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Experimental evidence for eukaryotic fossil preservation: Onion skin cells in silica solution

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ABSTRACT

Research on the origin of eukaryotes often focuses on the exceptional preservation found in silicified Precambrian fossils. Nuclei like subcellular structures in well-preserved fossil becomes confusion and arguments, which focus on whether the partial degradation of prokaryotes produces artifacts that resemble a 'nucleus', or fossilized nuclei of eukaryotes. In order to understand the mechanisms of silicification and identify the fossilized subcellular and microstructures in rocks, a series of laboratory controlled experiments were performed for simulating the silicification process. The effects of different silica solutions in eukaryote fossilization were studied in our experiments by exposing onion skin cells (epidermis) to silica solutions. Onion skin provides a good experimental model because of its well characterized cellular structures which are easily observed. The designed experiments revealed that the possibility of onion cell preserved as a "fossil" with nuclear structures, the first week fossilization, or mineralization as rapid as 1 week is important. And the experiment also revealed interactions between silica and the onion skin cell wall surface functional groups were weak. The preservation of nuclei in the onion skin model was due to precipitation in highly supersaturated silica solutions rather than simply the high silica concentration. When the silica gel precipitates slowly at low supersaturation states, the nuclei were not well preserved, but the rapid precipitation at high supersaturated silica conditions preserved nuclear structures. A better understanding of the processes involved in onion skin fossilization will further contribute to issues concerning the silicification of other eukaryotic materials.

1. Introduction

There are many well-preserved silicified fossils in earth history, which provide us with evidence for the evolution of life (e.g. Walsh and Lowe, 1985; Schopf and Packer, 1987; Schweitzer et al., 2007). In Precambrian studies, the origin of eukaryotes is one of the most important research areas to understanding the evolution of life (Embley and Martin, 2006; Rivera, 2007; Knoll et al., 2006). Eukaryotic plant cells are characterized by the presence of membrane-bound organelles, including chloroplasts, mitochondria and nuclei. The most compelling interpretations of Proterozoic cells as eukaryotic have been drawn on the basis of morphology by the identification of structural features known to be found in eukaryotic groups but not amongst bacteria or Archaea (Javaux et al., 2003). Both microfossils found in Precambrian chert and the botanic materials obtained from Phanerozoic hot spring depositions preserve good cellular structures (e.g. Westall et al., 2001; Taylor and Taylor, 1997), specially from well preserved and documented microfossils in chert from Belcher Group (Hofmann and Jackson, 1969; Hofmann, 1976; Bennett et al., 2007), and Bitter Spring Formation (Schopf and Oehler, 1976; Christopher et al., 2000; Schopf et al., 2005). Some of the fossils' subcellular structures are very similar to nuclei. For example, there were small blebs of organic-like matter found within microfossils from Central and South Australia, which might be the preserved remnants of nuclei or other organelles (Schopf, 1968). However, some confusion and arguments focus on whether the partial degradation of prokaryotes produces artifacts that resemble a 'nucleus' (Hofmann, 2004). Bacteria do not have a membrane-bound nucleus, but sometimes an 'artificial nucleus' can appear during fossilization due to the condensation of the cytoplasm in the course of degradation and mineralization. The resulting clot may be fossilized thus creating a 'false nucleus' (e.g. Francis et al., 1978b; Westall et al., 2001). Again, however, both field observations (Golubic and Barghoorn, 1977) and laboratory experiments (Knoll and Barghoorn, 1975) showed that organic remnants within fossils are mostly collapsed and partially decayed cytoplasm. To understand the mechanisms of silicification of eukaryotes and thus to identify if it is possible for subcellular detail such as nuclei

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to be fossilized, a series of laboratory controlled experiments in this paper were designed to simulate the silicification of eukaryotic cells.

In generally, the process of fossilization has been thought to require a considerably long interval of time; perhaps as long as several million years (e.g. Siever, 1972). However, it has only been appreciated in the past decade or so, that much more rapid fossilization is also possible under suitable chemical and physical conditions (e.g. Akahane et al., 2004; Channing and Edwards, 2004).

Many silicification experiments have been conducted to elucidate the processes involved in the silicification of organic material. Early laboratory studies suggested that aqueous silica is precipitated onto organic surfaces via hydrogen bonding at the molecular level (e.g. Oehler and Schopf, 1971; Schultze-Lam et al., 1995). Nevertheless, other experiments indicated that the interaction between silica and organic surface functional groups was weak, and demonstrated that dilute concentrations of aqueous silica did not readily absorb onto some organic surface (e.g. Yee et al., 2003). The experimental result of Toporski et al. (2002) showed that high silica concentrations resulted in better preservation of cellular detail, and that the silica concentration was more important than the duration in silica solution. Our new experiment (Chen et al., 2007) complements that of others attempting to silicify microbes in controlled laboratory conditions based on different organic materials and different silica solutions (e.g. Francis et al., 1978a,b; Phoenix et al., 2003; Benning et al., 2004). Regardless of the type of silicifying agent, silicification of respective organisms was achieved in all studies with varying degrees of fidelity in terms of the preservation of cellular details. But most of the silicification experiments have used cyanobacteria and other bacteria as study materials. Only a few silicification experiments have been conducted to explain the preservation of subcellular structures in eukaryotes (e.g. Francis et al., 1978b; Westall et al., 1995).

