Chemical methods for searching for evidence of extra-terrestrial life

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This paper describes the chemical concepts used for the purpose of detecting life in extra-terrestrial situations. These methods, developed initially within the oil industry, have been used to determine when life began on Earth and for investigating the Moon and Mars via space missions. In the case of Mars, the Viking missions led to the realization that we had meteorites from Mars on Earth. The study of Martian meteorites in the laboratory provides tantalizing clues for life on Mars in both the ancient and recent past. Meteorite analyses led to the launch of the Beagle 2 spacecraft, which was designed to prove that life-detection results obtained on Earth were authentic and not confused by terrestrial contamination. Some suggestions are made for future work.

Keywords: biomarker; carbon skeleton; isotope

1. Historical studies

There are a variety of concepts involved in using chemical methods to detect evidence of life during laboratory studies of samples on Earth or via instrumentation on planetary lander spacecraft. They comprise indentifying exact chemical structure, measuring precise stable isotope composition or a mixture of both.

2. Introduction

Perhaps, the second most important molecule concerning living things on Earth is (i) chlorophyll-a. It fulfils the function of translating the energy carried by sunlight into chemical energy stored as carbohydrates. In doing so, it removes carbon dioxide from the atmosphere and replenishes the oxygen. The identification of the chlorophyll nucleus dioxyphylloerythroetioporphyrin in sediments and oils was a

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landmark in realizing that the study of organic matter in the geosphere could be a method for determining when life began on our planet or by extrapolating the detection of extra-terrestrial biology.

3. Chemical fossils, carbon skeletons and biological markers

Studies of the occurrence of the porphyrin nucleus in petroleum samples first performed in the 1930s, by Alfred Treibs [1], soon led to the concept of the ‘chemical fossil’—an organic molecule with a ‘carbon skeleton’ having stability over geological time scales. However, to be recognizable as a fossil the skeleton needs to be sufficiently closely related to a known biological precursor. The structural changes that have occurred in the transition from the biosphere to the geosphere need to be explicable in terms of simple processes involved in diagenesis and maturation of sedimentary rocks.

On this basis, the porphyrin nucleus, central to the structure of chlorophyll, is a perfect example of a chemical fossil. Straight chain terpenoid hydrocarbon molecules, such as (ii) pristane and (iii) phytane, which clearly reflect the phytal side of chlorophyll, are also regarded indisputably in the same category.

The multiple ring systems, associated with naturally produced steroids and terpenoids, when the functional groups are removed, can lead the sterane and triterpene hydrocarbons that are similarly recognized as excellent chemical fossils. Perhaps more surprising is the acceptance of normal straight chain aliphatic hydrocarbons in this respect. These simpler hydrocarbons in low maturity oils [2] have odd to even predominance because they are the remnants of fatty acids biosynthesized from two carbon units—building blocks provided by acetyl coenzyme A. Thus, the straight chain hydrocarbons are the product of a
decarboxylation, and the chain length both reduces and loses its odd number preference by thermal cracking as samples undergo further maturation, so that mature oils have an almost Gaussian distribution about a mean chain length.

It can easily be appreciated that a carbon skeleton, appropriate as a chemical fossil, readily becomes what is described as a ‘biological marker’ where the specificity of structure is thus considered as indicating the intervention of some kind of biological agency. In contrast, it can be appreciated that, because carbon is tetravalent, a collection of 25–30 carbon atoms could combine abiogenically in an enormous number of ways; in theory, they could combine randomly to generate more different molecules than the number of stars in the observable universe.

Some of the molecules suggested as biological markers are not necessarily the best species to use for detecting ancient life, terrestrial or extra-terrestrial. Amino acids, possibly the most biologically significant molecules on Earth, are not good chemical fossils because they have carbon skeletons that are not readily retained in sediments over long periods of time. Their multiple heteroatom functionality greatly reduces stability. Polycyclic aromatic hydrocarbons (PAHs) are very stable and can resemble steroids and triterpanes, but a simple route from identifiable biological precursors is not obvious. The fact that PAHs are observed widely in extra-terrestrial environments [3] would tend to argue for an abiogenic provenance.

