Structural and chemical investigations on shells and pearls of nacre forming salt- and fresh-water bivalve molluscs

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Abstract

To investigate the shell structure of some pearl-forming bivalve molluscs the following techniques were used: light microscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD), texture goniometry, scanning force microscopy (SFM), energy dispersive X-ray fluorescence (EDXRF) and fourier transform infrared spectroscopy (FT-IR).

X-ray diffractograms were taken to determine the identity of the minerals forming the shell. They were identified as aragonite in the mother-of-pearl layer and as calcite in the prismatic layer.

The X-ray diffractograms also showed that the orientation of the crystallites forming the nacreous layer of freshwater mussels is different from those of saltwater oysters. In pictures taken by SEM the aragonite crystals of all the investigated mussels except *Hyriopsis schlegeli* show a pseudo-hexagonal shape. Investigations by texture goniometry indicate the crystals to be single crystals, so they are not twins like their inorganically grown counterparts.

A theory of nacre growth is proposed and its application would help to decrease the amount of waste pearls. It was found to be possible to influence the colour of the pearls without using artificial treatment.

Attempts were made to explain the crystal growth by means of high resolution scanning force microscopy (SFM) which enables examination of the surface of the aragonite microcrystals. The observed structures on top of these crystals could be the so-called organic matrix that strongly influences nacre growth.

The chemical investigations showed that there is little difference in the amount of minor and trace elements in the nacre and pearls from different localities. However, it is possible to distinguish between the origins of freshwater mussels due to their contents of manganese and potassium. The strontium contents of some saltwater oysters are presented but are not a diagnostic feature.

Introduction

Investigating the shell and shell growth of nacre forming molluscs (e.g. bivalvia (mussels)) is not only of gemmological interest because of their capability to form pearls. The shell growth is a biomineralization process which is related to similar processes in the human body (skeleton) (Handschin and Stern, 1992). Compared to human bones, the shell of a mussel is a simple model where mineralization processes can easily be studied (see also...
Caseiro, 1993). The aim of this study was to perform a structural and chemical comparison of shells and pearls from different origins. In order that one may understand the observed structures it is important to know how the mussel grows and to recognize the main parts of the shell.

Because the growth process is essentially the same in shells and pearls, the results of the shell investigations can be used to supplement the knowledge of the process of pearl formation, but this work does not attempt to explain the initiation of pearl growth.

Figure 1d shows a schematic cross-section through the type of shell used in this study. The bottom of the sketch represents the outer surface of the shell. This is a hydrophobic organic skin called the periostracum. It covers the hard parts of the shell and serves as a defence against erosion and enemies. On the inside of the periostracum is the prismatic layer, the first inorganic part of the shell. It is formed by small prisms of calcite crystals (CaCO$_3$, trigonal). The prismatic layer is followed by the mother-of-pearl layer, or nacreous layer, which is formed from tabular aragonite crystals (CaCO$_3$, orthorhombic).

All these components of the shell are produced by a soft part called the mantle (Figure 1), the part of the animal next to the shell. On the opposite side of the hinge (that is where the two shells are connected) the mantle forms a fold (x in Figure 1a). The cells in this fold produce molecules which grow to form the periostracum (Lowenstam and Weiner, 1989) which is the first part of the shell that is formed. It builds the substrate on which the prismatic and mother-of-pearl layers grow. There is also evidence for another organic phase besides the periostracum playing an important role in the formation of the shell. It is called an organic matrix (Weiner and Traub, 1984) and may influence strongly the crystallization of the calcite and aragonite crystals. More details follow in the section 'Morphological structures'.

Figure 1 shows the growth stages of the type of shell investigated. The shell always grows from the centre towards the edge. At the hinge the shell does not spread but thickens with increasing age. The growth of the shell is a consequence of the growth of the inner soft parts, in other words of the animal itself whose growth centre is the mantle. There are two types of mantle cell that secrete the Ca$^2+$ and CO$_3^{2-}$ ions (1 and 2 in Figure 1). The formation of the crystals as calcite or aragonite is determined by the

**Background**

This section includes not only the results of earlier work but also some of our own

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**Fig. 1.** Schematic cross-section through the shell, showing the mineral components of the shell and the soft parts of the animal (mantle tissue, periostracum) that produces them. From a to d the cross-sections represent increasing ages.
age of the cells. Young cells (1) near the 
border of the shell produce calcite and 
older cells (2) towards the inner part of the 
shell form aragonite.

