Microstructure Physics and Metal Forming
Prof. Dr. D. Raabe

Mesostructure, Microstructure and Anisotropy of the Lobster Cuticle

P. Romano, A. Al-Sawalmih, C. Sachs, D. Raabe, H.-G. Brokmeier[#]

[#]GKSS-Forschungszentrum, Max-Planck-Str., 21502 Geesthacht, Germany

Max-Planck-Institut für Eisenforschung GmbH
Düsseldorf/Germany

Introduction

Crustaceans are a remarkable group of animals because of their ability to elaborate cyclically two kinds of calcified biomineralizations: an unstretchable exoskeleton (or cuticle) and also, for many species, depending of the way of life of the considered animal, transitory calcium deposits [1]. The lobster cuticle or exoskeleton contains some amorphous calcium carbonate associated with the organic matrix of chitin-microfibrils and proteins assembled into a lamellar structure. These studies have been carried out in a small piece of the inner part of the cuticle [2].

In general most of the mollusc cuticles are composed of two well ordered interpenetrating networks: the chitinoproteinic matrix first deposited, and the calcite mineral which progressively confers its hardness to the exoskeleton [3]. The organic component of the exoskeleton consists of a-chitin microfibrils which are embedded in a protein matrix and are arranged in a helicoidal pattern [4]. Chitin is a polysaccharide chain composed of repeating units of N-acetyl-D-glucosamine, approximately 18 to 25 of these molecular chains assemble in narrow and long crstalline units [5].

Sample Preparation

POLISHED SAMPLES. Polished with SiO_2. Coated with gold.

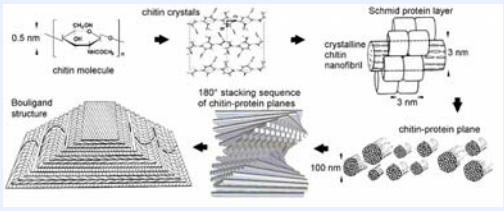
TRANSMISSION ELECTRON MICROSCOPY SAMPLES. Fixed with glutaraldehyde, decalcified with EDTA and stained and fixed with OsO_4. Final staining with uranyl acetate and lead citrate for contrast. 60 nm ultramicrotoming.

X-RAY DIFFRACTION AND SYNCHROTRON SAMPLES. Sections 2x5 mm of the claw were studied with beam size 1x1 mm, and wavelength 0.196 Å.

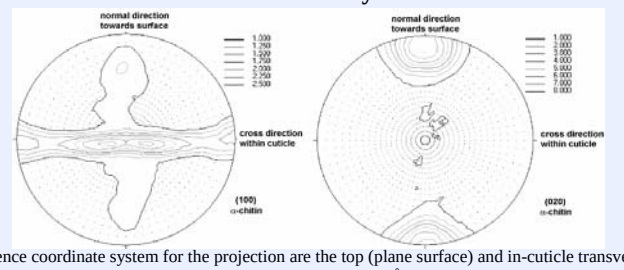
Equipment

SCANNING ELECTRON MICROSCOPY. Jeol 6500 F High Current Field Emission Gun SEM.
TRANSMISSION ELECTRON MICROSCOPY. Hitachi H600 fitted with a Gatan camera.
X-RAY DIFFRACTION. Laboratory-scale x-ray diffraction set-up using a CoKα1 beam.
SYNCHROTRON at HASYLAB/DESY. Hamburg Synchrotron Laboratory.

Hierarchical elements of the arthropod's exoskeleton



Texture analyses



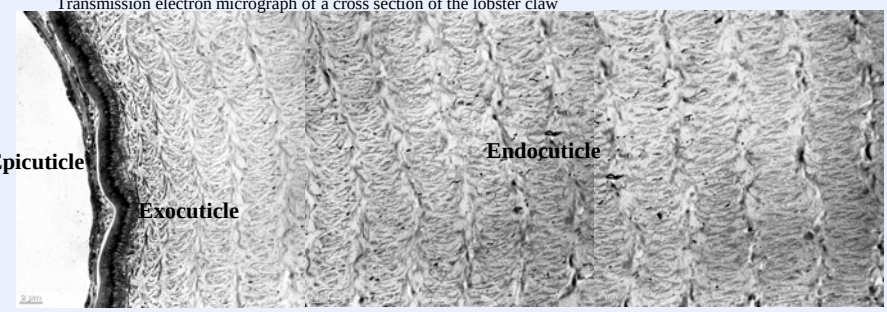
The reference coordinate system for the projection are the top (plane surface) and in-cuticle transverse directions of the claw; l=0.196 Å.