Over the years since Treibs’ seminal studies, the subject of chemical fossils and biological markers has mushroomed so that reviews of the field occupy volumes rather than large papers [4].

4. The origin of life on Earth

The study of chemical fossils/biological markers involves solvent extraction followed by gas chromatography (GC) and mass spectrometry (MS) and combined as GC–MS. One of the first applications of the concept was an attempt to answer the question of when life began on Earth. The idea hinged on co-locating biological markers with discrete microstructures seen by electron microscopy in Precambrian rocks to corroborate the interpretation that the latter features were indeed fossil algae/bacteria. The problems presented in recognizing and authenticating ancient microfossils were highlighted during a recent Royal Society Discussion Meeting [5]. On numerous occasions appropriate organic compounds have been found in the fossiliferous rocks but it has been recognized that the soluble organic compounds could have been mobilized to these locations by fluid transfer. Thus, an organic compound identified in an ancient sediment could be much more modern. The putative fossil and the biological marker might not be cogenetic and interpretations of what the data mean in terms of the origin of life are questionable.

5. Life on the Moon?

About the time that work was taking place on the Earth to recognize the planet’s earliest life forms (the mid- to late-1960s), an international community of organic geochemists applied for and received samples returned by the Apollo lunar missions for studies that aimed to answer age-old questions about the Moon.
If the lunar Mare were dried-up seas, they might reveal vestiges of life processes from a time before that covered by the terrestrial geological record. In order to have a meaningful study, the material allocated was processed in the lunar receiving laboratory with a great deal of effort expended to exclude terrestrial contamination by using a system of glove boxes and a giant vacuum system. In some cases, the samples were handled in laminar flow cabinets fed with dry filtered nitrogen. Thereafter, the groups recruited for the study all devised ways of minimizing the possibility of contamination of the samples from terrestrial biological sources in their own laboratories. Note that the worst problem associated with extra-terrestrial life detection by chemical methods is the spectre of terrestrial contamination. The methods envisaged to exclude the possibility of obtaining erroneous results from a terrestrial source tend to dominate planning for the necessary work.

At the University of Bristol, the lunar samples [6], which had been transported from Houston triple-bagged, were opened in a clean room on a clean air bench and transferred rapidly into a sealed and precleaned glass system designed to allow all the operations normally applied when studying terrestrial specimens. A variety of purified solvents and reagents (needed to make less-volatile compounds, e.g. fatty acids, into derivatives that could be vaporized at moderate temperatures) could be introduced from selectable reservoirs. When any liquid/solution needed to be transferred or reduced in volume, the necessary actions were achieved by diverting a pressurized flow of pure nitrogen.

With all the above precautions in place, it was shown that a 10g sample of the Apollo 11 soil did not contain molecules accepted as biological markers, at a level above 1–2 ppb [6]. Other groups using their own versions of the above technique confirmed the findings. One group [7] searched for the existence of porphyrins and, although trace amounts were found, the results were generally considered to be contamination.

The lunar soils from all the missions which returned specimens from the Moon, that is, six manned Apollo expeditions and all three Russian robotic probes, however, contain carbon up to a maximum of about 200 ppm. An interpretation of the preliminary results from an Apollo 11 sample erroneously described the lunar regolith as ‘like a lean Earth soil’, implying a possible organic carbon contribution. Later, detailed experiments, including the analysis of fractions of sieved soils (sometimes called finest fines), showed that the carbon (and the nitrogen) in the regolith is surface area-correlated. The two elements exist primarily as atoms implanted into the outer few nanometres of mineral crystals by the flux of ions emanating from the Sun, known as the solar wind [8].