This means that young cells situated on 
the inner side of the shell always produce 
calcite (type 1 cells). These young cells are 
followed by older cells (type 2 cells), which 
were former type 1 cells. Because the cells 
do not change their places relative to the 
shell, but change the mineral phase they 
produce, there will be a certain time when 
they deposit aragonite over calcite 
(Gutmannsbauer, 1992). This growth 
behaviour is summarized in Figure 1.

The knowledge of this theory could be of 
great importance for the cultured pearl 
industry. The production of large amounts 
of reject cultured pearls, e.g. – pearls that 
consist of, or are partly overgrown by, 
calcite – could be prevented. Since cul-
tured pearls will have the same colour as 
the mother-of-pearl grown by the original 
mantle cells, pearl culturers can influence 
the colour of the end product by carefully 
choosing the mantle cells from the right 
location in the bivalve sacrificed for its 
mantle tissue.

Material and methods
In this study the shells and pearls of the 
main pearl-forming oysters and mussels 
were of interest. Cultured as well as 
natural pearls were investigated and a 
main point for our sample acquisition was 
that the shells and pearls were not treated 
in any way, so that there could be no falsi-
fication of the chemical and structural data. 
The examined shells and pearls are listed 
with their origins in Table I.

The following techniques were used to 
investigate the structures of the shells: light 
microscopy (LM), scanning electron 
microscopy (SEM), X-ray diffraction 
(XRD), texture goniometry and scanning 
force microscopy (SFM).

Chemical analyses were carried out by 
energy dispersive X-ray fluorescence (ED-
XRF). Fourier transform infrared 
spectroscopy (FT-IR) was used for struc-

| Table I. Shells and pearls examined in the present study. |
|------------------------------|------------|-------------|-----|
| Saltwater oysters            | Quantity   | Origin      | Pearls |
| Pinctada margaritifera       | 10         | Tahiti      | yes  |
| Pinctada martensii           | 8          | Japan       | yes  |
| Pinctada maxima goldlipped   | 3          | Philippines | no   |
| Pinctada maxima goldlipped   | 4          | Australia   | no   |
| Pinctada maxima goldlipped   | 1          | Thailand    | no   |
| Pinctada maxima silverlipped | 1          | Burma       | no   |
| Pinctada maxima silverlipped | 12         | Australia   | no   |
| Pteria penguin               | 2          | Okinawa     | yes  |
| Pteria penguin               | 2          | Philippines | yes  |
| Pteria penguin               | 3          | Australia   | yes  |
| Pteria penguin               | 3          | Thailand    | yes  |
| Freshwater mussels           | Quantity   | Origin      | Pearls |
| Anodonta plicata             | 5          | China       | yes  |
| Hyriopsis schlegeli          | 6          | Japan       | yes  |
| Unio margaritifera           | 1          | USA         | yes  |
us to the theory of shell growth outlined above in the 'Background'. The pictures were taken on polished cross-sections of shells and pearls, both natural and cultured. Figure 2 shows a cross-section through the shell of the saltwater oyster *Pinctada margaritifera* from Tahiti. The layers seen compare favourably with those in Figure 1d. The black colour is due to the organic phases, conchiolin and porphyrin (Miyoshi et al., 1987, 1989). The difference of colour in the aragonite layer may be caused by either a seasonal change or variation in food availability.

The influence of the age of the tissue causing the growth of a pearl is shown in Figure 3 (cf. Figure 1). The bead of the cultivated Japanese saltwater pearl (the oyster *Pinctada martensi*) in Figure 3a is the nucleus for a deposit of a thin layer of calcite prisms. Figure 3b shows a large core of calcite prisms in a natural saltwater pearl. In both cases, the calcite layers are followed by aragonite layers. The identity of the calcite prisms was checked by FT-IR (Farmer, 1974).

