The potential of Raman spectroscopy for the analysis of diagenetically transformed carotenoids

BY CRAIG P. MARSHALL* AND ALISON OLCCOTT MARSHALL

Department of Geology, University of Kansas, Lawrence, KS 66045-7613, USA

Recently, carotenoids have received much attention as target compounds for astrobiological prospecting principally because they are a group of molecules that display unique diagnostic Raman spectra that can be assigned to organic material of unequivocal biological origin. However, no work has been performed on assessing the potential of Raman spectroscopic detection of carotenoids from fossilized microbes. Here, we report the first Raman spectra acquired from ‘perhydro’ derivatives of $\beta$-carotene and lycopene formed by hydrogenation of the polyene chain during diagenesis, resulting in much less specific fossil hydrocarbons such as $\beta$-carotane and lycopane, respectively. We propose here that diagenetically altered carotenoids formed by hydrogenation reactions during the fossilization processes also provide unique diagnostic spectra that can be interpreted as a biological signature.

Keywords: Raman spectroscopy; carotenoids, diagenetically altered carotenoids; astrobiology; Mars

1. Introduction

If microbial life ever appeared and evolved on Mars, whether extinct or extant it would produce biomolecules that might be preserved and detectable in Martian rocks. The search for organic compounds of unequivocal biological origin is one of the major goals of astrobiological exploration programmes of the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA). Both NASA and ESA have designed payloads with miniaturized Raman spectrometers for the in situ identification of both organic and inorganic compounds relevant to life detection on the Martian surface or near subsurface. Therefore, it is crucial to construct a Raman spectroscopic database of molecules that are exclusively of biological provenance using Earth-based Martian analogues.

Photoprotective pigments are an important group of biomolecules which are produced as a response to harmful UV radiation and act directly as UV-screening compounds (e.g. parietin and scytonemin), or indirectly as

*Author for correspondence (cpmarshall@ku.edu).

One contribution of 12 to a Theme Issue ‘Raman spectroscopic approach to analytical astrobiology: the detection of key geological and biomolecular markers in the search for life’.
antioxidants (carotenoids) to quench the reactive oxygen species responsible for cellular damage. Significantly, these compound classes can only be synthesized by biological processes. Additionally, Raman spectra acquired from these photoprotective pigments are unique diagnostic spectra that cannot be assigned to any other organic material thus fulfilling the astrobiological criteria of unequivocal spectral proof of life. Consequently, much work has been focused on carotenoids biosynthesized from extant extremophile microbes as target biological compounds of interest for life detection. For example, the pioneering studies of Wynn-Williams & Edwards (2000a,b) and Edwards et al. (2004, 2005a,b) investigated near-infrared Fourier transform Raman spectroscopy of various pigmented cyanobacteria.

Hypersaline brines, evaporite deposits and regions where water existed for a significant period of time, such as palaeolakes, are not only important terrestrial analogues for Mars, but also areas that tend to have a high concentration of carotenoids. Recently, Marshall et al. (2007) reported on the identification of carotenoids in halophilic archaea by resonance Raman spectroscopy using 514.5 nm excitation wavelength. The choice of this excitation wavelength is useful when analysing carotenoids owing to its coincidence with an electronic transition in the chromophore portion of carotenoids (π−π*) resulting in the Raman resonance effect, thus significantly enhancing the Raman signal. More recently, Vítek et al. (2009) and Jehlička et al. (2009) investigated the lowest β-carotene content in powdered gypsum, halite and epsomite detectable by Raman spectroscopy using excitation at a wavelength of 785 nm. Importantly, their work determined the lowest concentration of β-carotene that will allow detection in a selected evaporitic matrix alone or under the monocystal of gypsum and epsomite. This was undertaken to simulate the analysis of β-carotene incorporated inside evaporitic minerals, which have recently been identified on Mars by the NASA rovers Spirit and Opportunity (Squyres et al. 2004; Squyres & Knoll 2005; Wang et al. 2006).

While Raman spectroscopy has proven very powerful to detect carotenoids in their biological form, no work has been performed to assess the potential of Raman spectroscopy to identify residual diagenetically altered carotenoids from fossil microbes. Here, we report the first Raman spectra acquired from diagenetically altered carotenoids as well as the interpretation and relevance of these spectral signatures for astrobiological prospecting.

