Review of Palaeobotany and Palynology 210 (2014) 62–68

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Reexamination of cell contents in Pennsylvanian spores and pollen grains using Raman spectroscopy

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ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form 23 July 2014
Accepted 27 July 2014
Available online 11 August 2014

Keywords:
Intracellular inclusion
Nucleus
Permineralization
Coal ball

ABSTRACT

Permineralization offers exceptional structural preservation to the level of cellular components, many of which are found within fossilized plant specimens. In some cases, structures within plant cells, particularly those found within spores and pollen grains, have been interpreted as nuclei. Although these structures have been studied morphologically and ultrastructurally, little is known about their composition and origin. This study uses petrographic thin sections and ultraviolet (UV) Raman spectroscopy to reexamine cellular inclusions within three types of Pennsylvanian spores and pollen grains with the intent to interpret the molecular composition and therefore, the identity of these structures. Thin sections are examined using Raman spectroscopy, which indicates the presence of disordered carbonaceous material and calcite. There is no significant difference in the chemical composition between the intracellular inclusions and surrounding areas within each specimen. This study represents one of the first applications of Raman spectroscopy in which the internal cell contents of fossil plant taxa are analyzed. Results do not support the presence of nuclei, although further examination is recommended to elucidate the origin and identity of the intracellular inclusions.

1. Introduction

Historically, paleobotany has relied on several techniques to evaluate fossil plants, and to extract the greatest possible information from each specimen that can be applied to a variety of sub-disciplines (e.g., whole plant reconstructions, systematics, and paleoecology). The standard preparation technique for permineralized material was initially petrographic thin sections, which provided a wealth of information about cell types and tissue systems, especially those preserved within Carboniferous coal balls (Williamson, 1893; Hass and Rowe, 1999). Beginning in the 1950s, acetate peels were more frequently used to produce serial sections, which both accelerated the sectioning process and reduced the overall loss of material. Today, preformed cellulose acetate sheets are routinely used in the study of permineralized plant material from numerous stratigraphic levels (Galtier and Phillips, 1999). In recent years, however, it has become apparent that in some instances more detailed information is gained from thin sections, depending on the focus of the study (Taylor et al., 2011).

In some instances permineralization offers extraordinary preservation and allows for the interpretation of a variety of cell contents, some of which have been described as nuclei, chromatin granules, and mitotic chromosomes (Baxter, 1950; Schopf, 1968; Brack, 1970; Brack-Hanes and Vaughn, 1978; Bonde et al., 2004; Bomfleur et al., 2014). These studies utilized transmitted light, scanning electron microscopy (SEM), and synchrotron radiation X-ray tomographic microscopy (SRXTM), with descriptions based on the relative size, shape, and consistency of cell contents within a population of cells of a particular type. Consistency is an important consideration, as sections of three-dimensional structures generally do not provide information for the contents of every cell present due to the focal plane in which the specimen is viewed. In some cases cell contents have been examined at the ultrastructural level; however, these studies have not provided additional information about the identity of cell contents (Taylor and Millay, 1977a).

Several techniques relatively new to the field of paleobotany have been used in recent years to enhance the study of fossil plants. These techniques have provided new information, and clarified concepts including cell types and organ reconstruction, cell wall composition, and the effects of taphonomic processes such as diagenetic transformation. Today scanning and transmission electron microscopy, spinning disc confocal microscopy, and X-ray microtomography are frequently used to gain further insights into the specimens investigated (e.g., Taylor et al., 2004; Schopf et al., 2006; Friis et al., 2007). The majority of these relatively new techniques emphasize the analysis of morphological features of a specimen.

Some analytical techniques recently applied to paleobiology offer the possibility of extracting more complex data from fossils. These

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http://dx.doi.org/10.1016/j.revpalbo.2014.07.005
0034-6667/

approaches enable the observation of a broad range of chemical profiles from both the matrix and the fossil. Although such techniques have provided compositional information, they have not always offered data specific enough to define the systematic affinities of some fossils (e.g., Taylor et al., 2004). In some cases, however, the information obtained from these techniques gives insight as to the origins of a fossil or a questionable structure (Marshall et al., 2010, 2011; Nasdala et al., 2012). Raman spectroscopy in particular offers information about molecular structures, fossil biogenicity, and thermal maturity of specimens (Olcott Marshall et al., 2012). Originally developed for the use in the field of chemistry, Raman spectroscopy has become increasingly prominent in the field of paleobiology. Its applications range from the study of putative fossil microorganisms to the examination of extant organisms and biological molecules such as DNA and proteins, as well as the potential use for the investigation of possible biomolecules on Mars (Schopf et al., 2005; Tarcea et al., 2007; Marshall et al., 2010).

Several authors have stressed the importance of combining morphological data with chemical and molecular structural information to more confidently assess the biogenicity and origins of fossil material (Pflug and Jaeschke-Boyer, 1979; Schopf et al., 2005). The intent of this contribution is to analyze the contents of Carboniferous spores and pollen grains using ultraviolet (UV) Raman spectroscopy, and to determine the identity and molecular composition of these internal cell structures.

2. Materials and methods

2.1. Locality and geological background

The thermal maturity and geologic setting of specimens are useful when considering molecular composition. Because structural changes occur at the molecular level with exposure to heat and pressure, knowing the history of a specimen can help us interpret Raman data with more certainty. The specimens examined in this study all occur within calcium carbonate coal balls or concretions from several localities in North America.

