HRTEM INVESTIGATIONS BETWEEN MINERALS, FLUIDS AND LITHOBIONTIC
COMMUNITIES DURING NATURAL WEATHERING

Progress Report

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W. W. Barker and J. F. Banfield

Department of Geology and Geophysics
Weeks Hall 1215 West Dayton Street
The University of Wisconsin - Madison
Madison, Wisconsin 53706
jill@geology.wisc.edu; barker@geology.wisc.edu

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Abstract: As sites for biochemical and inorganically mediated weathering, sorption, and desorption, complex and poorly understood mineral-organism interfaces control the distribution and transport of natural as well as anthropogenic chemicals in the surface and subsurface. Physiochemical and biogeochemical weathering of calcic and riebeckitic amphibole in the Stettin syenite complex near Wausau, Wisconsin, has been examined by HRTEM, and LM. Fungal hyphae from two crustose lichens, *Rhizocarpon grande* and *Porpidia albocaerulescens* penetrate the mineral to > 10 mm. Fungal growth produces cleavage-bounded fragments as small as 5 μm which accumulate in the lower thallus.

Bacterial microcolonies are common. Extracellular microbial polymers completely coat all mineral surfaces, creating a microenvironment where mineral transformation proceeds via a complex dissolution-precipitation mechanism. High-resolution images demonstrate that dissolution produces no surface leached layers. Following complete loss of Ca, K, Al, Mg, Mn, and Ti, whether metabolized or lost in solution, remnant Fe and Si crystallize onto exopolymers as non-topotactic iron phyllosilicate. Biogeochemical weathering culminates in polymer-bound masses of clay minerals. In contrast, physiochemical weathering involves a combination of dissolution and structural inheritance. The calcic amphibole weathers to a topotactically oriented Ca smectite, whereas riebeckite transforms entirely to nanocrystalline goethite.

Objectives: The objective of our research is to explore the hypothesis that reactions occurring at the mineral:lichen interface are strongly impacted by biochemical processes and thus differ in their rates and products from those reactions occurring at distance from this interface. The first year of the project has been devoted to developing and testing a sample preparation protocol. We succeeded in overcoming significant sample preparation obstacles. In addition, we have made excellent progress towards the research goals: 1) taxonomic identification of lichens, 2) characterization of some critical intact organo-mineral interfaces, and 3) defining the process of biogeochemical weathering.

Project description: The role of microorganisms in mineral degradation long has been a subject of intense scientific debate. Historically, scientific opinion ranged between critically important to insignificant in near-surface mineral transformation reactions. A number of research studies investigated the extent to which members of lithobiontic communities, primarily lichens, participate in mineral degradation and formation of new phases. The transmission electron microscope (TEM) has not been widely applied to this question. The few instances of its use involved destructive sample preparation techniques such as chemical digestion of the organic material followed by ultrasonic disaggregation to isolate the mineral phases for study.

In our research, we use high-resolution transmission electron microscopy (HRTEM), energy dispersive x-ray spectroscopy (EDS) and selected area electron diffraction (SAED) to characterize intact lichen-mineral interfaces. Light microscopy (LM), X-ray diffraction (XRD) and SEM are also used when appropriate. These approaches provide millimeter to sub-nanometer-scale morphological, chemical and structural information.

Specifically, we are characterizing the contacts between one species of foliose and two species of crustose saxicolous lichens and ferromagnesian minerals in the Stettin amphibole syenite (1.6 Ga intrusive complex near Wausau, Wisconsin). We have also commenced study on a diverse lichen assemblage living on amphibolites from near Eau Claire, Wisconsin. A species list from this locality is included in Appendix 1.

For comparison, samples were collected from within weathering rinds, and thus not subjected to direct attack by lichen-derived compounds.

Sample Preparation: We have successfully adapted biological fixation and embedment protocols and used them to prepare rock samples encrusted with intact lithobiontic communities. Initially, all samples are essentially treated as biological samples in that they are fixed, postfixed, dehydrated, and finally infiltrated and embedded in epoxy resin.

Sample fixation protocols are detailed in Appendix II. Once the epoxy has cured, the samples are diamond sawed, mounted on petrographic microscope slides with acetone-soluble adhesive, and prepared as standard geological thin sections. A low viscosity heat-cured epoxy (M-Bond) is applied as necessary to the thin section to stabilize it during mechanical grinding. Areas of interest are removed from the slide, mounted on 3 mm TEM hole grids and Ar ion-milled to electron transparency. Duplicate material containing mineral/organism interfaces of interest is re-embedded in plastic and ultrathin sections are prepared using diamond knife ultramicrotomy. A complete sample preparation flow chart is given in Appendix 2.

