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The composition of the exoskeleton of two crustacea: The American lobster *Homarus americanus* and the edible crab *Cancer pagurus*

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Abstract

The exoskeletons of the American lobster *Homarus americanus* and of the edible crab *Cancer pagurus* were analysed with structural and chemical methods. The exoskeletons consist of crystalline magnesian calcite in the form of nanocrystals (domain size about 20 nm), amorphous calcium phosphate (ACP), and α-chitin. The composition varies among different parts of the skeleton and also between the two species. Differences are related to the mechanical requirements and biological escape behaviour of the animals. The finger and claw are strongly mineralized and very hard. The shell of the body (the carapace) is less mineralized and more elastic. The lobster, as a mobile, fast-swimming animal, typically escapes from a predator whereas the crab clings to the ground and burrows into the sand. Consequently, the shell of the lobster is less mineralized (and therefore lighter and less hard) than the shell of the crab.

Keywords: Biomineralization; Calcium carbonate; Crustacea; American lobster; *Homarus americanus*; Edible crab; *Cancer pagurus*

1. Introduction

Crustaceans constitute a widespread class of organisms of both marine and land-dwelling species. They possess an exoskeleton [1] that stabilizes the whole body of the animal and also serves for protection against predators. In the case of lobsters and crabs, the claws are part of the exoskeleton optimized to serve as a cutting tool. The exoskeleton of crustaceans [2] is a biomineralized [3–6] structure which consists of an organic matrix (in this case α-chitin) together with an inorganic mineral, in this case mostly calcium carbonate [7]. This composite has a distinct microstructure which gives it an optimal performance with respect to mechanical strength (for protection) and flexibility (for movement). Raabe et al. have analysed this microstructure and found a strongly hierarchical twisted plywood structure [8–10] and pronounced crystallographic textures (preferred orientation distributions of calcite and chitin [11]. This paper reports the chemical composition of these exoskeletons, following our study of the exoskeleton (cuticle) of land-dwelling woodlice [12,13].

2. Materials and methods

The American lobster *Homarus americanus* and the edible crab *Cancer pagurus* are in the class Malacostraca (higher crabs). The two animals are shown in Fig. 1. The claw, the finger and the carapace (Fig. 2) of the animals were investigated.

The composition of the shell parts was determined by thermogravimetry. As shown earlier with bone [14] and cuticles of woodlice [13] it is possible to separate the components water, organic matrix and calcium carbonate because the corresponding weight losses occur at different temperatures. A typical thermogravimetric curve is shown in Fig. 3. The calcium and magnesium content were determined by AAS measurements carried out sixfold each in a Solaar MSM2 by Thermo E (Dreieich, Germany) with graphite furnace technique (previous dissolution in concentrated HCl; no solid residue). Phosphate analyses were performed with a UV–visible-spectrophotometer Cary 1 Bio (Varian, Palo Alto, USA) as phosphate-molybdenum-blue complex. TGA-DTA measurements were performed under dynamic oxygen atmosphere (50 ml min^{-1}) in a TGA V STA 409 PC (Netzsch, Selb, Germany). The samples were filled into Al_2O_3-crucibles (typical sample weight: 60–110 mg). A heating rate of +2 K min^{-1} was applied. Powder diffraction was carried

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Fig. 1. The two crustacean species whose exoskeleton (shell) was studied: *Homarus americanus* (left) and *Cancer pagurus* (right).

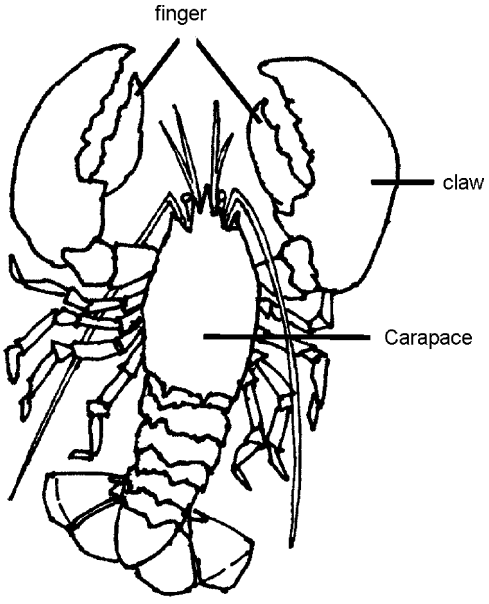

Fig. 2. The different parts of the exoskeleton that were analysed for the lobster: claw, finger and carapace. The corresponding equivalent parts were analysed for the crab.