What do we learn from the structures of these pearls?

The piece of mantle that was inserted with the bead, to build up the tabular aragonite crystals (nacre) around it, was cut from a little too near the edge of the victim bivalve. It was not old enough to produce aragonite from the beginning. After producing calcite prisms for a while, it switched to aragonite. This explains the calcite rim around the bead followed by the aragonite layer.

Initial growth of the natural pearl also took place near the edge of the shell in very young mantle cells which deposited calcite for a long time before changing to aragonite production. Like others (e.g. Scarritt, 1987), we also have observed both natural and cultivated pearls built totally out of calcite prisms. These pearls show none of the desired optical iridescence effects and most are of no commercial value.

tural as well as chemical investigations.

We will not give any technical details of the well-established methods such as SEM, FT-IR, XRD and texture goniometry, (a further development of XRD), but a short introduction is given to the less well-known method of energy dispersive X-ray fluorescence (EDXRF) and to scanning force microscopy (SFM), which is relatively new.

**Morphological structures**

**Light microscopy (LM)**

The essential observations were made with normal light microscopy, which led
Fig. 3a. Cross-section through a Japanese bead-nucleated pearl cultured in saltwater (Akoya). The bead (left) is followed by a small layer of calcite prisms that is followed by the mother-of-pearl layers (aragonite). 40x

Fig. 3b. Cross-section through a natural saltwater pearl with a large core formed of calcite prisms. The prismatic layer is followed by the mother-of-pearl layers. 30x

Fig. 4. SEM micrograph of the pseudo-hexagonal aragonite crystals forming the mother-of-pearl layer in the shell of the saltwater oyster 
*Pinctada maxima* (Australia). Length of one white line segment: d = 10μm.

Fig. 5. SEM micrograph of the aragonite crystals forming the mother-of-pearl layer in the shell of the freshwater mussel *Hyriopsis schlegeli* (Japan, Lake Biwa). Nearly all crystals show screw dislocations. Length of one white line segment: d = 10μm.

Fig. 6. SEM micrograph of the pseudo-hexagonal aragonite crystals forming the mother-of-pearl layer in the shell of the saltwater oyster *Pinctada margaritifera*. The crystals show hillocks on their surfaces. Length of one white line segment: d = 10μm

Fig. 7. SEM micrograph of a spiral formed by many aragonite crystals in the mother-of-pearl layer of the saltwater oyster *Pinctada margaritifera* (Tahiti). Length of one white line segment: d = 0.1mm
Scanning electron microscopy (SEM)

The aim of the SEM investigation was to characterize the aragonite and calcite crystals and perhaps some organic relics such as the organic matrix mentioned in the introduction (for a short review of SEM see Postek et al., 1980).

For the SEM investigations three specimens of each species were chosen. At least three samples were taken from every single shell. In this way, we hoped to gain an overview of the internal diversity of the shell. Indeed, we observed that the habits of the aragonite crystals vary between the different species and in the shells themselves. However, there is one particular habit of the aragonite crystals that can be found in every species. These are tabular crystals with a thickness of 400 nm to 1500 nm that have a pseudo-hexagonal shape (see also Mutvei, 1980, and Erben, 1970). Figure 4 shows a top view on the mother-of-pearl layer which is built of these pseudo-hexagonal aragonite crystals, from the shell of the saltwater oyster Pinctada maxima (Australia).

The nacreous layers from the shell of the freshwater mussel Hyriopsis schlegeli (Biwa pearls, Japan) and the saltwater oyster Pinctada margaritifera (Polynesian black pearls, Tahiti) show some features that distinguish them from the other shells. Figures 5 and 6 show SEM micrographs which exhibit two totally different kinds of growth. The Hyriopsis schlegeli grows by adding carbonate in spirals initiated by structural dislocations. The term dislocations describes changes in the symmetry of the array of atoms as they adhere to a crystal at its growing surface. Nearly every aragonite crystal in the nacreous layer shows this phenomenon. In contrast, the aragonite crystals of the Pinctada margaritifera (Figure 6) exhibit a kind of hillock on every crystal. We think that these ‘growth hills’ could be the initial stages of the crystals forming the next nacreous layer.