2. Carotenoids and subsequent diagenesis

Currently, there are more than 600 different carotenoid structures known. All photosynthetic eukaryotes, bacteria and halophilic archaea, and a large variety of non-photosynthetic organisms, can biosynthesize carotenoids (e.g. Britton et al. 1995). Carotenoids function as accessory pigments in the light harvesting complex of phototrophic organisms, and are important for photoprotection, phototropism and the coloration of plants and animals. Many functionalized carotenoids extracted from living organisms and recent sediments have been used to obtain information about biological origins, evolution and ecology (e.g. Britton et al. 1995).
Carotenoids are \(\pi\)-electron-conjugated carbon-chain molecules and are similar to polyenes with regard to their structure and optical properties. Structurally, these molecules have a linear, chain-like conjugated carbon backbone that consists of alternating carbon single (C−C) and double bonds (C=\(C\)) with varying numbers of conjugated double bonds and number of functionalities in various positions such as keto, aldehyde, ester, hydroxy, methoxy and glycoside groups. For example, the molecular structures of \(\beta\)-carotene and lycopene are shown in figure 1. Carotenoids are strongly coloured as they have an allowed \(\pi\)−\(\pi^*\) transition that occurs in the visible region of the electromagnetic spectrum. This colour is dependent on the number of conjugated double bonds in the main linear chain. Red shifting of this \(\pi\)−\(\pi^*\) absorption band indicates an increase in the conjugation length, which is reflected in the colour, progressing from yellow to orange to red.

Carotenoids and their diagenetically altered versions were long considered not useful as biomarkers owing to their sensitivity towards oxygen and low preservation potential in sediments (Brassell 1993). However, in recent years several studies have used solvent extraction and GC/MS analysis to indicate the presence of both intact and diagenetically altered carotenoids in sediments dating back as far as the Miocene (5.3–24 Ma; e.g. Sinninghe Damsté & Koopmans 1997). Of specific interest for organic geochemists are carotenoids diagnostic of past environmental conditions, such as chlorobactene and isorenieratene indicating anoxic and sulphidic (euxinic) conditions in the photic zone of the water column (e.g. Brocks et al. 2005). In general, concentrations of intact carotenoids, especially oxygen-containing ones, decrease rapidly with depth and only under anoxic conditions can substantial amounts of intact carotenoids be preserved in sediments. However, diagenetic products of the prokaryotic carotenoids have been found in sediments as old as the Mesoproterozoic (1.6 Ga) by GC/MS analysis (Brocks et al. 2005).

Over the geological time scale, the polyene chain can be altered by a number of reactions, which include saturation or hydrogenation, cyclization, aromatization, expulsion, sulphurization, and C−C bond cleavage. The most dominant reaction during senescence, sedimentation and early diagenesis is hydrogenation of the polyene chain (figure 1). For example, ‘perhydro’ derivatives of \(\beta\)-carotene and lycopene are formed by hydrogenation of the polyene chain forming much less specific-fossil hydrocarbons such as \(\beta\)-carotane and lycopane, respectively (figure 1).
3. Experimental

(a) Carotenoids

Raman spectra were collected on commercial carotenoid standards. β-Carotene and lycopene were purchased from Sigma-Aldrich and diagenetically transformed ‘perhydro’ carotenoid standards β-carotane and lycopane were purchased from Chiron.

(b) Raman spectroscopy

Raman spectra were obtained using near-infrared excitation at 1064 nm using a Bruker RFS 100 spectrometer and a Nd3+/YAG laser in the macroscopic mode with a spot diameter of 100 μm at the surface of the sample and a laser power of 60 mW. Spectra were recorded at a 4 cm⁻¹ spectral resolution with the co-addition of 456 scans over a wavenumber range of 50–3500 cm⁻¹.

4. Results and discussion

(a) Raman spectroscopy

Representative Raman spectra collected for β-carotene and β-carotane, and lycopene and lycopane are shown in figures 2 and 3, respectively. The spectra acquired from the carotenoids are simple, and are exclusively dominated by bands at 1512–1518 cm⁻¹ assigned to ν(C=C) in-phase stretching, 1155–1160 cm⁻¹ assigned to ν(C=C) stretching, and 1010 cm⁻¹ assigned to a combination of δ(C=CH) methyl in-plane rocking and δ(C–H) out-of-plane bending modes. By comparison, the ‘perhydro’ carotenoid derivatives have by far more complicated spectra. Most notably, an intense band at 1455 cm⁻¹ is the dominant mode and is assigned to δ(CH2) scissoring mode of methylene. Additionally, the spectra contain eight bands between 1390 and 1000 cm⁻¹ which are assigned to ν(C=C) stretching modes from the main hydrocarbon chain, and six bands between 1000 and 800 cm⁻¹ that are assigned to a combination of δ(C=CH) methyl in-plane rocking and δ(C–H) out-of-plane bending modes.

By and large, the spectral differences between the ‘perhydro’ carotenoid derivatives β-carotane and lycopane are slight (figure 4). The eight bands between 1390 and 1000 cm⁻¹ show only very subtle differences in band intensity for the two spectra acquired from β-carotane and lycopane, whereas the six bands between 1000 and 800 cm⁻¹ show subtle differences between band intensity and position (shown by the dashed lines in figure 4). However, while the spectra acquired from both the ‘perhydro’ carotenoid derivatives β-carotane and lycopane are similar, nonetheless they are distinct, indicating that the fossil hydrocarbons formed as a result of hydrogenation of the main polyene chain of carotenoids can be distinguished.

