Raman spectroscopic analysis of arctic nodules: relevance to the astrobiological exploration of Mars

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Abstract The discovery of small, spherical nodules termed ‘blueberries’ in Gusev Crater on Mars, by the NASA rover Opportunity has given rise to much debate on account of their interesting and novel morphology. A terrestrial analogue in the form of spherical nodules of similar size and morphology has been analysed using Raman spectroscopy; the mineralogical composition has been determined and evidence found for the biological colonisation of these nodules from the spectral signatures of cyanobacterial protective biochemical residues such as scytonemin, carotenoids, phycocyanins and xanthophylls. This is an important result for the recognition of future sites for the planned astrobiological exploration of planetary surfaces using remote robotic instrumentation in the search for extinct and extant life biosignatures and for the expansion of putative terrestrial Mars analogue geological niches and morphologies.

Keywords Raman spectroscopy · Nodules · Extremophiles · Scytonemin · Carotenoids · AMASE

Introduction

The search for life signatures in early evolutionary processes informs the origins of life arising from a prebiotic world [1]. In our terrestrial geological history, the recognition of the presence of fossilised relic cyanobacterial organisms through their morphological traces is a key factor in the so-called ‘top-down’ approach to an understanding of the early biological colonisation and the prebiological chemistry of the evolving geosphere.

Cyanobacterial colonisation of geological substrates has been identified in our earliest terrestrial geological formations, extending from about 3.8 Gy, and it is clear that an understanding of the survival strategies and protection mechanisms being adopted by these organisms can provide some important clues as to the conditions that were operating on early Earth and in their adaptation to the changing evolutionary conditions [2, 3].

The survival of cyanobacterial extremophiles in stressed terrestrial environments is fundamentally dependent upon their synthesis of a specialised suite of protective biochemicals in response to desiccation; low-wavelength high-energy radiation insulation; extremes of pH, temperature and pressure and high concentrations of toxic heavy metal ions [4–6]. Our understanding of the roles of these chemical protectants reveals a sophistication in design for specific functions; for example, the ultraviolet radiation screening effectiveness of scytonemin in cyanobacterial sheaths which selectively absorbs in the UVA, UVB and UVC regions of the electromagnetic spectrum whilst permitting the transmission of the longer-wavelength
photosynthetically active radiation (PAR) that is necessary for the operation of chlorophyll in photosynthetic colonies. Other protectants, such as trehalose, which is synthesised in Antarctic cyanobacterial mats colonising salt-rich lakes [5, 6], are dualistic in their roles; trehalose is used as a water-replacement intracellular molecule which also combats osmotic stress caused by abnormally large concentrations of soluble salts such as calcium chloride. Similarly, a range of carotenoids can serve as light-harvesting pigments and also as cellular DNA damage repair agents under stressed conditions [4].

It is generally accepted that life started on Earth 3.8 Gya, when the environmental conditions on the surface were drastically different to the current situation. At that time, it was predicted that Mars had similar conditions to those pertaining on Earth, and this has led to the idea that life could also have appeared on Mars [7–10]. As the external conditions were changing on early Mars, as on Earth, organisms needed to adapt to new habitats and colonise even the most extreme niches using a range of response strategies. Those organisms that were able to survive in such hostile environments can be considered to have left signatures of their presence on Mars; it is fundamentally important, therefore, to the understanding of these complex processes to analyse the terrestrial analogues of possible Mars scenarios [11–14].

Cold deserts, such as Antarctica or the Arctic, have been proposed as the closest Martian analogues on Earth [15]. The extremely low temperatures, dry atmospheres, strong UV-radiation isolation, low nutrient availability or long periods without sunlight have not been obstacles in the adaptation and proliferation of several different types of microorganisms. The survival adaptations of microorganisms in these environments necessarily use both inorganic and organic strategies [16–18], especially involving a wide range of radiation protective pigments [19, 20].