6. Viking missions to Mars

It was not really surprising that the Moon showed no evidence of any biological processes. However, the next attempt to detect life in the solar system was entered into with considerably higher hopes. In 1976, two Viking spacecraft landed on Mars. Both had been sterilized by heating to nearly 130°C for over 50 h before take off. Each was carrying a package of three experiments which were designed to reveal the presence of living micro-organisms from the recognition of metabolic waste products. When these experiments were carried out there was evidence of
some kind of ‘activity’ within the Martian soil. Just as for the preliminary results from the Moon, the immediate temptation was to claim a positive identification of something akin to life. Fortunately, Viking carried a back-up geochemical experiment that took samples of Martian soil and heated them, the evolved gases being fed into a GC–MS. Analysis of the carrier gas effluent showed no evidence of the organic molecules that would have been expected from the decomposition of microbiological material. Without evidence of a ‘body’, living or dead, it was reluctantly concluded that the very surface material on Mars had a property whereby a chemical effect was possibly able to mimic a biological one. The experimenters had not anticipated that the surface materials on Mars might be highly oxidizing, although, with hindsight, the fact that Mars has always been known as the Red planet should have provided a clue that iron-containing minerals would be in a ferric rather than a ferrous state.

7. Further work on the origin of life on Earth

To overcome the possibility of geologically derived contamination (material from a modern epoch overprinting an older generation of rocks) to pursue the quest for the origin of life on Earth, organic geochemists focused on high-molecular-weight organic macromolecules that can be considered as immobile. Such material in sediments is often termed ‘kerogen’ and is formed because the thermal processing of biological molecules, during gradual maturation, leads to the cross-linking of smaller soluble molecules into ever larger ones by the combination of free radicals produced by defunctionalization. With increased geological processing, kerogen more and more closely approximates to elemental carbon, with a few heteroatoms. The latter effect makes kerogen useless in one respect for origin of life investigations because while pyrolysis (heating to temperatures ca 500°C and greater) of the macromolecule can regenerate small organic molecules, their structures are far removed from the carbon skeletons of the original biomolecules and the chemical fossils.

Kerogens from the Earth’s oldest sedimentary rocks, however, can be used in a different way to indicate the existence of an ancient active biology [9]. Thanks to an enormous body of work by Schidlowski, it has been recognized that kerogens carry a carbon isotopic signature of life processes. By studying the relative carbon isotope compositions\(^1\) of carbonates and organic debris in sediments of ages from contemporary to ca 3.9 Byr ago, Schidlowski has recognized that the isotopic composition of the carbonates closely approximates the \(^{12}C/^{13}C\) ratio in the terrestrial atmosphere while the organic component tends to concentrate the \(^{12}C\) (lighter) isotope of carbon (figure 1). In parallel studies, Schidlowski demonstrated that various biosynthetic routes lead to different \(^{12}C/^{13}C\) abundances but always tending towards enrichment in \(^{12}C\). A compilation of Schidlowski’s results (figure 1) demonstrates that the difference measured for coexisting carbonates and kerogens in rocks can be described as a biological marker.

\(^1\)Carbon isotopic compositions are measured relative to a known standard carbonate, thereby different carbon-containing entities can be compared with each other. Results are expressed as a delta (\(\delta\)) function in parts per thousand (‰). A positive value means enriched in \(^{13}C\); a negative value enriched in \(^{12}C\).
The difference is observed for all the samples he studied (ca 10000), and interpretation of his data suggests that life on Earth began almost as soon as the planet was ready to support it.

Schidlowski’s test for the existence of biology [10] can be applied to extra-terrestrial situations. For example, any aqueous fluid with dissolved carbon dioxide would be expected to precipitate carbonates during percolation through rocks. The dead bodies of micro-organisms associated in the water would be expected to be incorporated into the mineral phase. The situation described is a microscopic version of the production of organic-containing carbonate sediments formed by the settling of detritus in a water column.

