Procedures for Obtaining Optimal SEM Images of Coccolithophore Debris in Coccolith Limestones

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Procedures for obtaining optimal SEM images of coccolithophore debris in coccolith limestones

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Coccolith debris in fossil zooplankton faecal pellets and the mode of its preservation are the unique source of data on the mode of feeding and digestion by ancient zooplankters. The animals are virtually absent in the fossil record in another form than their coprolites. However, minute structural details of coccospheres and their debris in the coccolith limestone are much less legible than in modern sediments. This paper presents how clear SEM images of details of coccolith plates in complete and dismembered coccospheres from fossil zooplankton faecal pellets can be obtained from thin sections of coccolith limestone. The images allow us to study the structural and compositional details of coccolith plates as well as their arrangements within the coccospheres and fossil faecal pellets.

Introduction

The details of minute components of coccolith debris in fossil zooplankton faecal pellets are difficult to study using the standard microscopic methods. The images of structural details and spatial arrangement of coccolith debris are a unique source of information in studies the feeding and digestion mode of ancient zooplankters (e.g., Haczewski, 1989; Chambers et al. 2000; Pearson et al. 2004; Lees et al. 2004; Bour et al. 2007). Mechanical disintegration of rock, in order to obtain the coccoliths debris, generally leads to the destruction of the coccospheres (if preserved in the studied sediment) and other primary structures.

This paper presents a technique which enables to observe the structural details of coccolith debris—coccospheres and individual coccoliths plates—in microlaminated limestone using well polished thin sections. The technique being applied to visualisation of coccolithophore debris is known in the literature as charge contrast imaging (CCI). This imaging technique have been applied to obtain novel structural data from bio- and geo-minerals (e.g., Griffin 2000; Toth et al. 2003; Clode 2006; Taylor et al. 2007), but it seems to be undervalued in the study of coccolith debris in limestone. CCI provides structural information as unique contrast variations in secondary electron image (SE), obtained by adjusting operating parameters of the Environmental Scanning Electron Microscope (ESEM). The CCI technique is highly complex and the phenomenon of the generation of these unique contrast variations is still a matter of study and discussion (e.g., Griffin 2000; Toth et al. 2003; Clode 2006) and will not be discussed here.

Institutional abbreviation.—ZNG PAN, Institute of Geological Sciences, Polish Academy of Sciences, Kraków Research Centre, Poland.

Other abbreviations.—BSE, backscattered secondary electrons; CCI, charge contrast imaging; ESEM, Environmental Scanning Electron Microscope; HV, high voltage; LFD, large field detector; SE, secondary electrons; SS, scan speed; WD, working distance (between the sample surface and the detector).

Methods

The images of coccosphere debris presented in this paper were acquired using standard well polished thin sections of microlaminated coccolith limestone. Thin sections glued with araldite (epoxy resin) on a glass microscope slide were polished using diamond suspensions; first with grain size of 3 μm, and then of 1 μm, until approximately 35 μm thick and were not covered with glass. Any detraction from the optimum thickness and from proper polishing compromised the quality of the images. The samples were mapped before the SEM study by determining coordinates of matched points on thin sections and on their photographs made under optical microscope. This technique enables a precise location of obtained images within the repetitive structures such as microlaminae and faecal pellets; see the supplementary online material (http://app.pan.pl/SOM/app55-Ciurej_SOM.pdf).

The thin sections were studied under ESEM (FEI Quanta 200 FEG) housed at the Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Kraków, Poland. The same areas of the samples were studied in SE (secondary electrons) and BSE (backscattered secondary electrons) mode, first without any coating and later after coating with approximately 20 nm of carbon. These procedures allowed to compare the quality of images and to cross-check the results.

Acceleration voltage was set at 15 keV. The environment used was the one of water vapour at a pressure of 100 Pa. WD was approximately 10.0 mm (9.9 mm to 10.2 mm) and SS for SE, 37 s/frame, for BSE, 32 s/frame. Working parameters are given at every image.

Observations

The images, obtained from non-coated and well polished thin sections using the CCI technique in SE mode in ESEM in low