Laser Raman spectroscopy is being adopted by National Aeronautics and Space Administration (NASA) and European Space Agency (ESA) as a part of an analytical instrumentation suite for Mars surface exploration, including the ExoMars mission scheduled for 2018 which will deploy one or two rovers on the surface of Mars; the rover(s) will sample the Martian atmosphere, searching for water and interrogating the subsurface geology with ground-penetrating radar and will be using a variety of analytical instrumentation for the recognition of extinct and extant life signatures in association with a detailed geological and mineralogical survey. The particular characteristics of Raman spectroscopy that make this technique a valuable instrument for the unambiguous identification of biological, biogeological and geological signatures [21] are primarily those of non-destructive analysis of specimen, ease of sample examination that does not involve any physical or chemical surface preparation and an ability to collect data from both biological and mineralogical entities in association.

Although, specifically, there is no single terrestrial Mars analogue site, the examination of a range of closely related extremophilic scenarios is therefore of potential relevance to the diverse situations that may be expected on Mars. The current study was undertaken on the geological and possible biological composition of spherical nodular structures found in sedimentary terrestrial rocks; the presence of residual protective biochemicals in such an ecological niche could be indicative of early life signatures [22] in extraterrestrial morphologies such as the ‘blueberries’ found in Gusev Crater on Mars.

Experimental

Raman spectroscopy

Spectra were collected using a Renishaw inVia Raman Spectrometer with a dedicated Leica DMLM microscope, using ×20 and ×50 objectives. The laser wavelength used was in the near-infrared at 785 nm for analysing both the host minerals and the organic protectant biomolecules produced by the cyanobacterial colonies. With a 10-s exposure time, a range of between 20 and 80 accumulations were achieved for improving signal-to-noise ratios in the spectra. The laser power was operated at values between 0.1 to 10 mW, depending on the compound analysed; for those specimens where there was a high risk of thermal degradation occurring, such as iron oxides, hydrated minerals or biomolecules, the lower laser power and spectral irradiance were used, whereas when there was a low risk of inducing structural changes, higher laser powers and spectral irradiance were selected for collecting spectra to minimise the acquisition times.

Specimens

Samples were collected from the Ebbaelva sandstone formation in Billeffjord (Svalbard, Norway), during the AMASE 2004 expedition (Arctic Mars Analogue Svalbard Expedition). The main outcrop shown in Fig. 1 was made up of a white laminated sandstone, which was dotted with small hemispherical depressions of around 1 cm diameter and 0.5 cm in depth; a sample from this outcrop containing fresh surfaces and these spherical structures (which we termed “negative nodules”) were analysed; a second sample collected from the underside of a ledge from the sandstone (Fig. 2) contained ‘positive nodules’ which appeared as
spherical convex structures together with a brownish weathered crust. In this second sample, the fresh host rock, the brownish crust as well as the inside of one of these positive nodules were analysed.

Finally, from the same outcrop, another sample was collected (Fig. 3), and this exhibited one endolithic (organisms living inside a host rock) and two more superficial chasmolithic (microorganisms living inside a rock fissure) communities. The endolithic organisms appeared as a continuous bluish-green band, at about 1 cm depth below the surface, while the two chasmolithic communities were present as patches of yellowish-green and red-coloured communities covering small cracks in the rock close to the endolith band.

Results and discussion

Host rock substrate and brown crust

The main component of the fresh rock was quartz, with characteristic Raman bands at 205, 261, 355 and 463 cm$^{-1}$ (Fig. 4, A); in addition, signatures at 141, 200, 393, 511 and 636 cm$^{-1}$ are characteristic of anatase (Fig. 4, D); the 701- and 262-cm$^{-1}$ bands have been assigned to muscovite and those at 506 and 477 cm$^{-1}$ to a feldspar. Raman spectra of small brown crystals, which appeared dispersed in the fresh rock matrix, showed signatures at 170, 290, 721 and 1092 cm$^{-1}$ and thus were unambiguously assigned to ankerite, a calcium-iron carbonate (Fig. 4, C). Furthermore, a white powdery phase was found under microscopic examination, filling the rock pores and acting as a cement; its Raman spectrum showed signatures at 413, 491, 617, 668, 1007 and 1135 cm$^{-1}$ which are unambiguously assigned to gypsum (Fig. 4, B).
In this sample, one spectrum shows two broad signatures at 1335 and 1590 cm$^{-1}$, which have been attributed to amorphous carbon. Since there are coal mines in Spitsbergen, it is possible that these particles may be a consequence of aerial contamination, but their presence could also be the result of the degradation of organic carbonaceous compounds.

