RAMAN SPECTROSCOPIC INVESTIGATIONS OF BURGESS SHALE–TYPE PRESERVATION: A NEW WAY FORWARD

ALISON OLCOTT MARSHALL,1* RANDOL L. WEHRBEIN,2 BRUCE S. LIEBERMAN,1,2 and CRAIG P. MARSHALL1

1Department of Geology, University of Kansas, Lawrence, Kansas 66045, USA, olcott@ku.edu, wehrbein@ku.edu, bieber@ku.edu, cpmarshall@ku.edu; 2Biodiversity Institute, University of Kansas, Lawrence, Kansas 66045, USA

ABSTRACT

Study of the Burgess Shale-type deposits of the Cambrian has greatly enhanced scientific understanding of early animal evolution, but as the mechanisms by which these deposits formed are still unclear, here we outline and present data from the application of a new analytical approach, Raman spectroscopy, that can be used to characterize fossils from these deposits. Although these deposits present exceptional views into a diverse, nonbimineralized to lightly bimineralized biota, this taphonomic regime mostly disappears from the fossil record after the Cambrian, with a few notable exceptions. Numerous detailed taphonomic and chemical studies have provided significant insight into modes of fossil preservation in these deposits, although there is still significant debate regarding the preservational and diagenetic mechanisms that may be involved. Compared to previous electron microscopy-based elemental mapping approaches, which have identified the elemental components of similar fossils, Raman spectroscopy allows a determination of the chemical phases and specific mineralogy at the molecular level, as well as the thermal maturity, of these fossils. This approach therefore provides new types of data, such as hematite phase, that may prove helpful for elucidating some of the mechanisms responsible for the exceptional types of preservation found in these deposits, and potentially helps to resolve the existing taphonomic debates.

INTRODUCTION

Cambrian strata host more localities preserving nonbimineralized-bodied fauna than any other rocks in the Phanerzoic (Allison and Briggs, 1993). Although these deposits of nonbimineralized fauna, often referred to as Burgess Shale-type (BST) deposits, have greatly enhanced our understanding of some of the key events in animal evolution (e.g., Briggs and Fortey, 1989; Gould, 1989; Edgecombe, 1998; Babcock, 2001; Hou et al., 2004), the mechanisms by which they formed are still debated. Burgess Shale-type preservation is additionally interesting as it is the most important pathway for the preservation of nonbimineralized fossils in the Cambrian but it mostly disappears from the fossil record thereafter (Butterfield, 1995; although see Van Roy et al., 2010, and Yuan et al., 2011).

Although there have been numerous, detailed studies of the chemistry of BST deposits (e.g., Orr et al., 1998; Gabbott et al., 2004; Moore and Lieberman, 2009; Lin and Briggs, 2010) which have provided significant advances in our understanding of the mechanisms behind the preservation of these distinctive deposits, the details of how these deposits were preserved are still unclear. Here we present a new approach to study the chemistry of these enigmatic deposits based on the use of Raman spectroscopy to analyze fossils from multiple deposits. As Raman spectroscopy reveals the nature of molecular bonding in materials which can then be used to infer the chemical composition, this methodology sometimes allows identification of the mineral phases present and delineation of the thermal maturity and molecular structure of the organic carbon (Marshall et al., 2011). Further, the spatial relationship between the organic carbon and the mineral assemblages in samples from different ages, depositional environments, and thermal maturity can be determined. This information is crucial for revealing the pathways of preservation and weathering that the fossils have undergone. Previous approaches could not always provide the level of detail about these pathways that Raman spectroscopy affords.

PREVIOUS CHEMICAL ANALYSES OF BST DEPOSITS

Researchers have investigated the precise elemental and mineralogical composition of individual specimens from multiple BST fossil sites. The chemistry of the Lower Cambrian (Series 2) Chengjiang biota (China; Gabbott et al., 2004; Zhu et al., 2005), Kinzers biota (Pennsylvania, United States), Balang Formation (China), Latham Shale (California, United States), and Metalline Formation (Washington, United States; Gaines et al., 2008) have been investigated, as have the Early and Middle Cambrian (Series 2–3) Poche Shale (Nevada, United States; Moore and Lieberman, 2009) and Kaibii biotas (China; Gaines et al., 2008; Lin and Briggs, 2010), the Middle Cambrian (Series 3) Wheeler Formation (Utah, United States) and Spence Shale (Utah, United States; Gaines et al., 2008), as well as specimens from the Middle Cambrian (Series 3) classic Burgess Shale (Canada; Orr et al., 1998; Gaines et al., 2008; Page et al., 2008; Lin and Briggs, 2010). In all of these studies, electron microscopy-based energy-dispersive X-ray spectroscopic (EDS) elemental mapping was used to delineate the chemical composition of the fossils. elemental data are very valuable for indicating the distribution of elements within a sample, which allows inferences to be made about minerals preserved, although without additional information it is not possible to precisely identify minerals. Thus, while previous work has resulted in a detailed understanding of the elemental composition of these 10 sites, more detailed molecular and mineralogical data could prove useful. Furthermore, because EDS elemental mapping is a relative difference technique, not all samples show distinctions in elemental maps due to compositional homogeneity of the host rock and fossil. For instance, in carbonaceous compression fossils, such as those from BST deposits, a distinctive carbon signal may not easily be shown in the fossils if the host rock matrix also contains a high level of carbon, and thus fossils that are visible to the eye as an area darker than the matrix are indistinguishable from the matrix in the corresponding elemental map (Gaines et al., 2008).