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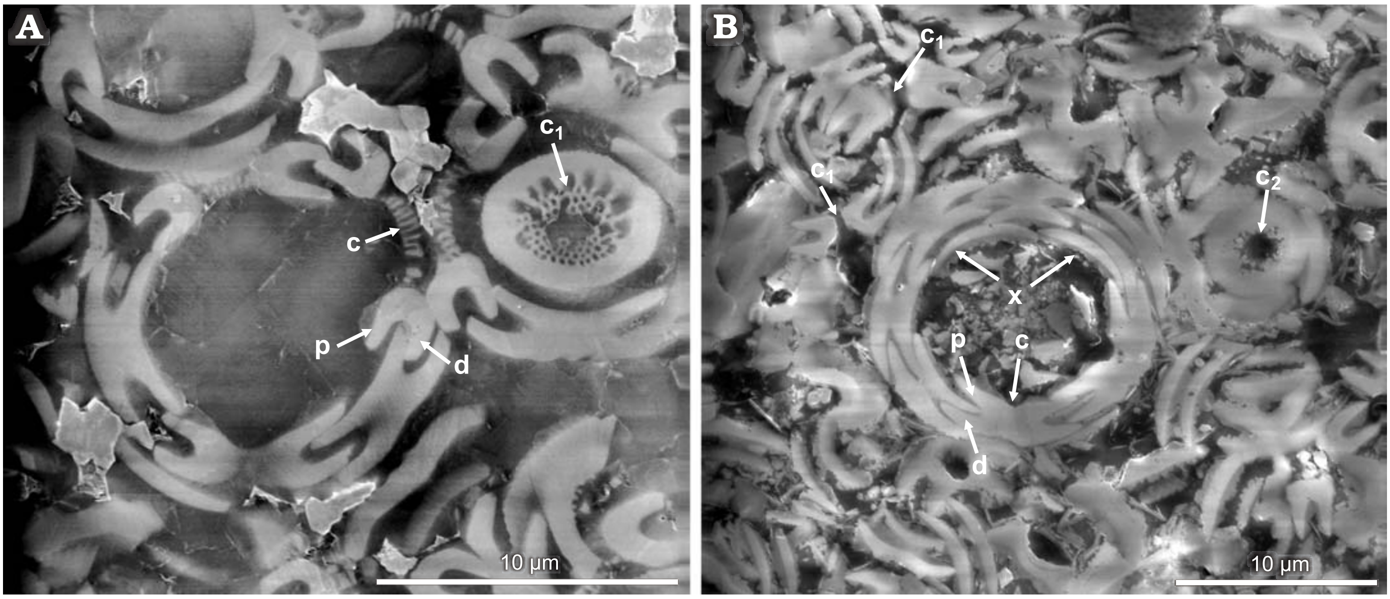

Fig. 1. CCI images of coccolith material in different stages of fragmentation within faecal pellets in coccolith limestone. Cross-sections of complete and dismembered, variously oriented, coccospheres allow to observe their structures. **A**. Perpendicular cross-section of a coccosphere with well visible distal (d) and proximal (p) shields of placoliths joined by parallel calcite crystallites in wide central area (c). Interlocking of adjacent edges of placolith shields is particularly well visible. The structure of the central area is well visible as a net in horizontal cross-section of the plate in the right (c_1), ZNG PAN B-III-75/3. **B**. Perpendicular cross-sections of coccospheres clearly show distal (d) and proximal (p) shields of placoliths, and central areas (c). The central areas are seen as narrow massive or open structures, depending on the orientation of cross-sections (compare c and c_1). Note the very tight interlocking of adjacent edges of placolith shields and additional placoliths (x) within the coccosphere. The structures closing the central area are seen as a narrow open structure in horizontal view of placoliths (c_2), ZNG PAN B-III-75/5. Both images were obtained in ESEM using SE under low vacuum, from non-coated polished thin section at WD, 10.1 mm, SS, 37 s/frame.

vacuum, reveal structural details of complete and dismembered coccospheres in cross-sections as shown in Fig. 1. The variable orientations of the cross-sections allow one to see such structures as distal and proximal shields and rims, central areas and the mode of their development, interlocking of adjacent edges of coccolith plates within coccospheres. Such structural details of coccospheres and separate plates have been hitherto well studied only on modern samples (Young et al. 1997; Winter and Siesser 2006; Taylor et al. 2007). The last mentioned authors have presented cross-sections of frozen coccospheres. More CCI images of coccolith material are shown in supplementary online material (http://app.pan.pl/SOM/app55-Ciurej_SOM.pdf).

Coccolith debris may become hardly recognisable in BSE images obtained from non-coated thin sections in low vacuum or illegible and unrecognisable after coating with carbon, both in SE and BSE mode, in low and high vacuum. For a detailed description of such cases, see the supplementary online material (http://app.pan.pl/SOM/app55-Ciurej_SOM.pdf).

Conclusions

The images obtained from well polished, non-coated thin sections, using charge contrast imaging (CCI) enable to visualise fossilised coccolith debris and the mode of its preservation in fossil zooplankton faecal pellets better than in any earlier published images of ancient coccolith limestone (see Chambers et al. 2000; Pearson et al. 2004; Lees et al. 2004; Bour et al. 2007). This technique is highly suited to the study of morphological features of complete and dismembered coccospheres in variously oriented cross-sections. The structural details of coccolith plates, such as the central area, distal and proximal shields, and their way of interlocking within coccospheres etc., become well discernible when this method is used.

The technique described here may be useful for samples for which mechanical disintegration fails to provide any data, because of the advanced degree of cementation. An important advantage of this technique is that we can study the skeletal elements within the undisturbed primary structure of the host microlaminated limestone. Diagenetic features, such as cement within and around coccospheres, as well as various stages of diagenetic alteration of coccolith plates can be assessed.

The study of the same polished thin sections under optical microscope and ESEM enables precise location of the studied details in the context of the sequence of microlaminae and their internal elements. These comparative images are important, because precise location of the observation fields at high magnifications is a difficult task in the microlaminated coccolith limestones, in which the mezoscopically apparent dark and light laminae are the result of subtle variations in concentration and size of pyrite framboids. The variable modes of coccosphere preservation in various pellets and in various parts of laminae provides a base for analysing the skeletal material within the framework of seasonal changes in the environment.

This imaging technique may be suitable for studies on the structural and compositional details of various carbonate microskeletons and their arrangement within their host rocks.

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