In the brownish crust, again, quartz and gypsum were detected, but no signatures of ankerite were observed; however, new Raman bands at 224, 292, 408 and 612 cm$^{-1}$ appeared in the spectra (Fig. 4, E) are characteristic of haematite, which could be responsible for the red colour. One of the spectra collected from this crust gave a rather weak band at 1050 cm$^{-1}$, which can be assigned to a lead carbonate (cerussite or hydrocerussite). The absence of ankerite on the rock surface could be ascribed to weathering processes which could have converted the ankerite to haematite.

Red chasmosolithic community

The red chasmosolith was a small patch (Fig. 3) of 2 cm wide situated on a crack around 5 mm below the surface crust but not in contact with it. Several analyses were carried out at points across this area, and despite the clear presence of a red colouration in the surface crust, no Raman signals of haematite were found in this red chasmosolith area; however, limonite, FeO(OH)\text{n}(H$_2$O)$_n$, was clearly identified by its Raman signatures at 204, 295, 391, 550 and 692 cm$^{-1}$. It is interesting to point out here that, despite a large number of analyses that have been carried out in our laboratory involving different endolithic and chasmosolithic organisms, we have not found in our studies any of those communities in the rock matrix itself directly related with haematite. It appears that if haematite was originally present in the bulk rock matrix before colonisation, it has always been chemically mobilised to external areas around the colony [16, 23]. In the specimens we have previously studied, as found here, the crust is often enriched with haematite itself and/or goethite, showing a different colour to that of the fresh rock. It has been already reported that haematite, Fe$_2$O$_3$, acts as an UV radiation screen [23] and, since this sample was collected at 79° North, this strategy could have been useful for avoiding DNA damage caused by the high UV radiation insolation levels experienced near the terrestrial poles. It is not clear why limonite should be associated with the microbial communities and what role this mineral plays, if any, for the survival of the endolith/chasmosolith communities. It is possible that limonite is a weathering product of the haematite observed in the surface crust, a process that may have been enhanced by the biological colonisation and that it may be a step in the mobilisation process for iron through the rock.

In terms of organic molecules in the chasmosolith, the presence of the 1515; 1154; 1002 and 980 cm$^{-1}$ Raman bands were assigned to canthaxanthin (Fig. 5, A and B). Although the position of the stretching mode C=C double-bond band at 1515 cm$^{-1}$ could be also related with betacarotene, analyses carried out in our laboratory with commercial standard betacarotene showed that there was no Raman band at 980 cm$^{-1}$ for pure betacarotene, and this weak broad signature at 980 cm$^{-1}$ only appears when this compound has been degraded because of its exposure to atmospheric conditions. As this signature appears in the Raman spectra of the red chasmosolith and always together with the 1515-cm$^{-1}$ band, we therefore prefer a canthaxanthin assignment. Apart from canthaxanthan, the peaks at 1509; 1153 and 1005 cm$^{-1}$ are attributable to a different carotenoid, probably astaxanthin (Fig. 5, A). A very broad signature, centred at 1330 cm$^{-1}$, which always appears associated with these carotenoid bands in the Raman spectra, can be assigned to chlorophyll.