RAMAN SPECTROSCOPY

Raman spectroscopy provides information about the nature of molecular bonding in materials that can then be used to infer the chemical composition, in contrast to most other methods. This technique allows rapid and non-destructive in situ detection of the minerals present with a spatial resolution as small as 1 μm. When the Raman spectrometer is interfaced with an open microscope stage, large fossil slabs can be analyzed. Raman spectroscopy involves irradiating a
sample with monochromatic radiation in the UV, visible, or near-IR region, and the light may be scattered either elastically (Rayleigh scattered) or inelastically (Raman scattered). Raman scattered light is shifted from its original incident frequency corresponding to a molecular vibration within the sample. Thus, the Raman shift is a function of the chemical bonds and symmetries of molecules, and provides a fingerprint for the identification of both organic and inorganic molecules.

Although Raman spectroscopic data are collected over the full region of 100–4000 cm\(^{-1}\) (or a Raman shift), specific smaller regions are often examined for structural information of different materials. For instance, the mineral fingerprint region (100–1800 cm\(^{-1}\)) is the portion of a Raman spectrum that contains diagnostic bands used to identify most minerals. The Raman spectrum of carbonaceous material can be divided into first- and second-order regions, depending upon the degree of crystallinity of the carbonaceous material (e.g., Marshall et al., 2010 and references therein). The carbon first-order region (800–1800 cm\(^{-1}\)) is sensitive to the degree of two-dimensional ordering present in carbonaceous material, whereas the carbon second-order region (2200–3400 cm\(^{-1}\)) is sensitive to the three-dimensional ordering of the carbonaceous material, and indicates the degree of its graphitization (Lespade et al., 1982). The carbon first-order region generally contains two broad bands from carbonaceous material; the first, commonly referred to as the D band, is seen at ~1350 cm\(^{-1}\), and is assigned to an \(A_{1g}\) symmetry mode that becomes Raman active with disorder. The second, referred to as the G band, is seen at ~1600 cm\(^{-1}\), and is assigned to the \(E_{2g}\) symmetry mode.

Raman analysis can also be used for paleothermometry, as it allows discrimination between carbonaceous material that has undergone thermal rearrangement, which is especially useful as carbonaceous material does not undergo retrograde metamorphism (Beyssac et al., 2002; Pasteris and Wopeneka, 1991). The D and G bands found in the carbon first-order region can be deconvoluted into component Gaussian-Lorentzian bands representing vibrational modes that become Raman active due to structural disorder (Marshall et al., 2010). These bands include the D2 band, reflecting lattice disorder, the G band, an \(E_{2g}\) symmetry mode representing in-plane C=C stretching within an aromatic ring, the D3 band, reflecting the presence of amorphous carbon, the D1 band, assigned to an \(A_{1g}\) symmetry mode that becomes Raman active due to disorder in the sp\(^2\) carbon network, and the D4 band, representing sp\(^3\) bonds, or C–C and C=C stretching modes in a polynene-like structure (Marshall et al., 2010). It has been shown that the ratio (R) of the band area of the D1 band to the sum of the areas of the D1, the G, and the D2 bands acts as a proxy for the total amount of thermal alteration experienced by the sample (Beyssac et al., 2002). Furthermore, experiments have revealed that this R-value can be used to obtain an absolute temperature of alteration, through the equation (Beyssac et al., 2002):

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T(\text{C}) = -445R^2 + 641(r^2 = 0.96)
\]

The ability to determine mineral composition in tandem with thermal alteration allows an assessment of whether the minerals in the sample are primary or later thermal alteration products; the latter has been suggested for many of the minerals within the Burgess Shale (e.g., Butterfield et al., 2007; Gaines et al., 2008; Moore and Lieberman, 2009).

