Application of back-scattered electron imaging to the study of the lichen–rock interface

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Summary

In the study of the lichen–rock interface, light microscopy, scanning electron microscopy (SEM) in secondary emission mode and transmission electron microscopy are the most commonly used techniques. As these methods have some limitations, there is a need to explore other techniques for observation of the lichen–substrate interface. One of the most promising methods is the application of SEM in the back-scattered electron (BSE) emission mode.

The thallus of *Aspicilia intermutans* (Nyl.) Arn. growing on granitic rock was examined by SEM in BSE mode. The detailed preparation of transverse sections of the lichen–rock contact zone is presented. The BSE scanning images of the lichen–rock interface obtained present new insights into the ultrastructural features of the biological components, providing more information about the biogeophysical and biogeochemical weathering of rock.

Introduction

Investigation into the contact zone between the lichen thallus and substrate has been the subject of scientific interest for several decades. In recent years, the need to investigate possible biodeterioration produced by lichens and other living organisms on historical monuments has revived interest in this field. The zone of contact between the lichen thallus and the rock presents a very high grade of heterogeneity as a consequence of the presence of rock components, microdivided particles, laminar minerals, inorganic compounds like oxalates and organic crystalline compounds such as lichen acids. Living organisms including the myco- and photobiont of lichen thalli, bacteria, cyanobacteria, algae or fungi

are also present. Viles (1987) has expressed the idea that lichens may be best viewed as one component which in some circumstances plays a dominant role in a complex weathering system.

Taking into account the complex composition of this contact zone, the study of interaction between lichens and rocks requires the application of very different techniques. According to the available literature, the combination of direct observation techniques such as light microscopy (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and also microanalytical techniques are most extensively used in the study of the interaction between lichens and rocks. Detailed reviews of methods used in the investigation of this zone are given by Ascaso & Wierzechos (1994).

Light microscopic (LM) examination of transverse sections of the lichen–rock interface was the principal technique used in the first investigations in this field. Although LM contributed sufficient information in the macroscale of observation and permitted good distinction between the different minerals of the rock in polarized light, the low spatial resolution did not permit determination of all the components and details from the lichen–rock interface. Moreover, LM is not suitable in conjunction with a microanalytical apparatus.

Hallbauer & Jahns (1977) first used SEM, and subsequently many other SEM investigations of the interface zone have been performed in secondary electron mode. Some of the most recent of these investigations include Wilson *et al.* (1981), Ascaso *et al.* (1982), Adamo & Violante (1989, 1991), Pinna & Salvadori (1992) and Nimis *et al.* (1992). Although in each of these studies new and interesting observations were presented, unfortunately, many biological and mineralogical materials do not produce a very high yield of secondary electrons and are non-conductive substances; these materials must therefore be coated with a layer of heavy metal. However, a heavy metal coating renders the specimen unsuitable for

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subsequent analysis by X-ray emission. Moreover, topographic images of the surface of transverse sections of the contact zone can contain artefacts resulting from the detachment of small particles during section preparation and from the obstruction of these particles by biological material.

Transmission electron microscopy (TEM) of ultra-thin transverse sections of the lichen-rock interface was discussed by Ascaso & Ollacarizqueta (1991). This technique, combined with energy-dispersive X-ray spectrometry (EDS), gave ultrastructural information about the symbionts and permitted analysis of the inorganic deposits present among the algae and hyphae of lichen thallus. However, this technique has inherent limitations. First, only material from the upper part of the contact zone can be investigated, because the presence of rock in lower layers of the lichen-substrate zone makes it very difficult to obtain ultra-thin sections. Another limitation is the small size of the observed area (1–2 mm²).

Therefore, we need to explore other techniques for the direct observation of the lichen-substrate interface that will permit observation of a wider surface area (over several centimetres, as in LM), with a high spatial resolution (approaching that of TEM techniques), allowing study from macro- to submicroscopic range, and the possibility of instantaneous qualitative and quantitative *in situ* microanalysis of the inorganic constituents.

One of the most promising tools, complementary to other techniques, is the scanning electron microscope operating in back-scattered electron (BSE) emission mode with an auxiliary EDS microanalytical system. The emission of BSE is strongly dependent on the mean atomic number of the target, and, for homogenous compounds, on the concentration of the element (White *et al.*, 1984; Robinson, 1987; Joy, 1991). This feature of BSE mode enables us to distinguish the components within the thallus and in the lichen-rock contact zone. Another potential advantage of the BSE mode for non-conductive specimens is that these electrons have high energy and are essentially unaffected by localized charging of the specimen surface, thus removing the need for surface coating with heavy metals.