Three representative structures were analyzed. *Peltastrobus reedae* is represented by a single specimen collected from the Petersburg or Alum Cave coal (Springfield Coal; Middle Pennsylvanian) from Booneville, Indiana (Baxter, 1950). This coal is part of the Petersburg Formation, and underlies the Alum Cave Limestone Member of the Dugger Formation (Guennel, 1952). Evidence suggests a fluvial channel depositional environment, where peat deposition occurred in fresh water adjacent to the channel (Eggert, 1982). Due to this non-marine depositional environment, these particular coals have a lower sulfur content than adjacent coals of the same seam underlying strata of marine origin (Treworgy and Jacobson, 1986). The Indiana No. 5 coals are directly overlain by black shales containing calcareous and pyritic concretions (Maples, 1986). Coal samples taken from the Petersburg Formation were found to have vitrinite reflectance values ranging from 0.388 to 0.655% (Willard et al., 1995). These values indicate diagenetic alteration on the order of immature to mature thermal maturity for hydrocarbon generation.

Flemingites schopfii is represented by a single cone collected from the Copland Coal Member (Middle Pennsylvanian) from Lewis Creek, Kentucky (Brack, 1970). This coal is part of the Breathitt Formation, thought to be composed of deposits from a coastal zone or alluvial plain (Brack, 1970; Aitken and Flint, 1995).

Two representative specimens of *Lasiostrobus polysacci* originate from the Calhoun Coal Member (Upper Pennsylvanian) of the Berryville, Illinois locality (Taylor, 1970). This coal comes from the Mattoon Formation and underlies the Bonpas Limestone (Willard et al., 2007). The Calhoun Coal Member is of non-marine delta or channel origin (Wanless et al., 1969), and samples have been found to have vitrinite maceral contents of approximately 82–83% (Harvey and Dillon, 1985).

2.2. Fossil material

All specimens were initially characterized using acetate peels. Specimens were prepared as paleontological thin sections without cover slips so that a greater degree of three dimensionality could be evaluated and spectroscopic analyses could be completed. Fragments of the specimens preserved in coal balls were smoothed using 600 grit silicon carbide and cleaned with ethyl alcohol. Specimens were mounted to standard microscope slides using Hillquist 2-part epoxy, then cut and ground using a Buehler thin section machine. Specimens were then ground to a desired thickness of approximately 35–50 μm; in some instances digital images were taken and the specimen subsequently ground thinner. All specimens and slides are included in the Paleobotanical Collections, Natural History Museum and Biodiversity Institute, University of Kansas (Lawrence, KS). Thin sections include CB 15003 P1 TS1, CB 15003 P3 TS1, CB 2124 C2-1 TOP TS1, CB 2124 C2-1 TOP TS2, CB 2198 E2 SIDE TS1, and CB 902 E1B SIDE TS1. These slides were made from specimens CB 15003 P1, CB 15003 P3, CB 2124 C2-1 Top, CB 2198 E2 Side, and CB 902 E1B side.

Images were produced using a Leica DC500 CCD attached to a Leica DM5000B compound light microscope. Focal stacking of these images in Adobe Photoshop and Helicon Focus was used to further enhance detail and three-dimensional features.

Cell contents were examined from three taxa from the Carboniferous of North America. Although there are many examples of cell contents preserved in permineralized plants, those found in spores and pollen grains perhaps provide the best opportunity to examine the chemical components due to their abundance, uniformity, and ease of identification. These include the spores of *Peltastrobus reedae*, a sphenophyte, spores of the lycopsid *Flemingites schopfii*, and the pollen grains of *Lasiostrobus polysacci*, a cone of gymnospermous affinity. In section view, not every spore or pollen grain contains an intracellular inclusion, because in some grains these structures were lost due to the process of sectioning or are not visible due to the plane of section. All of the materials come from the original specimens upon which the taxa are based. Structural dimensions are based on measurements of 100 spores each from *P. reedae* and *F. schopfii*, each represented by a single cone, and 40 pollen grains from *L. polysacci*, represented in this study by two specimens.

2.2.1. Peltastrobus reedae

Peltastrobus reedae is a sphenophyte cone consisting of whorls of peltate sporangiophores (Baxter, 1950). Within the sporangia are ellipsoid, monolete spores that are 26–42 μm in length and 16–23 μm in width. The spore wall thickness is 0.3–1.2 μm with an average thickness of 0.66 μm. In cross section, rounded structures protrude from the spore wall, and in some cases may project from the anterior and posterior poles. In the dispersed state the spores are most similar to the spore type *Columnisporites ovalis* (Courvoisier and Phillips, 1975).

Some spores contain well-defined central inclusions that range from spherical to ovoid in form, although several appear more irregular or partially fragmented (Plate I,10–12). They are nearly always more opaque than both the spore lumen and the spore wall. The inclusions frequently appear grainy, and some appear to contain more defined filamentous structures; however, this may be an artifact of the focal plane. Inclusion size ranges from 1.7 to 6.1 μm in diameter with an average of 4.0 μm. These structures account for 1–10% of the area of the spore lumen, and on average occupy 3% of the lumen space. They occur one per spore and are abundant throughout the sporangia of *P. reedae*.

2.2.2. Flemingites schopfii

Flemingites schopfii (formerly *Lepidostrobus schopfii*) is a bisporangiate lycopsid cone (Brack, 1970). This study considers only microspores that contain inclusions. Immature spores occur in tetrahedral tetrads; in some sporangia individual spores are separated from the tetrad. Of the spores studied, 55 were found in tetrads and 45 were solitary. They are