(b) Implications for astrobiology

Investigations of endolithic communities from extreme habitats on Earth have demonstrated that Raman spectroscopy is an excellent tool to detect various types of biomolecules that have been produced by particular microorganisms as
Fig. 2. Stacked Raman spectra of β-carotene and β-carotane. Modes at 1518 cm\(^{-1}\) and 1155–1160 cm\(^{-1}\) are due to in-phase C=C \((\nu_1)\) and C−C stretching \((\nu_2)\) vibrations of the polyene chain in carotenoids, and in-plane rocking modes of CH\(_3\) groups attached to the polyene chain coupled with C−C bonds occur in the 1010 cm\(^{-1}\) region which are observed in the spectrum of β-carotene. The spectrum acquired from β-carotane has an intense band at 1455 cm\(^{-1}\) assigned to δ(CH\(_2\)) scissoring mode and, additionally, the spectrum contains eight bands between 1390 and 1000 cm\(^{-1}\) which are assigned to ν(C−C) stretching modes from the main hydrocarbon chain, and six bands between 1000 and 800 cm\(^{-1}\) that are assigned to a combination of δ(C=CH) methyl in-plane rocking and δ(C−H) out-of-plane bending modes.

part of their survival strategy in extreme environments (e.g. Wynn-Williams & Edwards 2000a,b; Edwards et al. 2004, 2005a,b; Villar et al. 2005; Villar & Edwards 2006). Photoprotective pigments provide an important group of biomolecules which are produced as a response to harmful UV radiation and act as UV-screening compounds (e.g. parietin and scytonemin), or indirectly as antioxidants (carotenoids) to quench the reactive oxygen species responsible for cellular damage. Due to recent identification of evaporites on Mars by the NASA rovers Spirit and Opportunity, it is reasonable to assume that halophilic microorganisms could have flourished in the Martian environment. Based on these results, it is conceivable that brine pools may have been relatively common on the surface of that planet. Brines or evaporites may be suitable habitats for extant halophilic microorganisms or the last refuge for an extinct life (Mancinelli et al. 2004), thus potentially bearing a fossil record. Here we have demonstrated for the first time that diagnostic Raman spectra can be collected
Figure 3. Stacked Raman spectra of lycopene and lycopane. Modes at 1512 cm\(^{-1}\) and 1155–1160 cm\(^{-1}\) are due to in-phase C=C (\(\nu_1\)) and C–C stretching (\(\nu_2\)) vibrations of the polyene chain in carotenoids, and in-plane rocking modes of CH\(_3\) groups attached to the polyene chain coupled with C–C bonds occur in the 1010 cm\(^{-1}\) region which are observed in the spectrum of lycopene. The spectrum acquired from lycopane has an intense band at 1455 cm\(^{-1}\) assigned to \(\delta(CH_2)\) scissoring mode and, additionally, the spectrum contains eight bands between 1390 and 1000 cm\(^{-1}\) which are assigned to \(\nu(C–C)\) stretching modes from the main hydrocarbon chain, and six bands between 1000 and 800 cm\(^{-1}\) that are assigned to a combination of \(\delta(C=CH)\) methyl in-plane rocking and \(\delta(C–H)\) out-of-plane bending modes.

from the preserved/fossilized ‘perhydro’ derivatives of \(\beta\)-carotene and lycopene, i.e. \(\beta\)-carotane and lycopane. Significantly, this work contributes the first fossil ‘perhydro’ carotenoid diagenetic alteration compound of a biological origin and nature for an astrobiological Raman database for the potential evidence of past life that can be used in the astrobiological prospecting of Mars.

5. Conclusions

These results highlight the spectral differences between the functionalized and diagenetically transformed carotenoids. From an astrobiological perspective it is desirable to target organic molecules that are clearly distinguished from the abiogenic compounds that are widely distributed throughout the cosmos. Clearly, the Raman spectroscopy of functionalized carotenoids unequivocally fulfils this objective as they are only synthesized by biological processes and
Raman analysis of ancient carotenoids

Figure 4. Stacked Raman spectra of β-carotane and lycopane both showing an intense band at 1455 cm$^{-1}$ assigned to $\delta$(CH$_2$) scissoring mode and, additionally, the spectra contain eight bands between 1390 and 1000 cm$^{-1}$ which are assigned to $\nu$(C−C) stretching modes from the main hydrocarbon chain, and six bands between 1000 and 800 cm$^{-1}$ that are assigned to a combination of $\delta$(C=CH) methyl in-plane rocking and $\delta$(C−H) out-of-plane bending modes.

display unique diagnostic spectra. We propose here that likewise diagenetically reduced carotenoids can only be formed by the fossilization of biological material and moreover they also provide unique diagnostic spectra.

C.P.M. would like to thank the Australian Research Council (ARC) for funding.

References


Edwards, H. G. M., Moody, C. D., Villar, S. E. J. & Wynn-Williams, D. D. 2005α Raman spectroscopic detection of key biomarkers of cyanobacteria and lichen symbiosis in extreme...

Phil. Trans. R. Soc. A (2010)