The first attempts to take back-scattered electron scanning images (BESI) of lichen material were reported by Chisholm *et al.* (1987) and Purvis *et al.* (1987, 1990). They examined sections of lichen thallus containing lichen acid and oxalate compounds. They did not obtain a sufficiently bright response in their BSE images for the micromorphological study of biological material as a result of the preparative procedure, the rough nature of the specimen and coating of the observed surface by Au/Pd.

We describe the first attempt to study the lichen-substrate contact zone with SEM in BSE emission mode, with the idea of obtaining sufficient resolution to enable ultrastructural observations of either the thallus or other

biological elements such as cyanobacteria or algae. Considerable attention was given to the preparation procedures for transverse sections of the lichen-rock interface.

Material and methods

Material sampling

Aspicilia intermutans (Nyl.) Arn. was collected from granitic rocks at Bustarviejo de la Sierra, near Madrid, Spain. A previous petrographic study of the rock carried showed the presence of quartz, orthoclase and plagioclase as essential minerals, and biotite, tourmaline, zircon and apatite as accessory minerals (Ascaso, 1985). Pieces of rock with thalli were hand-cut using a hammer and chisel.

Preparative procedure of samples for SEM (BSE) study

For optimal polishing and fixation of biological material from weak contact zones, the following preparation method was developed. Small (a few cm³) blocks were carefully cut with a diamond saw in the transverse direction. The surface of the lichen-rock interface (several cm²) was dry-polished with waterproof silicon carbide pregrinding papers, starting with 320 grains/mm² — paper (no. 320) and ending with 800 grains/mm² — paper (no. 800). The transverse surface was then cleaned with purified compressed air. Afterwards the pre-polished transverse surfaces were fixed with glutaraldehyde/osmium tetroxide and dehydrated through a graded series of ethanol according to a conventional TEM procedure for lichen material (Ascaso *et al.* 1988). In order to raise the cohesion of the material, the transverse surfaces were then impregnated in a vacuum chamber with LR White (medium grade) resin. This low-viscosity acrylic resin was used to ensure maximum saturation of the micropores. After polymerization in an oven (24 h, 333 K), dry hand-polishing of resin-impregnated surfaces was continued using the pregrinding papers (starting with no. 320). When the contact zone surface was approached, papers no. 800 and no. 1200 were used. Dry polishing was continued by hand with silicone carbide abrasive sheets with grain diameters of 15, 9 and 3 µm. Final polishing used liquid diamond polishing compound on napped cloth with diamond particle sizes of 1 and 0.25 µm in an oil-based lubricating fluid. The transverse surfaces were finally washed with petroleum ether and dried with pure compressed air. The final polished surfaces were stained with lead citrate (Reynolds, 1963) for 10 min. Post-fixation in OsO₄ and staining with lead citrate enhanced the resolution of ultrastructural aspects in BSE emission mode. After washing in distilled water and air drying, the surfaces of polished blocks were coated with evaporated carbon. The samples were then examined with a SEM Zeiss

960) DSM equipped with a scintillator (Robinson variety) type BSE detector and Link ISIS EDS microanalytical system. Optimum conditions of observation were obtained at an accelerating voltage of 25 kV, a working distance of 8 mm and a specimen current of 10^{-8} A.

Results and discussion

Figure 1(a) shows a general view (low-magnification) of the contact zone between *Aspicilia intermutans* thallus and granitic rock. Note that the algal, medulla and cortex zones of thallus can be distinguished readily as a result of the greater absorbance of the fixing and staining heavy metals in algal cells. In this example the BSE images show contact between the hyphae of the lower part of the thallus and the substrate. The hyphae detach parts of the granitic rock and encrust all the surface minerals which are microdivided and embedded (see arrow in Fig. 1a). This image shows that the lichen thallus acts mechanically upon the surface of the rock.