Problems and Solutions: Sample preparation which preserves the intact mineral-organism interface is exceedingly difficult, especially on weathered geological materials. We have confronted and solved three major difficulties so far in this research.

1. Sample disintegrates during mechanical grinding. This problem is especially prevalent either in minerals that have perfect cleavages or in material which is extensively weathered. For example, orientation of an amphibole grain with the c-axis perpendicular to the plane of the thin section exposes the perfect {110} cleavage to substantial lateral force during grinding, leading to plucking of diamond shaped cleavage fragments and in extreme cases, complete loss of the amphibole from the slide. As physiochemical weathering in amphiboles is often cleavage-controlled, this plucking tendency is exacerbated in weathered materials. Additionally, weathering products concentrated in these channels can be selectively ground away during sample preparation. Our solution is to stabilize the samples with a very low viscosity heat-cured two part epoxy which is marketed under the trade name of M-Bond. The epoxy is brushed onto thin sections at the first sign of deterioration and may be reapplied as necessary. The low viscosity allows it to penetrate cracks and cleavages which develop during grinding. M-Bond does not appear to interfere with optical characteristics of minerals. It is insoluble in acetone and stabilizes the mineral slice after removal from the petrographic slide. Further, M-bond is amorphous to electron diffraction, has no elemental signature (except C), and is reasonably stable under electron bombardment.

2. Biological material preferentially mills during Ar ion bombardment. We have pursued several strategies including liquid nitrogen cooling, differential sample stage rotation and masking to circumvent this problem. At this point, Ar ion milling will effectively clean all the biological material from a hyphae-filled crack before any thin edges suitable for HRTEM examination are produced in mineral edges. Of even greater concern is the apparent complete destruction of cellular ultrastructure during ion bombardment. Therefore, examination of diamond knife ultramicrotomed material supplies the bulk of our data concerning intact mineral-organism interfaces.

3. Mineral grains shatter and fall out during diamond knife ultramicrotomy. Careful orientation of the mineral grain in question so that cleavages parallel the plane of the diamond knife edge as it passes through the sample has greatly reduced this problem. This limits the crystallographic data obtainable from these types of samples.

Results: In collaboration with Professor J.W. Thomson, we have applied microcrystal chemical tests based on identification of lichen substances to identify *Porpidia albocaerulescens* and *Rhizocarpon grande* as the dominant species of crustose lichens developed on the Stettin Syenite. *Xanthoparmelia somloensis*, a foliose saxicolous lichen was also studied for comparison purposes. These lichen substances are extracted with acetone, crystallized, and the habit of the resultant crystallites are studied optically and compared with picture atlases for identification. Due to the chelation abilities of these compounds, previous workers have postulated that they may be involved in mineral

reactions. However, we do not consider these substances to be especially important in mineral dissolution as they are sequestered in the upper part of the thallus and are almost absent in the region immediately adjacent to the rock surface. The probable role of these substances is to deter predation.

Optical microscopy of thin sections made from embedded samples shows that the fungal component of a lichen, the mycobiont, exploits cracks, cleavages, and grain boundaries to penetrate the surface of minerals to a depth of at least 10 mm. Characterization of ion-milled and diamond knife ultramicrotomed TEM samples prepared from these thin sections reveals continued fungal growth shoulders grains apart, resulting in cleavage-bounded mineral fragments as small as 5 μm . These mineral fragments are lifted away from the underlying substrate and together with possibly subaerially deposited particles, accumulate within the lower thallus. All mineral surfaces are completely coated in extracellular polymers from fungal and bacterial sources. This organic coating differentially stains with Pb, U, and Os, indicating a strongly electronegative character. Staining patterns appear to show that electronegative zones are concentrated near mineral surfaces. Clay minerals, often as small as three or four unit cells thick in the (001) direction are enmeshed in this organic coating. The clay particle orientation is tangential or face-to-face with respect to both the parent mineral surface and other clay particles as well. We have observed a complete progression from polymer-coated pristine amphibole surfaces through to a remnant packet of polymer-bound face-to-face oriented clay minerals which incorporate the remnant polyhedra left from the dissolution of the parent mineral phase. The intermediate form is a grain of amphibole, rounded by dissolution, and mantled by a rind of tangentially-oriented clay minerals (Figure 1). While we originally interpreted these polymer-bound clay packets as fecal pellets, they are clearly the result of a complex biologically controlled reaction involving complete dissolution of amphibole and reprecipitation of an iron phyllosilicate. Contrary to predictions based on previous studies of experimental mineral dissolution and the fate of mineral dusts in the human lung, dissolution does not appear to involve leaching. The smallest mineral fragments are crystalline and retain their chemical identity. Even the sodic amphibole fragments completely enclosed by extracellular polymers retain measurable Na. Lattice imaging of the amphibole-polymer interface clearly shows lattice fringes extending to the edge of the grain. There is no amorphous or leached layer on the surface. Although other studies that employed chemical digestion of organic material have reported that lichens are capable of completely extracting all the cations from some minerals (chrysotile, for example), leaving only silica, no such siliceous relics have been identified in our samples.