8. Martian meteorites

Although following Viking there was a hiatus in terms of space missions to Mars, the search for evidence of life on the Red planet was able to continue through the serendipitous discovery of Martian meteorites. The realization that samples of Mars were available on Earth came about because a small family of meteorites called the SNC meteorites (after the initial letters of the type specimens of their subgroup, Shergotty, Nakhla and Chassigny) had crystallization ages that suggested they derived from a celestial body larger than the Moon. Thereafter, it was found that one of the 12 samples constituting this family, a meteorite found on Antarctica in 1979 (EETA79001), had shock-produced glass inclusions that contained trapped gases, which could be matched (1:1 correlation) [11] with the composition of the Martian atmosphere measured by the Viking landers during their descent to the planet’s surface (figure 2).

The relationship between this single meteorite and others of the SNC clan was sought, without success, by searching for other examples containing trapped gas. However, it has been categorically shown, by high-precision measurement of
the three isotopes of oxygen ($^{16}$O, $^{17}$O and $^{18}$O) within the silicate portions of different SNC meteorites, that the samples are genetically related and thus all Martian. Oxygen isotopic measurements have subsequently increased the number of Martian meteorites for study on Earth to over 40 [13].

While the efforts to identify a trace of atmospheric gas within SNC meteorites other than EETA79001 were unsuccessful, an experiment with the Nakhla meteorite (a specimen observed to fall in Egypt in 1911 and almost immediately collected) recognized small amounts of carbonate with a high (non-terrestrial) $^{13}$C abundance [11]. Despite having been studied by petrological microscope for more than 100 years, no carbonates had ever been observed and these meteorites were considered to be of igneous origin. The existence of carbonates was not readily accepted until carbonate-rich pockets of ‘white druse’ were recognized by microscopic observation in EETA79001 [14].

Analysis of ‘white druse’ by combustion in a pure oxygen atmosphere demonstrated that the material was relatively carbon rich, whereas the host rock was relatively carbon poor. The richest specimens analysed were characterized by an increase in the heavier isotope $^{13}$C. The data are shown plotted in figure 3 and can be explained in terms of a two-component mixture, one of which must be the carbonate.

The nature of the other component and the isotopic composition of the carbonate can be discerned by conducting the combustion in a stepped fashion (raising the temperature of samples incrementally, removing the carbon dioxide produced and adding fresh oxygen at each step). By employing such an experiment, it was found that the second component was some kind of organic matter which burned at a lower temperature than that at which the carbonate decomposed.
An unexpected development occurred when it was discovered that the Antarctic collection of meteorites included another Martian sample, ALH84001, that had previously been overlooked. Significantly, ALH84001 contained very large amounts of carbonate minerals as veins throughout the meteorite. Dissolving samples of the carbonate in phosphoric acid suggested that massive alteration by a carbon dioxide-containing fluid at moderate temperatures (10–90°C) had occurred [15]. Such a low temperature would have been conducive to biological activity. Stepped combustion confirmed that the carbonate was heavy ($\delta^{13}C$ ca +43‰) and that the meteorite contained isotopically light ($\delta^{13}C = -25‰$) organic matter. Shortly after these data were published, an electron microscope study of ALH84001 revealed features that might be tiny (100 nm in size) fossils [16].

The isotopic composition of carbonate in ALH84001 can be used to offer an interpretation of the two-component mixture in white druse found in EETA79001. To have a material balance, either the amount of organic matter in the mix needs to be relatively high to counter carbonate of +43‰, or it needs to be of low abundance but isotopically highly enriched in $^{12}C$.

An intriguing result obtained with a minute amount of material from ALH84001 has some bearing on this argument: replicate stepped combustion of a small black speck picked out of the ALH84001 carbonate revealed the presence of a component that burned at a temperature similar to carbonate decomposition but with a $\delta^{13}C$ of around $-60‰$, i.e. rich in $^{12}C$ (figure 4).