Onion skin (onion epidermis) was chosen as an experimental model for eukaryotic material that is easily observed in our experimental design. Onion skin cells are generally from 0.2 to 0.8 mm in length, each contains a small, spherical nucleus. The cell wall of onion skin is composed of cellulose and other carbohydrate, which usually also form the main structure of other botanic cells. Onion skin is a good material for simulated experiments because of its well known and clearly displayed cell structure, further more, the results here may be significants to plant silicification experiments for the similar composition and structure. The experiments were designed to determine how the duration of exposure time and the degree of silica supersaturation affected the preservation of nuclei and subcellular details. A better understanding of the processes involved in bacterial fossilization will also contribute to our knowledge of the silicification mechanism of botanic tissues and to issues concerning the search for early eukaryotes.

2. Materials and methods

2.1. The preparation of onion skin cells

Onion skin was taken from fresh and healthy onion bulbs. The onion skins were cut into small slices (1 cm × 2 cm in size). The slices were immersed into distilled water for 1 h and then carefully washed to clean out the attached substances for further experimental use.

2.2. Silica sorption/precipitation experiments

A number of simulated experiments have been conducted on silicification processes (e.g. Yee et al., 2003; Phoenix et al., 2003; Toporski et al., 2002). These were designed with different silica concentration between 60 and 5000 ppm. Generally, natural silica solutions do not exceed 500 ppm, however, silica concentrations >750 ppm can occur in hotspring environments and may reach saturation levels with respect to silicic acid, resulting in the formation of colloidal silica or silica gels (Toporski et al., 2002). In our experiments 1000, 3000, 5000 ppm stock silica solutions (the same as in experiments of Toporski et al., 2002) were prepared with commercially available hydrous sodium silicate, containing ~20% SiO_2 and ~20% Na_2O. The pH of the solutions was adjusted to pH 8.0 using diluted hydrochloric acid. The error of pH measurement during the experiment was less than 0.5 pH unit. Water from a modern lake near Yangtse was used in our experiment because it contains common ions found in naturally occurring water systems encountered in environments where silicification can take place. Onion skin slices were placed in these solutions for time-dependent experiments: 24 h, 1 week, 1 month, 3 months. Each conical flask contained 200 ml silica solutions with five onion skin slices and were left at room temperature and ambient pressure.

We designed experiments to determine whether salinity will affect processes leading to the decay of cellular structures. To detect the salt effect on silica solutions, a 10‰ NaCl solution (the same NaCl concentration as in 5000 ppm silica solution which was prepared from Na_2SiO_3–$5H_2O$ mixed with HCl) was used to treat the onion skin cells. The experiments revealed that the amount of monomeric SiO_2 decreased rapidly in the first few hours after the silica solutions were mixed with Na_2SiO_3–$5H_2O$ and HCl (Yee et al., 2003). Two background experiments were conducted in our lab to test how the rapid decrease of monomeric silica gel (or SiO_2) can affect the preservation of subcellular structures.

Background experiment I: Onion skin slices were left in 5000 ppm silica solution which had previously been left to polymerize for 3 days.

Background experiment II: Onion skin slices were placed in freshwater after treatment with newly compounded 5000 ppm silica solution for 3 days.

The chemical compositions of our solutions are showed in Table 1, all the solution samples were filtered by medium-speed filter papers (pore size: 30–50 μm) before being analyzed by an Inductive Coupled Plasma (ICP) Atomic Emission Spectrometer. A summary of the samples in our silica precipitation experiments and the figures in which they are shown are given in Table 2.

2.3. Preparation for observation

After immersion in silica solution for the stated time, the onion skin slices were removed from the silica solution. Portions of onion skin slices were treated with Ziehl-Neelsen Stain and then observed with an optical microscope.

Additionally, LEO1530VP SEM was used for the EDS (energy dispersive spectroscopy) analyses of the silicified samples in our laboratory. All samples were rinsed several times with distilled water to wash off the attached silica solution, and then dehydrated through a series of ethanol solutions (2 min in each 30%, 50%, 75%, 85%, and 95% ethanol solution). Dried samples were then placed on SEM stubs, sputter-coated with gold, and analyzed on SEM operating at 5–15 kV. Qualitative precipitate chemistry was determined using an EDS system.

3. Results

Optical microscope photomicrographs of fresh onion skin cell samples are shown in Fig. 1. It can be seen that most of the fresh onion skin cells keep intact nuclei and no detached cytoplasm. The image in Fig. 1B shows that each onion skin cell has a spherical or oval nucleus that contains chromosomes.