HRTEM characterization reveals physiochemical weathering of calcic amphibole surfaces results in highly corroded channels, with surface topography defined by few-nanometer-wide serrations bounded by {110} planes. Reaction products include Fe-rich smectite and minor goethite. The conversion of the amphibole (approximately ferro-alumino-winchite) to smectite involves a partial loss of Ca (and K) and complete loss of Na. Although the smectite is topotactically oriented (c-smectite // a-amphibole; b-smectite // b-amphibole), the reaction is neither isochemical nor isovolumetric. Mass balance considerations indicate extensive dissolution and transport of released constituents. Similar results involving both riebeckite and acmite were recorded from a second locality. In this instance, however, the dominant weathering product is goethite. Initial reaction involves complete dissolution and a total loss of Na and Si accompanied by the formation of nanocrystalline goethite. There is no obvious topotactic relationship between these 3 nm goethite crystallites and either the acmite or riebeckite. Over time these crystals coarsen into much larger goethite crystals. Preliminary Debye-Scherrer XRD data indicate that biotite directly in contact with the lichen thallus (*R. grande*) is intimately interpenetrated by fungal hyphae growing along (001) cleavages and partially converted to vermiculite via the loss of interlayer K. The vermiculitization of biotite is also observed using HRTEM. This process appears to involve transformation of packets of 6-10 individual layers (along c) interleaved with unaltered biotite.

Summary: Biophysical and biogeochemical weathering of ferromagnesian minerals in two amphibole syenites associated with the Stettin complex near Wausau, Wisconsin, have been examined by HRTEM, SEM, LM, and XRD. The first rock, an acmite riebeckite syenite carries extensive colonies of the foliose lichen *Xanthoparmelia somloensis*. Two species of crustose saxicolous lichens, *Rhizocarpon grande* and *Porpidia albocaerulescens*, colonize the second rock type, a biotite hornblende. While a more intimate contact between crustose versus foliose thalli and the underlying mineral surface exists, fungal hyphae from all three species exploit grain boundaries, cleavages, and cracks to gain access to interior mineral surfaces and penetrate the rock surface to a depth of 10 mm.

Bacterial microcolonies are common and all mineral surfaces are completely coated in extracellular polymers (probably acidic mucopolysaccharides) from fungal and bacterial sources. Continued fungal growth shoulders grains apart, resulting in cleavage-bounded mineral fragments as small as 5 μm . Larger fragments, some of which may represent subaerially deposited mineral particles, accumulate within the lower thallus. At this point, the mineral grain is completely surrounded by extracellular organic material and begins a polymer-mediated dissolution-precipitation process. Dissolution does not appear to involve pervasive leaching, for even the smallest mineral fragments retain their chemical and structural identity. No siliceous relics have been identified. Early stages of biogeochemical weathering of a calcic amphibole produce a thin rind of intimately mixed extracellular polymers and an iron phyllosilicate. The lower thallus also contains abundant 5-10 μm diameter packets of polymer-bound clay minerals which represent the end product of biogeochemical weathering. The elemental composition of these clays is quite constant within an individual packet, indicating these clays are locally derived. They appear to represent the end products of polymer-mediated complete dissolution of the parent mineral phase and reprecipitation of elements which were not metabolized or lost in solution. For our purposes, polymer-mediated is interpreted to mean that microbial exopolymers both facilitate mineral dissolution and also serve as nucleation sites for reaction products formed during this process. Biotite directly in contact with the lichen thallus is intimately interpenetrated by fungal hyphae growing along (001) cleavages and is partially converted to vermiculite.