MATERIALS AND METHODS

Paleontological Specimens

An analyzed sample from the Spence Shale (Cambrian Series 3), Utah, United States housed in the University of Kansas Museum of Invertebrate Paleontology (KUMIP) is described herein. The specimen, KUMIP 314028, is referred to as *Leanchoilia superlata?* *Leanchoilia*, an arthropod, is known from several BST deposits. The genus has been previously considered in the context of BST deposit taphonomy (e.g., Butterfield, 2002). This particular fossil is also useful because juxtaposed on the same slab is a biomineralized *Amecephalus* trilobite (Briggs et al., 2008; Fig. 1) which provides a useful baseline for chemical comparisons. In the hand sample, the rock appears to have been altered to a slate. The geological setting of the Middle Cambrian of Utah has been considered in detail by, among others, Rees (1986), Robison (1991), Liddell et al. (1997), Elrick and Snider (2002), Gaines et al. (2005) and Babcock et al. (2007). The Spence Shale represents deposition on a marine, mixed carbonate-siliciclastic slope adjacent to a carbonate platform, and preserves both nonbiomineralized carbonaceous and biomineralized specimens (Briggs et al., 2008).

Raman Spectroscopy

Raman point spectra were collected using a Renishaw inVia Reflex Raman Microprobe with a Peltier-cooled charge-coupled device detector. The microscope optics for the Raman system are based on a Leica DM-LM microscope. A 514.5 nm 30 mW Ar\(^+\) laser and a 785 nm 250 mW air-cooled near-IR diode laser oriented normal to the sample.

**FIGURE 1**—Sample analyzed with Raman spectroscopy (KUMIP 314028). This fossil slab contains a BST-preserved *Leanchoilia superlata?* (left) and a biomineralized *Amecephalus* trilobite (right). Numbers mark localities of Raman spectra presented in the text.
FIGURE 2—Comparison stack plot of representative Raman spectra acquired from 1) host rock, 2) trilobite cephalon, 3) Leancholia head shield, 4) red spot, and 5) gut nodule. The Raman spectra were acquired with a 785 nm excitation source. The G band is assigned to in-plane C=C in an aromatic ring stretching vibrational mode with \( E_{2g} \) symmetry and the D band is assigned to an \( A_{1g} \) symmetry mode that becomes Raman active due to disorder in the \( sp^2 \) carbon network. The PAHs between \( \sim 1800 \text{ cm}^{-1} \) and \( \sim 1000 \text{ cm}^{-1} \) are attributed to the presence of large-ring clusters of polycyclic aromatic hydrocarbons (PAHs). \( \gamma \)-hematite has modes at \( \sim 220 \) and \( \sim 489 \text{ cm}^{-1} \) (\( A_{2g} \) symmetry modes), and \( \sim 289, \sim 402, \) and \( \sim 606 \text{ cm}^{-1} \) (\( E_g \) symmetry modes). \( \gamma \)-hematite bands are broad and not well-defined, which is indicative of the comparative low crystallinity of \( \gamma \)-hematite. The weak band at \( \sim 982 \text{ cm}^{-1} \) is assigned to phosphate stretching modes.

were used to excite the samples. The instrument was calibrated against the Raman signal for Si obtained from a silicon wafer reference at 520 cm\(^{-1}\). A refractive glass 100x objective lens was used to focus the laser on a 1µm spot to collect the backscattered radiation. Collection parameters for the scans varied from 30 to 60 s with 1 to 10 accumulations at 1 to 5 mW to obtain a good signal-to-noise ratio, and to minimize the photodamage experienced by carbonaceous material. Data were collected with both laser lines to better explore the preservation of the sample. As no downshifting of the D band or change in the D/G band ratio was observed with repeat analyses, no photodegradation of carbonaceous material occurred.