Yellowish-green chasmosolith

The yellowish-green chasmosolithic colony was found adjacent to the red chasmosolith described above but situated further away from the rock surface (Fig. 3). Apart from quartz and gypsum, the Raman spectra show signatures at 1524; 1155 and 1007 cm$^{-1}$ assignable to a carotenoid, possibly lutein (Fig. 6). Although numerous Raman spectroscopic analyses were acquired from this area, only this carotenoid was observed. However, in many spectra the clear signatures at 1438; 1388; 1325; 1285; 1225; 1185; 1069; 1048; 916; 756; 744 and 514 cm$^{-1}$ were characteristic of chlorophyll (Fig. 6). It is of interest that no signatures of any iron oxide were found in this region despite its closeness to the surface and
its direct contact with the red chasmolith where limonite has been clearly identified (Fig. 3).

Endolithic community

The endolithic community appeared as a continuous bluish-green band about 1 cm below the surface crust (Fig. 3). Two different types of carotenoid have been detected here, the first one with signatures at 1517; 1155; 1005 and 984 cm\(^{-1}\) is assignable to canthaxanthin, and the second carotenoid, with Raman bands at 1525; 1156 and 1004 cm\(^{-1}\), was identified as lutein. However, in contrast to the chasmolithic communities, in the endolith associated with both these carotenes, clear Raman bands at 1628; 1581; 1465; 1371; 1339; 1284; 1240; 1109; 1053; 972; 823; 667 and 505 cm\(^{-1}\) that were assigned to c-phycocyanin were identified (Fig. 7). C-phycocyanin is a light-harvesting accessory pigment important in photosynthetic processes when weak sunlight is a limiting factor, such as in dark niches; the synthesis of c-phycocyanin could therefore be a necessity for this particular endolithic community where only minimal polar sunlight reaches the colony at depths in the rock matrix.

Positive nodule

As in all other rock samples analysed in this study, quartz and gypsum (Fig. 8, B) were found as the main components also on the inside of the positive nodule; however, rutile and anatase were also identified (Fig. 8, C). It is interesting to note that, in the bulk rock, no signals of rutile were observed in any of the Raman spectra, whereas anatase was found.

However, among the organic compounds found on the inside surface of the convex positive nodule, apart from a carotenoid (probably lutein) and chlorophyll, for the first time in this set of samples, the cyanobacterial radiation protectant biomolecule scytonemin was unambiguously identified from its Raman bands at 1709; 1631; 1605; 1591; 1555; 1520; 1384; 1324; 1285; 1245; 1171; 1154; 1097; 983; 889; 830; 754; 677 and 569 cm\(^{-1}\) (Fig. 8, A).

Negative nodule

Finally, a series of analyses of the negative (concave) nodule (Fig. 1) was carried out. Compared with the rock matrix, the only newly identified phase here was calcite, with characteristic bands at 1086; 711; 281 and 154 cm\(^{-1}\). Together with quartz, rutile and haematite as mineral components, bands of a carotenoid (1524; 1157 and 1004 cm\(^{-1}\)) and chlorophyll indicate that these nodules were colonised by microorganisms. Not anatase, ankerite, or any of the other protective biomolecules noted above were detected in the Raman spectra of these negative nodules.

Conclusions

Raman spectroscopy is a powerful analytical tool that can be used to distinguish among a large variety of mineral
species even when they are in small amounts or just as small particles on rock surfaces; these particulars make Raman spectroscopy an ideal complementary payload to XRD/XRF or other mineralogical instruments on future Mars missions. However, what makes Raman spectrometers potentially an exceptional flight instrument for future “Search for life” missions to Mars is its high sensitivity to vibrations from organic molecules even in complex matrixes and—compared with other more destructive tools—the fact that it can be used in situ with little sample handling and preparation.

A major advantage of Raman spectroscopic analysis is the ability to identify simultaneously both organic and inorganic compounds in the same specimen without undertaking any chemical pre-treatment; in these studies, it was necessary to fracture the rock specimens to expose the endolithic strata and interior of the spherical nodules, but no other form of mechanical treatment such as grinding, polishing or etching was undertaken or necessary for the Raman analyses. The identification of the mineralogical and key biomolecular components of the rock matrix was unambiguous even for compounds in admixture.