Screw dislocations can be observed in nearly all shells, but to a much lesser extent than in the Hyriopsis schlegeli. Only a few crystals in the whole of the Pinctada margaritifera (Tahiti) shell have such dislocations, but these are transmitted to the overlying crystals, giving rise to a large spiral involving many single aragonite crystals without dislocations (Figure 7). The reason why the aragonite crystals of some shells have many dislocations while others have very few is not yet clear, but it will be an interesting subject for our further investigation.

The calcite crystals of the prismatic layer in the shells are quite uniform in shape through all the investigated species. They only vary in size. For SEM images of the prismatic layer see Doumenge et al. (1991).

X-Ray diffraction (XRD) and texture goniometry

Shell material of every species was investigated by XRD. It confirmed that the nacreous layer of every shell consisted of aragonite and the prismatic layer of calcite. Fibrous aragonite as described by Caseiro (1993) was not found in our samples.

More astonishing was the fact that the diffractograms of the nacreous layer of saltwater oysters looked different from those of freshwater mussels. The diffractograms of the aragonitic layers in freshwater mussels had some reflections that did not appear, or only to a much lesser extent, in those of saltwater oysters. We concluded that the orientations of the aragonite crystals in the nacreous layer of saltwater oysters are slightly different from those in the nacreous layer of freshwater mussels.

To confirm this theory, we used the method of texture goniometry, with which it is possible to determine the orientations of the crystal axes in a polycrystalline material. This very useful method works on the basis of X-ray diffraction, and a detailed description is given by Wenk (1985). These investigations showed that the c-axes of all aragonite crystals forming the nacreous layer are aligned perfectly
parallel to each other, and perpendicular to the inner surface. But the lateral axes of the aragonite crystals of the saltwater oysters were better aligned than those of the freshwater mussels. The reason for these different orientations are not known, but the factors of water temperature and concentrations of dissolved elements (which for freshwater mussels are usually lower than for saltwater oysters) may play a role.

Studies carried out by texture goniometry revealed other even more interesting results. It was clearly shown that the aragonite crystals forming the mother-of-pearl layer in all our investigated shells are single crystals. This is in agreement with some earlier studies (Towe and Hamilton, 1968; Wise 1970). However, it contradicts the findings of other authors who postulated that the pseudo-hexagonal habit of the aragonite crystals, arose as a result of twinning (Mutvei, 1970; Hänni, 1982).

Although one query has been solved a new one arises: how can an aragonite crystal with orthorhombic crystal symmetry have a hexagonal shape? The hexagonal shape and the spatial arrangement of the aragonite crystals in nacre has been described by Erben (1970) and Mutvei (1970) using SEM. Theoretically it is possible to have aragonite single crystals with orthorhombic crystal symmetry displaying a 'pseudo-hexagonal' habit (Graeser, 1992), and the question of whether these crystals are twins can possibly be answered by another result of the texture goniometry study. We detected a highly ordered phase in the mother-of-pearl layer that could not be correlated with any of the modifications of CaCO₃ (calcite, aragonite or vaterite) or with strontianite. This highly ordered phase may not be crystalline at all and the reflection could come from the organic matrix (Weiner and Traub, 1984) mentioned in the ‘Background’. The exact nature of the organic matrix, which presumably covers every single crystal in the shell, could not be observed on the SEM.

**Scanning force microscopy (SFM)**

With scanning force microscopy (SFM), whose lateral and vertical resolution is high enough to distinguish atoms, we were able to depict the surface of single aragonite crystals forming the nacreous layer in the shell of the Australian oyster *Pinctada maxima*.