The crystallographic orientation distribution of the chitin network is characterized by two main features:

1. It reveals a pronounced fiber texture with a very strong crystallographic <020> fiber axis normal to the surface of the lobster cuticle (see {020} pole figure). This means that the long b-axis of the elementary cell of the α-chitin crystals points towards the surface of the exoskeleton (in the crusher claw region from where the sample was taken).
2. The <020> fiber texture is not completely symmetric. The {100} pole figure shows two strong maxima of the (100) peaks misoriented by about ±15° and two weak maxima misoriented by about ±90° on the equatorian plane. The crystallographic orientation is, in the case of the α-chitin crystals, strictly equivalent to their topological orientation. This means that the chitin-protein network reveals not only a main fiber orientation parallel to the surface but also some degree of crystallographic order within the cross section of the overall network.

Microstructure

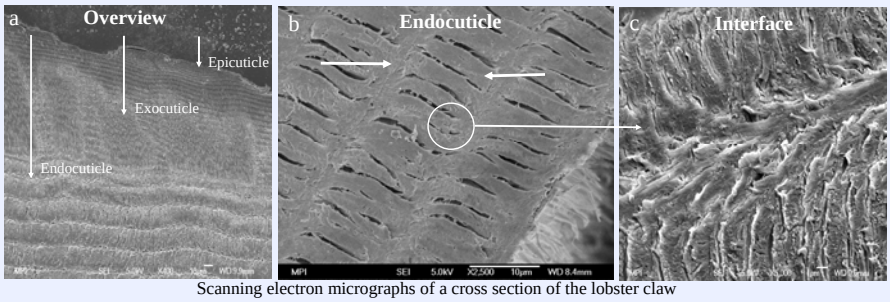
Transmission electron micrograph of a cross section of the lobster claw



The TEM micrograph shows a decalcified, deproteined section of the lobster claw. The network observed belongs to the α-chitin. The chitin fibers will act as a scafold to secure the crystalline calcite and the amorphous calcium carbonate that the structure contains.

When looking from the exocuticle towards the inner part of the endocuticle, it can be observed that the *chitin planes or stacks* become thicker. The density of the *chitin stacks* is different in the two regions and not homogeneous inside the layers. The exocuticle has a much finer Bouligand mesostructure than the endocuticle. In general, pronounced variations of the stacking density appear in the exocuticle, which can be due to local differences in the kinetics during the synthesis.

The SEM micrographs (a, b and c) show in detail the structure of the exoskeleton with all its constituents, α-chitin, protein and minerals. The main three layers are called epi, exo and endocuticle.
The *thick* layers, visible especially in the b micrograph (indicated by white arrows), are bounded by interfaces (detail in figure c), which are perpendicular to the arcs formed by the chitin-protein planes. These thick layers can be referred to as *Bouligand* or *plywood layers* [5, 6]. According to Bouligand [6] they consist of helicoidal stacks of chitin-protein planes that are gradually rotated about their normal axis. The thickness of the layers corresponds to the height of the chitin-protein planes that is required for an accumulated total rotation of 180° around their normal direction.



Scanning electron micrographs of a cross section of the lobster claw

Conclusions

Scanning and transmission electron microscopy as well as x-ray diffraction were used to study the structure and the crystallographic texture of the lobster, *homarus americanus*, exoskeleton. The analysis of the results reveals a pronounced hierarchical structure of the material and a strong crystallographic texture of the α-chitin-protein network. The matrix of biopolymer and proteins is charaterized by a very strong texture fiber. The longitudinal direction of the fibers is perpendicular to the long b axis of the crystal cell of α-chitin and to the surface of the cuticle.

Literature Review

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Acknowledgments

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