ON THE NATURE OF THE CALCIUM CARBONATE IN THE EXOSKELETON OF THE WOODLOUSE ONISCUS ASELLUS L. (ISOPODA, ONISCOIDEA)

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INTRODUCTION

Calcite, aragonite and vaterite, the three crystalline forms of calcium carbonate (Lippmann, 1973), have been identified in many biological systems primarily by x-ray diffraction, and, using infrared spectroscopy, it has been shown (Pobeguin, 1954) that an additional amorphous form is of widespread occurrence, being present in the majority of decapod Crustacea, some Diplopoda and the larvae of some Diptera.

Although the fine structure of the hindgut cuticle (Holdich & Mayes, 1975), exoskeletal epicuticle surface (Holdich & Lincoln, 1974; Schmalfuss, 1978) and the integument (Price & Holdich, 1980a and b) of the terrestrial Isopoda Oniscoidea has been frequently studied, the chemical composition of the exoskeleton has not been fully defined. The main components of the exoskeleton are chitin, protein and calcium carbonate (Sutton, 1972) and while the cuticle of the Oniscoidea, Porcellio laevis Latreille, 1804, P. lamellatus Verhoeff, 1931, and Armadillidium vulgare (Latreille, 1804) contains 80-85% by weight of calcium carbonate (Lagarrigue, 1968), the form of the latter has not been identified in Oniscus asellus L., 1758.

We report here that the calcium carbonate in the exoskeleton of Oniscus asellus is amorphous. It is maintained in this form by association with protein and not with phosphate or silicate (Pobeguin, 1954) and only crystallizes to calcite when mechanisms such as moulting disrupt this association. A comparison is made with the millipede Tachypodium nigerr (Leach, 1815).

MATERIALS AND METHODS

Oniscus asellus were collected from the grounds of The Macaulay Institute for Soil Research and cultured in biovessels containing sand and decomposing litter. Fresh exoskeletons consisting of the pereion region and shed exoskeletons were washed with distilled water.
Samples of the exoskeletons were ground in propan-2-ol, freeze-dried and specimens (0.5 mg) incorporated in 13 mm diameter KBr discs. Infra-red spectra were recorded on a Perkin Elmer 580B spectrometer.

Specimens of dried exoskeletons were attached to aluminium stubs with colloidal carbon and coated with either carbon or gold. The specimens were examined in a Cambridge S4 stereoscan instrument equipped with an energy-dispersive x-ray analyser. X-ray spectra were recorded from the carbon coated specimens, and the Ca$^{2+}$ distribution from the gold coated specimens.

RESULTS

In a naturally shed exoskeleton of *O. asellus*, the calcium carbonate occurs predominantly as calcite, its sharp infrared absorption bands at 2516, 1798, 1418, 872, 713 cm$^{-1}$, and the broad band at 315 cm$^{-1}$ (fig. 1a) matching closely those of an authentic sample of calcite (fig. 1c). The sharp calcite bands are much weaker in the spectrum of an exoskeleton from a freshly killed specimen (fig. 1b), and are replaced by much broader weaker carbonate bands. The striking difference between shed and fresh exoskeletons is even more clearly illustrated in the spectra of *T. niger* exoskeletons (fig. 1c and d), no calcite being detectable from the spectrum of the fresh skeleton. The broad carbonate bands agree well with those for a synthetic amorphous calcium carbonate (fig. 1f), also reported by Pobeguin (1954), indicating that the calcium carbonate in fresh exoskeletons of *O. asellus* is predominantly amorphous. This was confirmed by X-ray diffraction, powder patterns of the fresh exoskeletons showing only weak or non-detectable calcite reflections, in contrast to those of the shed skeletons which were dominated by sharp calcite lines. The possibility that other cations might be incorporated in the exoskeleton carbonate phase was excluded because X-ray microprobe analysis revealed only Ca$^{2+}$ (fig. 2a) which was uniformly distributed throughout the exoskeleton (fig. 3).

The transformation from amorphous calcium carbonate in the fresh, to well crystallized calcite in the shed exoskeleton is accompanied by significant changes in the other major skeletal components protein and chitin. Infrared spectra show that strong bands of protein at 1660 and 1540 cm$^{-1}$ in the spectra of fresh skeletons (fig. 1b and d) are very much weaker in those of the shed skeletons (fig. 1a and c) consistent with the conservation of nitrogen by the organism during the onset of ecdysis. This conservation mechanism can also be seen to operate for chitin, whose sharp pattern of absorption bands in the 900-1200 cm$^{-1}$ range in the spectra of fresh skeleton (fig. 1b and d) is replaced by the broader, more ill-defined pattern of N-acetyl-deficient chitosan on ecdysis (fig. 1a and c). These conclusions from changes in infrared spectra are fully supported by chemical analyses which show a marked decrease in protein N and glucosamine N contents from 3.1 and 0.9% respectively in the fresh, to 0.7 and 0.1% in the shed woodlouse exoskeletons. Similar changes in N contents were also obtained for the millipede exoskeletons.