The specimens studied in this work were all sampled from the same outcrop, but they showed clear differences both in the minerals and biological molecules present. For example, ankerite was only found in the bulk rock matrix; its absence from the surface crust or in places colonised by microbial communities could be attributed to weathering processes. The absence of ankerite signals could also be related to the presence of haematite on the surface, since ankerite could supply the iron necessary for the formation of haematite here. Furthermore, on the surface crust, and in association with the negative nodules, other carbonates have been identified, such as lead carbonate and calcite.

Another interesting observation not described hitherto is the distribution of the titanium oxide polymorphs. In the bulk rock, only anatase was identified even from the relatively large number of analyses carried out. Since both rutile and anatase give very strong and characteristic Raman signals, rutile is clearly either absent in the fresh rock or is present in only very low amounts. However, rutile was found in association with the both types of nodules, but, significantly, rutile appears along with anatase in the positive nodule whereas anatase was not identified in the negative nodule. The thermal transformation of anatase to rutile has been reported [24], but this can also be affected through hydrothermal conditions and catalytic reactions at high temperatures; it is noteworthy that hitherto there has been no reference to this conversion by microorganisms at environmental temperatures.

Haematite, a mineral often responsible of the reddish colouration of rocks and in our samples for the colour of the surface crust, was also present in the negative (concave) nodule, but it did not appear in the fresh rock matrix or in the red chasmolithic community, where limonite was the iron oxide identified based on the clear Raman bands.

Finally, in terms of biological signals, besides the often-reported chlorophyll and carotenoids, noteworthy are the two strategies adopted by microorganisms for protection from the harsh Arctic environment: (a) the bluish-green endolith community, situated about 1 cm below the rock surface, produced a light harvesting pigment to compensate for the lower level of PAR that reaches those depths inside the rock while (b) the microorganisms inside the positive nodule produce scytonemin, an UV protective pigment exclusively synthesised by cyanobacteria.

In our studies of samples collected during earlier AMASE expeditions to the Norwegian Arctic, we analysed biological and mineral signatures in dark basaltic rocks [20], in fossil travertine terraces [25] or in algae and their associated mineral consortia living on snow fields [26] and showed that similar protective biomolecules were present. Among these, we often identified specialised carotenoids (in many cases, the whole suite from betacarotene to astaxanthin), chlorophyll, scytonemin or c-phycocyanin. With the use of in situ analyses by Raman spectroscopy, some of these molecules were clearly identified even when no morphological microbial remnants were found by staining or high-resolution scanning electron microscopy [20]. Furthermore, Raman analyses carried out in our laboratory on endoliths from Antarctica also show similar biome molecules [5, 16–18, 21, 22]. In all cases, both the various bio- and geo-markers were unambiguously identified even when they appeared in complex mixtures.

In the current study, some of the biomolecules described in relation to the microorganisms found in the Ebbaelva sandstone samples were equivalent to previously reported biosignatures for microorganisms living under similar or other extreme environments [5, 6, 18–21]. In our case, the identified microbial signatures indicate that microorganisms survived in extreme cold, dry and high UV environments, and they have done this through various protective and adaptive strategies against the hazardous conditions they live in, for example, the use of haematite, carotenoids or scytonemin as UV radiation screen, carotenoids as free radical quenching molecules or c-phycocyanins as light harvesting compounds; similarly, in some cases, the microorganisms used mineral covers or the rocks themselves (i.e. Fig. 3) as protective micro-environments which, at the same time, act as a filter against harmful radiation and
protect the colony against wind, high UV, desiccation and low temperatures. These strategies allowed microorganisms to not just survive under these harsh conditions but actually to thrive in them, thus, the appropriate strategies adopted. But these microorganisms are considered highly relevant as terrestrial analogues for possible habitable actual or past Martian environments. In this context, such molecules are very highly sensitive for Raman identification even at low quantities and in small organisms and thus, Raman spectroscopy outstands as an advantageous technique for identification and characterisation of both minerals and biomolecules related with these extremophile microorganisms and prove to be a powerful astrobiological tool for future missions.

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References