Scanning force microscopy is essentially a further development of the scanning tunnelling microscope (STM) invented by Binnig *et al.* (1982). In contrast to the STM, which senses a tunnelling current, the force microscope senses forces between the probing tip and the sample surface. With the SFM it is possible to study surfaces of insulators as well as conductors, whereas the STM is restricted to conducting samples. Figure 8 shows a scheme for a scanning force microscope that is based on a force sensor, a displacement sensor, a feedback system which monitors and regulates the deflection of the cantilever, a mechanical scanning system and a computer system for acquisition of data and image processing. For recent reviews see Ruger and Hansma (1990) and Meyer (1992).

Two general modes of operation have been developed for SFM to sense short-range forces as well as long-range forces: the contact mode and the non-contact mode.

In this study only the contact mode was applied; therefore the description is restricted to this method. Using the contact mode, the probing tip (2) is brought close to the repulsive force range of the sample surface (3) (tenths of nanometers). In response to the repulsive short-range interatomic forces, which act on the probing tip, the cantilever (1) is bent. While scanning the sample surface with piezoelectric transducers, the deflection of the cantilever is kept constant by a feedback loop that adjusts the relative distance between sample surface and probing tip. The feedback signal is monitored as a func-
tion of the lateral position of the probe and the image of equiforce contours (termed force micrograph) is obtained. This relief is interpreted as the topography of the sample surface.

In this study a commercially available scanning force microscope, equipped with a beam deflection system for measuring the cantilever displacement was used. This system is widely used in commercially available microscopes because of its simplicity. A laser beam is collimated and focused on the rear side of the cantilever and there reflected off to a segment photo diode acting as a position sensitive detector (PSD). Due to the interaction between probing tip and sample surface, the cantilever is deflected elastically and thereby changes the reflection angle of the laser beam detected by the PSD (Figure 8).

Figure 9 shows a force micrograph of a single aragonite crystal from the nacreous layer. The crystal shows no re-entrant angles as twins sometimes do (Hurlbut, 1977). It has a thickness of about 600 nm and a diameter of about 5000 nm (5μm). According to Weiner and Traub (1984), the growth of these aragonite hexagons is strongly influenced by an organic matrix covering every single crystal. In confirmation of this theory, the crystals of the mother-of-pearl layer all look as if they are covered with organic matter. Figure 10 is a top view of the crystal in Figure 9. Particles with diameters of 80 to 100 nm can be seen. These particles probably form the organic matrix. They also often show hexagonal shapes which are taken on by the subsequent growth of aragonite crystals (Gutmannsbauer, 1993). It is also known that organic compounds can influence the shape of inorganic crystals. Uric acid causes NaCl crystals to crystallize as octahedra instead of the cubes usually seen. It is possible that a similar process between the organic matter and the arago-
Fig. 9. Scanning force micrograph of an aragonite crystal forming the mother-of-pearl layer in the shell of the saltwater oyster *Pinctada maxima* (Australia). Diameter of the crystal: d=5μm

Fig. 10. Scanning force micrograph of the top of the crystal in Figure 9. This is probably the organic matrix that covers every crystal in the shell. Diameter of the polygonal domed areas forming the matrix: d=80 nm.
Fig. 11. EDXRF analysis of the Mn and Sr contents in the mother-of-pearl layers of saltwater and freshwater shells.

Fig. 12. EDXRF analysis of the Mn and Sr contents in the mother-of-pearl layers of the shells of the freshwater mussels *Hyriopsis schlegeli* and *Anodonta plicata*.

Aragonite crystals influences the growth of the aragonite crystals. According to Lowenstam and Weiner (1989), there is evidence of organic matter inside the crystals.

The thicknesses of these aragonite microcrystals are within the range of wavelengths of visible light, which suggests that the rainbow colours seen on nacre are due to interference.

Chemistry

Energy dispersive X-ray fluorescence (EDXRF)

The chemical analyses of the shells were carried out by EDXRF. This is a spectroscopic analysis method for the determination of the chemical composition of usually solid matter. The sample is supplied with radiation energy from an X-ray tube. Due to this irradiation the atoms in the sample also emit X-rays of characteristic wavelength (line spectra) that are typical for every element. The fluorescence or energy is registered by a detector. The detection records simultaneously all major and minor elements with the atomic numbers from Z=11 (Na) to Z=92 (U).

