*Acta Mineralogica–Petrographica, Szeged, XLI, Supplementum B, 2000*

# DISCOVERY OF BOTANICAL IRON BIOMINERALS BY ELECTRON DIFFRACTION AND MICROSCOPY

GAJDARDZISKA-JOSIFOVSKA, M., SCHOFIELD, M.A. (Department of Physics, University of Wisconsin Milwaukee, USA), McCLEAN, R.G., KEAN, F.W. (Department of Geosciences, University of Wisconsin Milwaukee, USA) & SOMMER, C.V. (Department of Biological Sciences, University of Wisconsin Milwaukee, USA)

Introduction

Characterization of soil profiles often shows enhanced magnetism in the top soil, attributed to higher concentration of ferrimagnetic iron oxides (CORNELL & SCHWERTMANN, 1996). Two contrary routes, an inorganic (MAHER & TAYLOR, 1988) and a bacterial (FASSBINDER et al., 1990), have been proposed for the formation of ultra-fine magnetite in soil, and the soil magnetite controversy is yet unresolved. Soil by definition supports plant life, and it is known that plants, like animals, store iron in their cells. Based on magnetic studies of various plant material, McCLEAN & KEAN (1993, 1996) have proposed that plants can be contributors to the soil magnetism. These saturation isothermal remanence magnetization studies and hysteresis measurements were indicative of magnetite as the dominant magnetic mineral, in the grain size range of superparamagnetic to pseudo-single-domain, with minor antiferromagnetic components. This size range of magnetite is consistent with the findings of many transmission electron microscopy studies of sectioned plant cells, which have revealed micron sized phytoferritin agglomerates made of nanometer sized electron dense cores with ordered, semi-ordered and random self assembly (e.g. review by SECKBACK, 1982). However, the crystal structure of these phytoferritin iron cores has remained unknown, as biominerals are seldomly available in the quantities needed for characterization by X-ray diffraction. In our work we use electron diffraction to get the first determination of the plant iron biomineral phases.

Experimental

Leaf and stem clippings from normal grass (*Festuca* species) were harvested in August 1995 from a rural location in southeast Wisconsin. We used the biochemical procedure of HYDE et al. (1968) to extract and isolate phytoferritin, which was then dispersed on thin amorphous carbon films for studies with transmission electron microscopy (TEM: Hitachi H-9000 NAR, operated at 100 keV). Digitally recorded selected area transmission electron diffraction patterns (SAED) were used to determine the crystallography of the phytoferritin iron core. Two kinds of TEM samples were investigated: standard ultracentrifuged extracts with dispersed phytoferritin nanoparticles; and novel low-speed centrifuged and filtered extracts with micron-sized phytoferritin agglomerates. We used energy dispersive X-ray (EDX) spectrometry (Noran Voyager II) to discriminate between various sub-cellular extracts present in the low-speed centrifuged samples. Only those specimen areas that displayed characteristic Fe peaks in the EDX spectra were analyzed with electron diffraction and imaging. This approach allowed us to investigate the crystallography of both ferromagnetic and antiferromagnetic phases that might be present, as suggested by our magnetic measurements of plant clippings, ashes, and soil phytoliths.

Results

Detailed correlative analysis of 36 SAED patterns and their corresponding TEM images indicate that phytoferritin occurs as crystalline magnetite ($Fe_3O_4$), $\varepsilon$-$Fe_2O_3$, and hematite ($\alpha$-$Fe_2O_3$), with typical sizes of single crystallites in the 1–50 nm range and agglomerate grain sizes up to 4 μm. The three dimensional agglomerates are built with different biomineral nanocrystals in three distinct modes of biological self-assembly: 1) ordered magnetite; 2) semi-ordered mixture of magnetite and $\varepsilon$-$Fe_2O_3$; and 3) random hematite. These self-assemblies correspond to prior TEM reports of crystalline, paracrystalline and amorphous phytoferritin arrangements in sectioned cell samples. A fourth plant iron biomineral, tentatively assigned as calcium ferrate hexahydrate, has morphology and diffraction patterns distinct from the phytoferritin aggregates. We did not detect diffraction patterns that are consistent with the 2-line or 6-line ferrihydrite, the postulated form of phytoferritin based on analogies with the animal ferritin (BIENFAIT & VAN DER MARK, 1983).

These diffraction results mark the first discovery of crystalline iron biominerals in plants. Of these four new iron biominerals, three are antiferromagnetic, while magnetite is the only ferromagnetic phase. These results are consistent with the SIRM measurements of McCLEAN & KEAN (1993, 1996). The properties of the observed botanical magnetite are of particular interest in the context of its contribution to plant and soil magnetism. The majority of plant magnetite nanocrystals display cubo-octahedral shapes and narrow size distributions typical for intracellular boundary organized biomineralization processes (LOWENSTAM, 1981). The botanical magnetite nanocrystals are self-organized in ordered micron range agglomerates, distinct from magnetite strings in magnetotactic bacteria (BLAKEMORE, 1975, PETERSEN et al., 1986) and similar to some pedogenic magnetite currently attributed to inorganic processes (HOUNSLOW, M.W. & MAHER, B.A., 1996).

References

BIENFAIT, H.F. & VAN DER MARK, F. (1983). In "Metals and Micronutrients: Uptake and Utilization by Plants" (Robb, D.A. & Pierpoint, W.S., Eds.), Academic Press, New York, pp. 111–123.

BLAKEMORE, R.P. (1975). Science, **190**: 377–379.

CORNELL, R.M. & SCHWERTMANN, U. (1996). The Iron Oxides. VCH, Weinheim, New York.

FASSBINDER, W.E., STANJEK, H. & VALI, H. (1990). Nature, **343**: 161–163.

HYDE, B B., HODGE, A.J., KAHN, A. & BIRNSTIEL, M.L. (1963): J. Ultrastruct. Res., **9**: 248–258.

HOUNSLOW, M.W. & MAHER, B.A. (1996). Geophys. J. Int., **124**: 57–74.

LOWENSTAM, H.A. (1981). Science, **244**: 126–130.

McCLEAN, R.G. (1996). Plant magnetism, and its possible contribution to soil magnetism. M.S. thesis, University of Wisconsin-Milwaukee.

McCLEAN, R.G. & KEAN, W.F. (1993). Earth Planet. Sci. Lett., **119**: 387–394.

MAHER, B.A. & TAYLOR, R.M. (1988). Nature, **336**: 368–370.

PETERSEN, N., VON DOBENECK, T. & VALI, H. (1986). Nature, **320**: 611–615.

SECKBACK, J. (1982). J. Plant Nutr., **5:** 369.