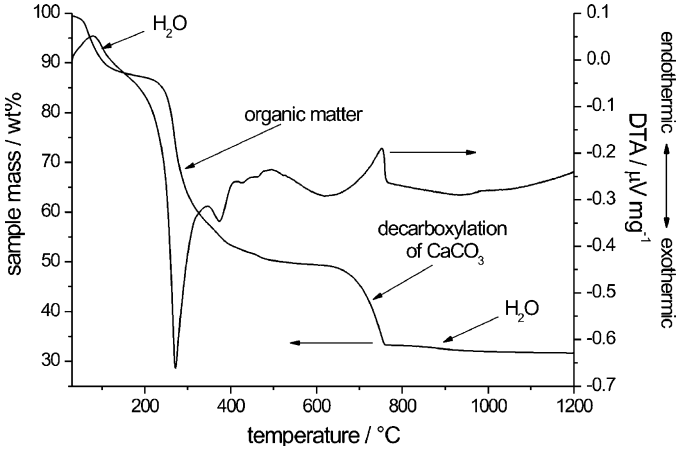

Fig. 3. Thermogravimetry and DTA under dynamic oxygen atmosphere of the carapace of the lobster *Homarus americanus*, showing the release of water (endothermic), the combustion of the organic matrix (exothermic), the decarboxylation of calcium carbonate ($CaCO_3$; endothermic) and the release of hydroxidic water from hydroxyapatite ($Ca_5(PO_4)_3OH$; endothermic).

out on the milled samples in Bragg–Brentano geometry with a Siemens Diffractometer D500 (now Bruker AXS GmbH, Karlsruhe) equipped with a Cu anode (1.54060 Å) and proportional counter.

The cuticles were obtained from deep-frozen adult animals which were obtained from a local store and taxonomically identified by a qualified zoologist. The cuticles were dissected with a sharp knife. All samples were ground in a ball mill (500 rpm) for at least 3 h at room temperature (due to their high mechanical strength, this treatment was necessary). To ensure that the material was not changed during this treatment, thermogravimetry of pieces of all samples was performed both before and after ball milling. This gave almost identical results; therefore we tentatively excluded preparation artefacts due to ball milling. The fact that the X-ray powder diffractogram showed nanocrystalline calcium carbonate, in good accord with electron microscopic data on untreated parts of the exoskeleton [10], also supports our assumption that no recrystallization of the nanocrystalline calcium carbonate to larger crystals occurred during ball milling. Elemental analysis (AAS) also gave the same results for ball-milled samples and samples which were ground with liquid nitrogen in a mortar. We therefore are confident that the overall composition of the exoskeleton was not changed by ball milling.

3. Results and discussion

All thermogravimetric results are summarized in Table 1.

X-ray powder diffraction revealed the crystalline components of the exocuticle (Fig. 4). This was mainly calcite with broad reflections, indicating a nanocrystalline structure. Application of the Scherrer equation gave an estimate of the size of the crystalline domains [14–16]. The reflections (1 1 0) at 36.2° 2Θ and (2 0 2) at 43.4° 2Θ, were analysed with a form factor of $K = 1$. The typical full-width at half maximum was 0.4–0.5° 2Θ. This gave an average crystallite size for calcite in the lobster exoskeleton of 19 nm and in the crab exoskeleton of 25 nm. The crystallite size was independent of the reflection and the part of the shell. This agrees with electron microscopic results reported by Raabe et al. for the skeleton of the American lobster (20–50 nm) [10].

By annealing of the cuticles to 1100 °C, the organic matrix and water are removed, calcium carbonate is decarboxylated

Table 1
Thermogravimetric results of all samples

Material (wt%)	H_2O	Organic matrix	Total mineral content	CO_2 from $CaCO_3$	H_2O from apatite	Remaining mass at 1200 °C
Sample						
Crab finger	1.0	10.4	88.6	36.0	1.0	51.5
Crab carapace	11.8	16.6	71.6	27.3	0.7	43.6
Crab claw	9.9	13.4	76.7	29.7	0.5	46.4
Lobster finger	7.3	19.7	73.0	23.1	1.5	48.4
Lobster carapace	11.8	38.2	50.0	16.1	1.6	32.3
Lobster claw	19.0	20.3	60.7	19.1	1.2	40.4

The temperature ranges were comparable for all six samples.

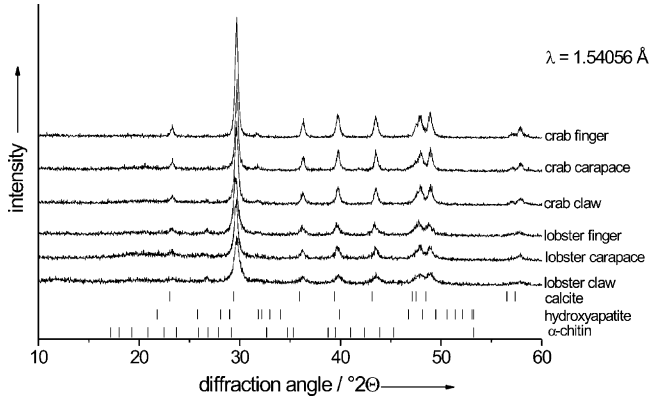

Fig. 4. X-ray powder diffraction on the exocuticles, showing only crystalline calcite and a broad peak of α-chitin at 26.8° 2*Θ*.