Because the power of the X-ray tube is not very high, no radiation damage is caused. For a detailed description see Hahn-Weinheimer *et al.* (1984).

With EDXRF we studied the chemical composition of the periostracum, the mother-of-pearl layers and the pearls of the different species that were available.

The results showed that the composition of the periostracum of all shell material contains a greater number of different elements and in higher concentrations than the corresponding mother-of-pearl layers and pearls. Among the elements found were Na, Mg, S, Cl, K and P. We suggest that these elements give some indication of the composition of the water the animals lived in, but because there is no gemmological relevance for these elements in the periostracum, we will not go into further details.

As earlier investigations have already shown (Farn, 1986), saltwater shells and pearls contain significant lower Mn than those from freshwater. Sr can easily be incorporated in the structure of aragonite instead of Ca, and all of the investigated mussels do incorporate Sr in their shell but not enough for the formation of the mineral strontianite. However, there is no significant difference in the concentration of Sr between salt- and fresh-water shells (Figure 11). The concentration of Mn differs even among the freshwater mussels themselves. The mother-of-pearl layer and pearls of the mussel *Anodonta plicata*
contain significantly less Mn than
the mother-of-pearl layer and pearls of the
mussel *Hyriopsis schlegeli* (Figure 12).
There is no statistically demonstrable dif-
ference between these two species with
regard to Sr content.

The mother-of-pearl layer and the pearls
of the freshwater mussel *Hyriopsis schlegeli*
are the only freshwater mussels that
contain no K.

The chemical analyses of mother-of-pearl
and pearls of the freshwater mussels origi-
inating from the Mississippi (*Unio
margaritifera*) showed that it is not possible
to characterize these in terms of their trace
elements. The values of the element concen-
trations overlap the values found from
all other investigated species. This can be
explained by considering the vast extent of
the Mississippi river system. The river
passes through many different geological
environments and the element concen-
trations of the water will vary to a large
degree.

It was not possible to find any difference
between the element concentrations in the
mother-of-pearl layers in shells from differ-
ent saltwater oysters. However, the pearls
of the saltwater oyster *Pinctada martensii*
have lower Sr concentrations than the
other saltwater pearls.

Fourier transform infrared spectroscopy
(FT-IR)

All the shells and pearls we received
were described as untreated. However,
nearly all Japanese saltwater grey to black
cultured pearls contained beads coloured
with an organic dye. Figure 13 shows an
Akoya cultured pearl of this kind. The dye
causes a series of peaks in the infrared
range between 2000 and 1300 cm⁻¹.

All our samples were tested using FT-IR
to determine the presence of calcite or
aragonite. A quick identification is possi-
ble (Speer, 1983) by checking the spectrum
for peaks either at 1492-1432, 879 and
706 cm⁻¹ (calcite) or 1504, 1492, 1080, 866,
711 and 706 cm⁻¹ (aragonite).

Conclusions

This study shows that formation of
mother-of-pearl and pearl is complex and
can only be investigated with the use of
specialized instruments. Although this
study took one year of research for one of
the authors (W.G.), many unsolved prob-
lems remain and some new questions have
arisen.

The study showed that it is not possible
to identify shell species and pearls using
only one method. On the contrary, only a
combination of techniques can lead to real-
istic interpretations. For example, the
determination of the bivalve that formed a
pearl is only possible by a combination of
results gained from structural and chemi-
cal investigations.

To detect and confirm any treatments
(e.g. dyeing) even more techniques are
required (e.g. FT-IR).

The investigation of mother-of-pearl is
not only of gemmological interest: the
growth process itself, including biominer-
alization is also of great importance in
medical research. The nacreous layer of
the bivalve molluscs makes available a rel-
atively simple model in which bio-
minalization processes can be studied.

Fig. 13. Akoya cultured pearl with a bead that is
treated with an organic dye. 30x
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The large amount of data and the many different analytical methods used mean that this work could not have been done by one single person.

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