of the Martian atmosphere at the landing site. On Earth, atmospheric carbon dioxide is depleted in the isotope carbon-13 relative to surface carbonates because of the presence of a large surface reservoir of isotopically light biologically derived organic carbon (21). Finding an isotopic offset between atmospheric carbon dioxide and regolith carbonates on Mars would provide indirect evidence for the existence of a reduced organic carbon reservoir, a finding that could be indicative of either past or present Martian biology, or the presence of organic matter derived from carbonaceous meteorite accumulation.

MOD: The Next Generation

An important aspect of amino acid analyses of Martian samples is distinguishing those produced abiotically from those synthesized by either extinct or extant life. Amino acid homochirality provides an unambiguous way of distinguishing between abiotic vs. biotic origins (see ref. 22 and references therein). Proteins made up of a mixture of D- and L-amino acids would not be efficient enzymes because they could not fold into bioactive configurations such as the α-helix. However, enzymes made up of all D-amino acids function just as well as those made up of only L-amino acids, but the two enzymes use the opposite stereoisomeric substrates. There are thus no apparent biochemical reasons L-amino acids would be favored over D-amino acids. On Earth, the use of only L-amino acids in proteins by life is probably simply a matter of chance. We assume that if proteins and enzymes were a component of extinct or extant life on Mars, then amino acid homochirality would have been a requirement. However, the possibility that Martian life was (or is) based on D-amino acids would be equal to that based on L-amino acids. The detection of a nonracemic mixture of amino acids in a Martian sample would be strong evidence for the presence of an extinct or extant biota on Mars. The finding of an excess of D-amino acids would provide irrefutable evidence of unique Martian life that could not have been derived from seeding the planet with terrestrial life (or the seeding of the Earth with Martian life). In contrast, the presence of racemic amino acids, along with nonprotein amino acids such as α-aminoisobutyric acid and racemic isovaline, would be indicative of an abiotic origin, although we have to consider the possibility that racemic amino acids were generated from the racemization of biotically produced amino acids (23).

A potential impediment to the search for life on Mars is the forward contamination of the planet by a spacecraft with either terrestrial organisms, or more likely terrestrial biomolecules. This problem would be of great importance in assessments of whether there are any amino acids indigenous to Mars. Because of the distinctive mixture and the L-enantiomeric signature of amino acids associated with terrestrial life, chiral amino acid analyses can be used to monitor the level of forward contamination of Mars that occurs during the course of planetary exploration. A long-range monitoring program would provide a critical baseline data set for assessing forward contamination during the eventual human exploration of Mars.

A relatively new technology that shows promise for spacecraft-based amino acid enantiomeric analysis is microchip-based capillary electrophoresis (μCE). With μCE, both the identity and enantiomeric composition of amino acids can be determined at subpart-per-billion levels. The μCE-based analyses are about an order of magnitude faster than analytical methods such as conventional CE and HPLC. In addition, μCE has a detection limit more than 3 orders of magnitude better than conventional HPLC. Thus, proportionally smaller samples ($\approx$100 picoliter or 10^{-10} liter) can be analyzed.

A μCE chip system has been used to explore the feasibility of using such devices to analyze for amino acid enantiomers in extraterrestrial samples (24). The test system consisted of a folded electrophoresis channel (19.0 cm long × 150 mm wide × 20 mm deep) that was photolithographically fabricated in a 10-cm-diameter glass wafer sandwich, coupled to a laser-excited confocal fluorescence detection apparatus providing subattomole ($<10^{-18}$ mole) sensitivity. The μCE analysis system consists of a stack of wafer scale components, which individually provide the liquid flow channels, the capillary separation zones, the electrophoretic controls, the fluid reservoirs, and the detection system. This μCE system is more than an order of magnitude smaller in size than conventional laboratory bench-top amino acid analytical instruments. Analysis times with μCE are on the order of a few minutes compared with almost an hour for HPLC-based analysis.

A critical aspect is that enantiomeric ratios can be rapidly and accurately determined by using the microfabricated CE chip instrument. Using a SDS/γ-cyclodextrin, pH 10.0 carbonate electrophoresis buffer and a separation voltage of 550 V/cm at 10°C, baseline resolution is observed for the enantiomers of valine, alanine, glutamic

acid, and aspartic acid in only 4 min (see Fig. 2). Enantiomeric ratios of amino acids extracted from sediments and the Murchison meteorite using this μCE chip system closely matched values determined by HPLC-based methods (24).