Physiochemical weathering of amphiboles in the Stettin syenite differs fundamentally from biogeochemical weathering. The cleavage-controlled reaction is neither isochemical nor isovolumetric. Textures suggest extensive dissolution and transport of released constituents. In the case of calcic amphibole, topotactically oriented smectite with minor goethite forms as a reaction product. The conversion of hornblende to smectite involves a complete loss of Na, K, and Ti, as well as a partial loss of Ca and Mn. Acmitic pyroxene and riebeckitic amphibole transform directly to non-topotactic nanocrystalline goethite which later coarsens to well-ordered larger crystallites. Thus, the initial chemistry of the parent mineral is an important controlling factor in reaction products formed during natural physiochemical weathering.

Our preliminary results demonstrate that fundamental and quantifiable differences exist between physiochemical and biologically-mediated weathering of amphiboles. Physical weathering involves growth of topotactically-oriented smectite or nanocrystalline goethite by a combination of structural inheritance, dissolution, and transport in a relatively open system. Biogeochemical weathering commences with mechanical fracturing and proceeds through a complex set of biogeochemical reactions which are possibly unique to each mineral type and perhaps each type of organism involved. During the coming year we will examine the interaction of other species of lichens and bacteria with plagioclase and potassium feldspar and pyroxenes. We expect to find an extremely complex heterogeneous system, the nature of which can only be studied if the intact organic-inorganic interface is preserved.

Figure 1 Microbial exopolymers (P) completely surround a grain of calcic hornblende (A) which is mantled by a rind of iron phyllosilicates (C). Passage of the diamond knife shattered the amphibole.

P

C

A

C

P

0.1 mm

Appendix 1- Species List

The following species of crustose saxicolous lichens have been collected from the amphibole syenites exposed in the Stettin Syenite complex near Wausau, Wisconsin:

Lecidea macrocarpa

Lepraria sp.

Porpidia albocaerulescens *

Porpidia crustulata

Rhizocarpon grande *

The following species of foliose saxicolous lichens also have been collected from this locality:

Cladonia cristatella

Cladonia grayi

Cladonia rei

Cladonia verticillata

Stereocaulon saxatila

Xanthoparmelia somloensis *

The following species of crustose saxicolous lichens have been collected from the amphibolites exposed at the Falls of the Eau Claire River, near Eau Claire, Wisconsin:

Acarospora fuscata *

Aspicilia caesiocineria *

Buellia punctata

Lecanora intricata *

Phycia dubia

Phycia subtilis

Lichen/mineral sample preparation protocol

PRIMARY FIXATION

2.5% glutaraldehyde/0.1M phosphate buffer 2 hr @ 20°C
or use Karnovsky fixative (glutaraldehyde/paraformaldehyde)

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BUFFER WASH

0.1 M phosphate buffer 3 x 15 min

|

|

POST FIXATION

2% OsO₄ / DDH₂O 2 hr @ 20°C

|

|

FINAL WASH

DDH₂O 2 x 10 min

|

|

DEHYDRATION

gradational dehydration series of 50,70,80,95,100% ETOH / DDH₂O
(can be stored overnight @ 80% if refrigerated)

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INFILTRATION AND EMBEDMENT

1:1 Propylene oxide (PO) / ETOH 10 min

|

100% PO 5 min

|

|

gradational series of PO / plastic (Epon-Araldite)

25% plastic; 0.5 hr, 50% plastic; 1 hr, 75% plastic; 2 hr,
95% plastic; 12 hr under vacuum,

100% plastic; 12 hr under vacuum,

place samples in embedding mold with fresh 100% plastic,
polymerize 24-48 hr @ 60°C.

Warning: Glutaraldehyde, paraformaldehyde, osmium tetroxide, and the chemicals comprising Epon-Araldite resin are hazardous. **This applies both to vapors and liquids. Always** work in a properly rated chemical hood and avoid skin contact at all costs. All contaminated objects must be disposed of properly as contaminated labwaste.

Depending on size, properly embedded and polymerized samples can be either:

1. Ultramicrotomed using a diamond knife and an ultramicrotome. The thinnest sections it is possible to cut are about 40 nm.
2. Larger samples can be slabbed with a diamond saw (Buehler Isomet).

Ultramicrotomed Thin Sections

Stain with uranyl acetate
and lead citrate

TEM examination

Diamond Saw Slabs

mount w/ cyanoacrylate
or crystal bond

standard thin sxn to
100 microns

Hand grind using diamond laps, pastes, tripod, etc. M-bond as needed.

PLM examination and photomicrography

Excision of selected areas *

Mount on C-coated grid of appropriate size (hole, slot, etc.)

PLM Photomicrography

Ar Ion mill

PLM Photomicrography

C-coat in vacuum evaporator

HRTEM Examination

*Selected areas can be removed and mounted in a Debye-Scherrer camera for x-ray diffraction or re-embedded for ultramicrotomy.

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