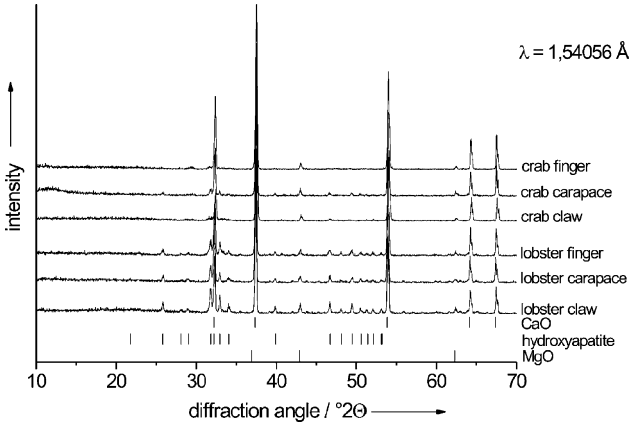

Fig. 5. X-ray powder diffractograms of the calcined cuticles (1100 °C), showing calcium oxide, magnesium oxide and hydroxyapatite, all well crystallized.

and any previously amorphous inorganic material should have recrystallized [14]. The corresponding powder diffractograms in Fig. 5 show calcium oxide, magnesium oxide and hydroxyapatite. The mineral of the cuticle contained magnesium as foreign ion either in calcite or in calcium phosphate. We prefer the first interpretation because magnesian calcite decomposes to calcium oxide and magnesium oxide as two separate phases [7,13]. However, it is possible that magnesium was present as an X-ray amorphous phase, i.e. as amorphous magnesium carbonate or as impurity in amorphous calcium phosphate.

Energy-dispersive X-ray spectroscopy found calcium, magnesium, phosphorus, carbon and oxygen as main components of the cuticles and sodium, chloride and silicon in minor amounts. The latter are probably due to residual NaCl from seawater and possibly to silicon dioxide from diatoms attaching to the shell.

Elemental analysis of the cuticles gave the contents of calcium, magnesium and phosphate. Together with the data from thermogravimetry, this permits a quantitative analysis of the mineral in the cuticles [13]. Table 2 summarizes all results.

The mineral content in the exoskeletons varies with the species and with the location in the exoskeleton. The ratios of calcium to magnesium and of carbonate to phosphate are also variable. Raabe et al. studied the cuticle of *Homarus americanus* by thermogravimetry and found a loss of water of 19.8 wt%, a content of organic matrix of 29.2 wt% and a loss of CO_2 of 36.5 wt%. By energy-dispersive X-ray spectroscopy, carbon,

Table 2
Summary of all results from elemental analysis (AAS and UV) and thermogravimetry (TGA)

Result #	Value determined	Method	Crab finger	Crab carapace	Crab claw	Lobster finger	Lobster carapace	Lobster claw
1	Ca (wt%)	AAS	27.8	22.0	23.5	23.0	17.3	18.5
2	Mg (wt%)	AAS	1.0	1.2	1.4	1.0	1.0	1.2
3	PO_4^{3-} (wt%)	UV	1.2	3.4	2.8	4.4	6.4	2.0
4	Water (wt%)	TGA	1.0	11.8	9.9	7.3	11.8	19.0
5	Organic matrix (wt%)	TGA	10.4	16.6	13.4	19.7	38.2	20.3
6	Mineral content (wt%)	TGA	88.6	71.6	76.7	73.0	50.0	60.7
7	Loss of CO_2	TGA	36.0	27.3	29.7	23.1	16.1	19.1
8	Ca:Mg = *n*:*n*	(From 1 and 2)	17.0:1	10.7:1	10.2:1	13.8:1	10.7:1	9.7:1
9	Ca:Mg = *m*:*m*	(From 1 and 2)	28.1:1	17.6:1	16.9:1	22.7:1	17.6:1	15.9:1
10	CO_3^{2-}:PO_4^{3-} = *n*:*n*	(From 3 and 7)	47.5:1	12.8:1	17.1:1	8.4:1	4.0:1	14.9:1
11	CO_3^{2-}:PO_4^{3-} = *m*:*m*	(From 3 and 7)	30.0:1	8.1:1	10.8:1	5.3:1	2.5:1	9.4:1

The uncertainty in the thermogravimetric results is estimated to be ±0.3 wt%. The standard deviation was ±0.2 wt% for Mg, ±2 wt% for Ca and 0.3 wt% for PO_4^{3-} ($N = 6$ for all samples and all methods). *n*:*n* denotes molar ratios and *m*:*m* denotes mass ratios.

oxygen, sodium, magnesium, phosphorus and calcium were found [10], in good accord with our results.

4. Conclusions

In both species, the mineral content increased from the carapace to the claw to the finger. This is explained by the different requirements for hardness. The finger is the movable part of the cutting device of the animal and must therefore be very hard. The claw is the fixed counterpart which must be more elastic, i.e. less mineralized. The carapace is the shell of the main body, i.e. it should be even more elastic to allow the movement and some bending of the animal. For comparable parts, the exoskeleton of the crab had a higher mineral content than that of the lobster. This is explained by the different escape behaviour. The lobster escapes rapidly and hides between rocks [17] and therefore should have a lighter, more elastic cuticle. In contrast, the edible crab clings to the ground or hides in the sand upon attack [18], and therefore needs a hard, highly mineralized shell. The fact that the lobster can swim whereas the crab can only walk supports that interpretation.

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