For spacecraft-based μCE chip analyses, a microfluidics-based sample processing system is required to deliver an amino acid extract suitable for analysis. In a design scheme presently being tested, amino acids are first extracted from a sample by heating in hot water for about an hour, a procedure similar to that used to extract amino acids from meteorites in the laboratory (13). The aqueous extract obtained by this procedure is frozen and then sublimed at Mars ambient pressure onto a cold finger. The sublimed ice/amino acid mixture is thawed and collected in a reservoir interfaced with a μCE chip instrument. With this design, no desalting is required, thus eliminating a procedure that requires reagents and ion-exchange chromatography.

Life on Extrasolar Planets

To extend our search for life to extrasolar planetary systems, we must rely on infrared-based remote sensing technology to search for key molecules like water and chemically incompatible gases such as methane, carbon dioxide, and ozone in the atmospheres of extrasolar planets. But, the intense, blinding light from the host star presents a difficult obstacle for remote sensing instrumentation. This obstacle can be overcome, however, using interferometer techniques (25). With coordinated telescopes working in tandem and broadband destructive interference methods, the central starlight could be blackened out, or nulled, while leaving the dim reflected planet's light unaffected.

To carry out spectral analyses of the atmospheres of extrasolar planets, infrared interferometer telescopes would need to be space-based systems. To reduce the background from our solar system's zodiacal light, it may be necessary to make these observations at orbital distances much greater than at near Earth orbit. All of this is very, very expensive, with price tags approaching the $500 million range, but, it should be worth it. A space-based infrared interferometry system looking at our solar system from a distant star would be able to detect most of the planets in our solar system, including Earth, and determine the chemical composition of their atmospheres. Just like the Galileo spacecraft observed when it passed by Earth, finding an extrasolar planet with an atmosphere containing both ozone (and by implication oxygen) and methane would be an indication that not only does life exist there, but that it has likely had a long evolutionary history. Finding an Earth-like extrasolar planet with an atmosphere rich in methane and water would suggest the possibility for the prebiotic chemistry needed to set the stage for the origin of life. Futuristic space-based interferometers consisting of perhaps a 100 or more coordinated telescopes might even be able to determine the type of organic material present on the surfaces of promising extrasolar planets (26).

Unfortunately, searching for the presence of primitive living entities that have not evolved beyond the stage of self-sustaining, autonomous replication on extrasolar planets is impossible even with the most advanced interferometer-based methods. In addition, interferometry will not allow us to search for evidence of life in the places where it may be the most abundant: rogue Earth-like planets in the emptiness of interstellar space that were ejected from their parent stars soon after accretion (27). Earth-like rogue planets should have an inventory of radioactive elements sufficient to heat and melt their interiors. Because they retained their initial dense hydrogen atmospheres, heat leaking out of their interiors would not be lost rapidly into space. As a result, surface temperatures could be above the freezing point of water. Water vented to the surface could condense and oceans could form.

With the atmospheres of these rogue planets rich in hydrogen, both methane and ammonia should be plentiful and conditions similar to those used in the Miller spark discharge experiment (1) could exist. The potential for prebiotic syntheses and chemical and biological (?) evolution would last for billions of years on these interstellar planets before the heat-providing radioactive elements decayed away. What would the forces of evolution produce? How do we search for life on these rogue planets?

Conclusions

In the coming decades, state-of-the-art spacecraft-based instruments that can detect key components associated with life as we know it on Earth will directly search for extinct or extant extraterrestrial life in our solar system. Advances in our analytical and detection capabilities, especially those based on microscale technologies, will be important in enhancing the abilities of these instruments. Remote sensing investigations of the atmospheres of extrasolar planets could provide evidence of photosynthetic-based life outside our solar system, although less advanced life will remain undetectable by these methods. Finding evidence of extraterrestrial life would have profound consequences both with respect to our understanding of chemical and biological evolution, and whether the biochemistry on Earth is unique in the universe.

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