Organic matter known on Earth to be rich in $^{12}C$ is produced by fixation of carbon from methanogenic bacteria. On Earth, the finding of a very isotopically light kerogen would be indicative of a biological event involving primitive organisms. The Schidlowski diagram (figure 1) shows that such material was analysed during Schidlowksi’s study. Unfortunately, no other black specks have ever been encountered in the ALH84001 carbonate.
Figure 4. Stepped combustion of a ‘black speck’ extracted from ALH84001 carbonate (carbon abundance and isotopic composition versus combustion temperature). Solid line, C ppm/C; line with filled circles, $\delta^{13}C$.

9. Evidence for Martian life in Martian meteorites

The discovery of nanometre fossils in ALH84001 led to a media storm about the possibility of life on Mars. Their existence, however, has been questioned even though similar but less conclusive features have been observed in the Nakhla meteorite, as have carbonaceous tubes [17].

All the various pieces of evidence that Martian meteorites might contain evidence of past life on Mars have been queried. One of the most popular alternative explanations is that any organic matter observed is the result of contamination in the terrestrial environment (either before collection or since storage), which is ubiquitous on Earth. The burden of proof that the results are not owing to contamination has fallen on those who believe that the results reflect events that occurred on Mars. Some arguments against contamination are as follows: both the Antarctic meteorites involved (EETA79001 and ALH84001) were collected in relatively clean (low biological activity) conditions on the polar ice cap. Nakhla had a short-term exposure before collection, i.e. it was an observed ‘fall’ rather than a ‘find’. Work done on Nakhla with respect to fossils was carried out on interior portions of a stone that had not previously been cracked open. A radiocarbon study of Nakhla has shown that 80 per cent of the organic matter present contains no $^{14}C$, indicating that it predates the weapon tests of the 1950s ($^{14}C$ in the Earth’s atmosphere is artificial, having been created by atomic bombs) [18]. Eighty per cent of the organic matter in Nakhla is therefore likely to be indigenous to the meteorite. All the specimens used in the combustion experiments carried out with samples of EETA79001 and ALH84001 were prepared under clean room conditions and all the equipment used was precleaned by combustion. In the case of the reaction vessels, the cleaning
step was carried out at temperatures over 1200°C. ‘Blank’ experiments routinely
give amounts of extraneous carbon no more than a few nanograms in total. There appears to be no logical reason why white druse would be preferentially contaminated when compared with the host rock.

10. Beagle 2 and past life on Mars

At the present time, searching for nanometre-sized fossils is not possible by a
robotic analysis of Martian rocks and soils in situ on Mars. However, remote
analysis by combustion on a ‘clean’ spacecraft would be a route to circumvent
criticisms regarding terrestrial contamination of the meteorites. The latter
rationale was the raison d’être behind the Beagle 2 lander flown with the
European Space Agency’s Mars Express mission in 2003 (The Complete Guide to
Beagle [19]; copies available from the author). Beagle 2 carried a magnetic sector
mass spectrometer optimized for isotopic measurement connected to a stepped
combustion sample-handling system, which could repeat on Mars the laboratory
experiments done on Martian meteorites to obtain both carbon abundance and
isotopic data for selected samples. To avoid the difficulties encountered by Viking
owing to possible oxidizing conditions on Mars, Beagle 2 carried a ‘mole’ capable
of collecting subsurface samples (from depths down to 1.5 m) and perhaps more
significantly by burrowing under boulders not moveable by the high winds,
which are a feature of atmospheric activity of Mars. Beagle 2’s instrument
package included a pair of multispectral cameras, a microscope with 4 μm
resolution and chemical (XRF) and mineralogical (Mössbauer) spectrometers.
Using these auxiliary instruments, any sample investigated for evidence of
organic matter and/or carbonates would have been characterized as to its
suitability. Among the parameters that would be established for the rocks were
crystallization and cosmic ray exposure ages using interior fragments obtained
by a corer/grinder.