Figure 1(b) shows the organization of algal and hyphae in the thallus. Note that satisfactory resolution of BSEI permits the distinction of organelles of algae and fungi. In the photobiont central chloroplast, starch granules about $0.5\text{ }\mu\text{m}$ in length and an oval-shaped zone (pyrenoid) can be distinguished. The contrast of the BSE images is the reverse of that of the LM or TEM images, but it is possible to invert this contrast electronically. Figure 1(c) shows the system of fissures and cracks in granitic rock which are filled with biological material. The fissures appear bright because of the high emission of BSE from absorbed heavy metals, as a result of the fixing and staining treatment on the biological material. Figure 1(d) was taken from the fissure marked by arrow 1 on Fig. 1(c). Free living, chasmoendolithic algae in the process of cell division were observed at a depth of up to 3 mm below the surface of rock. However, it is not clear whether the algae produce fissures in the rock or whether the algae exploit existing fissures. This point has to be investigated more exhaustively. In the vicinity of arrow 2 in Fig. 1(c), another type of structure was found (Fig. 2(a)); the excellent resolution of BSE images allows observation of two hyphal cells joined by a septum which occupies the available space in a fissure $4\text{ }\mu\text{m}$ in diameter. When the thallus covers a zone of rock rich in micaceous material, the plates of mica begin to be broken and separated by algae and hyphae, as is shown in Fig. 2(b) and in a more detailed view in Fig. 2(c). Indeed, some of the mica plates are completely separated by groups of algae (black arrow, Fig. 2b). Penetration of masses of hyphae on the surface of the rock, resulting in separation from the feldspar block and displacement of packed mica plates towards the left is apparent (Fig. 2b). The mechanical action of the lichen thallus upon the micaceous part of the rock is evident and the successive stages of this action can be distinguished. All

the observations presented so far have shown evidence for the mechanical action that the lichen thallus produces on the rock substrate (biogeophysical weathering).

In the interior of the *A. intermutans* thallus (Fig. 2b) we can distinguish a great quantity of microdivided minerals, probably originating from the rock surface (white arrows). Microdivision of these minerals is probably a result of the mechanical action of the thalli, but the existence of some type of chemical action cannot be discarded. The application of EDS to the study of the nature of the microdivided minerals would enable us to determine chemical changes in their composition and thus determine whether weathering has occurred.

The biogeochemical action of the thallus could also take place in the fissures. The application of SEM equipped with a BSE detector and an EDS microanalytical system permits detection and determination of the inorganic crystals found in the fissures of granitic rock (Fig. 2d). The rhombohedral (octahedral dipyrmaid in 3-D) form of the calcium oxalate dihydrate compound is recognizable (see arrow 1). Calcium oxalate dihydrate crystals can also be recognized as 'ghosts' (arrow 2). The shape of 'ghost' crystals is distorted because when the crystals grew they thrust the matrix aside to minimize the intercrystalline space. The morphological determination of calcium oxalate was confirmed by EDS microanalysis.

Conclusions

The results show that SEM using the BSE signal can be valuable in investigating the lichen-rock interface. BSE composition images of finely polished transverse sections from the contact zone may reveal additional information about the interactions between lichens and rocks, thereby increasing our understanding of the biogeophysical and biogeochemical weathering processes. This technique of direct observation has clear advantages over other techniques such as light microscopy, scanning electron microscopy in secondary electron emission mode and transmission electron microscopy. One of the principal advantages of the BSE method is the possibility of continuous observation from the macro- to submicroscopic scale. The back-scattered electron scanning images of polished transverse sections of the lichen-rock interface show good correspondence with the TEM and LM images. Moreover, determination of the components of this contact zone can be confirmed by an auxiliary microanalytical EDS system.

We would like to note that use of BSE to study the interaction between lichen and rock can also be applied to other fields where alteration of solid materials, such as metals, cements, plastic, ceramic, glass and construction materials, is caused by the growth of biological species. This technique can also be used to study the ultrastructure and

Fig. 1. (a) General view of the interaction between *Aspicilia intermutans* thalli and granitic rock. A, algal layer; C, cortex; F, feldspar; M, medulla; Q, quartz; arrow, microdivided minerals. (b) The photobiont and mycobiont arrangement in thallus. ch, chloroplast; M, mycobiont; P, photobiont; py, pyrenoid; s, starch. (c) General view of fissures in granitic rock filled with biological material. (d) Detail of the fissure marked by arrow 1 in (c). Algal cells in division.

Fig. 2. (a) Detail of the fissure marked by arrow 2 in Fig. 1(c). (b) General view of the interaction between algae and hyphae of thallins and the micaceous part of granitic rock. F, feldspar; H, hyphae; Mi, mica; black arrow, algal cells; white arrow, microdivided minerals. (c) Higher magnification view of (b). Algal cells between the mica layers. Mi, mica; P, algal cells. (d) Calcium oxalate dihydrate crystals in the fissures in granitic rock. Mi, mica; arrow 1, octahedral dipyrmaid Ca-oxalate crystals; arrow 2, 'ghosts' of Ca-oxalate crystals.

micromorphology of the biological tissue 'embedded' in solid material and can be used for the instantaneous micro-analysis of the inclusions occurring within the biological material.

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