RESULTS

Raman Data Collected at 785 nm

Raman point spectra were collected on the matrix, on the trilobite, and along the length of the Leancholia, including the head shield, the gut nodules, and a likely diagenetically altered red spot (Fig. 1). Within the mineral fingerprint region, the red spot (4 in Fig. 1) contains diagnostic vibrational modes at \( \sim 220 \) and \( \sim 489 \text{ cm}^{-1} \) (\( A_{2g} \) symmetry modes), and \( \sim 289, \sim 402, \) and \( \sim 606 \text{ cm}^{-1} \) (\( E_g \) symmetry modes), indicative of \( \gamma \)-hematite (de Faria et al., 1997; Fig. 2). Interestingly, within the mineral fingerprint region, the trilobite cephalon (2 in Fig 1), the Leancholia cephalon (3 in Fig 1), as well as the host rock (1 in Fig 1), contain bands at \( \sim 211, \sim 259, \sim 341, \sim 458, \sim 506, \sim 597 \text{ cm}^{-1} \), attributed to \( \gamma \)-hematite (magnhematite), a mineral formed by weathering of ferrous iron (de Faria et al., 1997; Fig. 2). In contrast to the sharp intense \( \gamma \)-hematite bands, the \( \gamma \)-hematite bands are broad and not well defined, which is indicative of the comparative low crystallinity of \( \gamma \)-hematite (de Faria et al., 1997). Although the gut nodules (5 in Fig 1) did not seem to preserve iron, they did contain a weak band at \( \sim 982 \text{ cm}^{-1} \), which is assigned to phosphate (Marshall et al., 1999; Fig. 2).

With the exception of the gut nodules, all samples contained D and G bands (Fig. 2), indicating the presence of carbonaceous material. In contrast, the gut nodules contained 5 intense bands with shoulders between \( \sim 1800 \text{ cm}^{-1} \) and \( \sim 1000 \text{ cm}^{-1} \), all attributed to the presence of large-ring clusters of polycyclic aromatic hydrocarbons (PAHs) (Mapelli et al., 1999; Fig. 2). This difference is likely due to the presence of phosphate within the gut nodules. Phosphate is known to hinder the large-scale aromatization of carbon, which occurs during the early stages of carbonization, and results in D and G Raman bands (Jehlicka et al., 2003; Marshall et al., 2010). In contrast, the spectrum of the trilobite sample is indistinguishable from the host rock; both show D and G bands and signs of \( \gamma \)-hematite bands, although the shape of these bands is different than those from the Leancholia. Intriguingly, although clays are Raman active (e.g., Frost, 1995; He et al., 2004), there was no clay found in association with the fossil.

Raman Data Collected at 514.5 nm

In addition to the data collected at 785 nm, Raman spectroscopic data were collected on the Leancholia fossil using the 514 nm laser to examine the thermal maturation of the samples. Typically, excitation sources emitting 514.5 nm or 532 nm are used to determine the thermal maturation of carbonaceous matter, as wavelengths in the red (e.g., 785 nm) and near-infrared (e.g., 830 nm, 1032 nm, and 1064 nm) cause a resonance effect on the D band, altering its shape and intensity.
The temperature of alteration, yielding an average temperature of 327°C (Fig. 3). The deconvoluted spectra were then used to calculate the order region only, then deconvoluted into the component bands (Marshall et al., 2010). These data were collected over the carbon first-hemihematite spot on the fossil. -hematite reveals an important role in other types of BST preservation (e.g., Orr et al., 1998; Anderson et al., 2011). However, our results are based only on a single specimen and it is thus too soon to say that clay minerals play no role in facilitating preservation in Spence Shale fossils. By contrast, iron may have played an important role in the special preservation of this particular fossil. For instance, the presence of γ-hematite reveals an oxidation event of primary ferrous iron separate from the later diagenetic event that created the red -hematite spot on the fossil.

Another hypothesis explaining how BST preservation occurs relies on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007). Again, on the important role of carbon, with clays inhibiting degradational enzymes (Butterfield, 1990, 1995; Butterfield et al., 2007).
discernment between this primary carbonaceous material and the potentially secondary carbonaceous material found on the body of the fossil and in the matrix. Thus, it is clear that carbon also plays a role in explaining this particular example of BST preservation.

Of course there are a host of other hypotheses aiming to explain BST preservation including sulfide brine fluids restricting diagenesis of the carbon (Parsons-Hubbard et al., 2008), and physical conditions restricting sediment porosity (Gaines et al., 2005). These specific hypotheses could not be considered solely by the data presented herein, but additional types of data, including biomarkers, in conjunction with Raman data, might ultimately prove valuable.

CONCLUSIONS

Ultimately, it is clear that determining the preservational pathways that resulted in the formation of these BST fossil deposits requires a detailed understanding of the chemical composition of the fossils themselves. This points to the value of approaches based on Raman spectroscopy, as they provide a degree of mineralogical and chemical precision not possible with previous approaches. It also allows for an understanding of diagenetic events. Thus, the availability of the Raman spectroscopic approach helps expand the toolkit available to paleontologists interested in teasing apart the mechanisms behind the preservation of these spectacular fossils. Further, it is likely that obtaining these types of data on a wide variety of BST fossils from a wide range of deposits could provide great utility in understanding this important Cambrian preservational pathway.

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