11. Beagle 2 and current life on Mars

Beagle 2 also carried a suite of environmental sensors to measure inter alia
wind speed, direction and atmospheric temperature. These would provide data in
support of an experiment designed to search for methane as a trace constituent in
the Martian atmosphere as an indicator of current life on Mars. The rationale for
this study is best explained by reference to Earth’s atmosphere where methane
occurs at the parts per million levels, although it should be absent because of
the oxidizing potential. The explanation is that methane is continuously
being replenished and the obvious source of fresh methane is biology. Even
methane produced by thermal degradation of kerogen from the geosphere
is ultimately of a biological origin because the kerogen is an amalgam of
biological detritus.

Beagle 2 included a system to process large volumes of Martian atmosphere
to concentrate methane for isotopic measurements. The experiment would have
been very valuable because the gas has been detected as an atmospheric species by
telescope observation from Earth [20]. While this clearly justifies the experiments
which were to be done by Beagle, the data obtained by telescope are only circumstantial evidence for a contemporary Martian biology; the latter cannot be proved by remote measurement at present. Additional data concerning the methane would be required. Carbon isotope and D/H measurements for the methane would be advantageous but not conclusive. The abundance and isotopic systematics of other species in the methane cycle, e.g. water, elemental carbon and atmospheric carbon dioxide, are needed to understand the underlying methane production method, biological or abiological. Such data were to be obtained by Beagle 2.

12. Exosolar planets

The study of methane in the Martian atmosphere has connotations for using this gas as an indicator of life on exoplanets via spectroscopic studies of their atmospheres. While the existence of methane in the atmosphere of planets in the habitable region of faraway stars would be thought-provoking, as has just been explained, for Mars methane data cannot be considered in isolation, they have to be considered in context.

13. The future

As has been discussed, there are very good reasons already for using isotopic measurements as a biological marker for organic matter contributed to the geosphere. There are two other areas where isotopic measurements can be expected to be important. The first concerns acquisition of isotopic compositions for individual organic compounds in an extra-terrestrial location such as Mars. This additional parameter might be able to distinguish indigenous molecules from terrestrial contamination, exactly as the carbonates in Martian meteorites may be considered as being generated wherever the meteorites come from because there are no carbonates on Earth having a similar isotopic signature. Such data might allow the recognition of organics from meteorites in fall expected to be present on the Martian surface because of proximity to the asteroid belt [21].

However, intermolecular isotope ratios cannot be used to provide other than circumstantial evidence of a biological origin. There is an alternative, intramolecular isotopic measurement, i.e. the distribution of isotopes with molecules. Such data are enormously difficult to acquire; the technique has been applied successfully only in the terrestrial laboratory [22] and was used to show that biochemical chain extension of alkanes (adding together units of acetyl coenzyme A) occurs by alternate carbon atoms having a different isotopic signature. The reduced and oxidized carbon atoms are easily distinguishable by different $\delta^{13}$ values. In consequence, if molecules from Mars (i.e. found during a robotic space mission or in a sample returned from the planet) had some non-random but repeatable isotopic pattern as opposed to a bland average composition, they could be considered as having a biological origin.
14. Conclusion

Finally, it should be said that using chemical methods for life detection has the advantage that every atom in all its forms can be used to provide meaningful information. The application of stepped combustion in tandem with high-precision isotopic measurements by mass spectrometer is an experiment that falls into this category. The fractionation of isotopes is governed by the laws of physics and therefore can be considered applicable throughout the Universe. Identification of specific molecules or classes of compounds is selective and there is nothing to justify a belief that biology in a location other than on Earth would be based on the same set of organic molecules as on our home planet. In retrospect, the metabolic experiments conducted by the Viking spacecraft were not universally applicable either. Only 2 per cent of terrestrial micro-organisms known on Earth can be grown or stimulated to multiply under laboratory conditions. Given that caveat, it would be fortuitous if the nutrients and growth conditions chosen for experiments on a planetary lander were coincidentally appropriate for unknown microbiology.

References

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