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Physicochemical characterization of biogenic calcium carbonate

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ABSTRACT

Biogenic minerals are widely studied materials for their particular properties derived from their hierarchical structure, using building blocks with sizes spanning several orders of magnitude. These special features can be assessed with different analytical tools, and it is important to know their capabilities and limitations. In order to determine the hierarchical structure of the shells, the nacre and prismatic layers of two marine animals were characterized by infrared spectroscopy, X-ray diffraction, and scanning electron microscopy. Based on these assessments, we found that the combination of these three techniques is useful to describe each structure level, and to explain some of the unique properties observed in these natural materials.

INTRODUCTION

Nowadays, the biomimesis of materials found in Nature is one of the main challenges in Material Sciences. Many of the unsolved technological issues are somehow solved by Nature, as the super-hydrophobicity, selectivity, biocompatibility, etc.

Materials in Nature are formed in well-optimized processes over thousands and millions of years, mostly based on the self-assembly of building blocks. This procedure is an amazing challenge to the Thermodynamic laws, as implies a huge loss of entropy by the system being self-assembled, which is compensated by strong interactions between the building blocks allowing for the release of a high amount of energy as heat that increase the entropy of the surroundings. Hence, this entropy-enthalpy compensation produces the net increase in the entropy of the universe, as required by the Second law of the Thermodynamics. The interesting point is that the first step in the assemblage process produces a building block which is further self-assembled in bigger building blocks that ends in the macroscopic material. This step-by-step created structures are known as

hierarchical structures [1] and remain in the final material as the evidence of the building process.

In the case of inorganic materials, the biogenic minerals offer different properties than the one obtained only by geochemical means, because the synthesis of the materials is directed by proteins that act as guides in the assemblage process. Though the inorganic phase of biogenic structures is the same, several features reveal the hierarchical structures which are based on. Biogenic calcium carbonate is formed in the marine environment, and constitute the shell of different molluscs and gastropods. Two crystalline forms are noticeable in the shell of some marine animals: the inner phase consisting in nacre, and the outer phase, known as the prismatic layer [2]. In the nacre layer, a hierarchical structure is evident at different building block unit sizes, ranging from the μm to the nm size values. The level 1, which is the nacre layer itself, up to a few cm and is formed through the self-assemblage of aragonite plaques [3,4], which have a length between 5 – 15 μm, and a width between 200 – 900 nm, and constitute the level 2 of the structure. Between the aragonite plaques, there is a space filled with proteins and β-chitin like polysaccharides (ca. 1 – 5 % in weight of the nacre). In this space, with a width of ca. 30 – 300 nm, the synthesis and growth of calcium carbonate crystals take place [5,6]. The plaques are formed by minute crystalline grains with sizes between 25 – 34 nm, which also form bridges between the aragonite plaques. These grains constitute the level 3 of the structure.

According to the different size ranges of the building blocks, not all analytical methods will give the same information. The aim of the present work is to provide the experimental evidence of the information obtained on the level assessed in the hierarchical structure of calcium carbonate found in the nacre of marine animals, and to compare these results with those of the prismatic layer. Based on these results, and on the existing theories that explain the stiffness of biominerals, the importance of our approach is to assess the potentiality of the above mentioned methodologies in order to provide information at the different hierarchical levels in the design of bioinspired materials.

EXPERIMENTAL DETAILS

Samples of the marine bivalve mollusc white pearl oyster (*Pinctada maxima*) and the marine gastropod green turban (*Turbo marmoratus*) were commercially acquired. After a thorough cleaning involving hot water (50 °C) and detergent, samples were dried in air for 2 days. Sub-samples of the internal face (nacre) and the external phase (prismatic layer) were obtained with a Nicholson® tool *(warning: masks should be used during this procedure to avoid inhalation of particulate material)* and stored in plastic tubes for further analysis.

X-ray diffraction analyses were obtained between $10° < 2\theta < 60°$, using Cu Kα radiation at 30 kV and 40 mA. Results were compared to the database included in Crystallographica Search-Match Version 3.1.0.2.

Fourier-transformed Infrared Spectroscopy (FTIR) were obtained using the method of the KBr pellet, and the spectra were registered at 10 scans, and a resolution of 4 cm^{-1} in the wavenumber range 4000 – 400 cm^{-1}. Signals were processed by employing a Happ-Genzel apodization, corrected from residual humidity and the presence of atmospheric CO_2, and smoothed with a Savitzky-Golay algorithm of 15 data points. Spectra were normalized for comparison.

Nacre samples for Scanning Electron Microscopy (SEM) were covered with a thin layer of gold obtained by vapour deposition at a constant current of 30 mA for 120 s. SEM micrographs were obtained at an accelerated voltage of 20 kV.

RESULTS AND DISCUSSION

Chemical information

The chemical composition of the shell parts (nacre or prismatic layer) is presented according to the phase analysed, i.e. inorganic or organic. XRD analysis of the inorganic phase unequivocally identified the samples from the chemical point of view, by comparing the diffraction pattern with standards (Figure 1). In all cases, no evidence of amorphous structure is deduced, confirming the high crystallinity of the samples, and that their biological origin corresponds to adult specimens [7]. For both samples, aragonite was identified as the single component in the nacre samples, while mixed calcium and magnesium carbonate was identified in the prismatic layer.

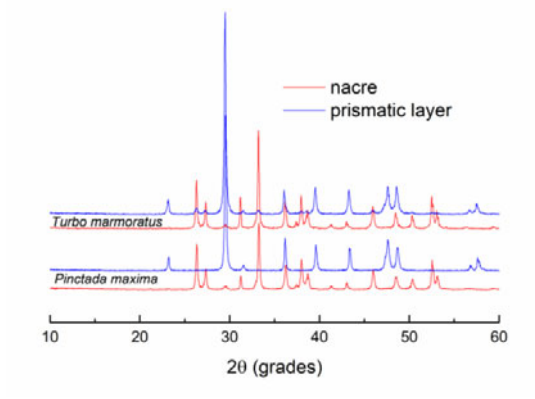

Figure 1. XRD patterns for the nacre and prismatic layers of *Pinctada maxima* and *Turbo marmoratus*.

Information from the level 2 of the hierarchical structure

The FTIR spectra for the nacre and prismatic layers of both marine animals are dominated by a strong adsorption at ca. 1427 cm^{-1}, assigned to the v_3 asymmetric stretching vibration of C-O bond. Other minor bands are centred at 713 cm^{-1} (δ_4 in-plane bending of O-C-O group), 876 cm^{-1} (δ_2 out of plane bending of CO_3 group), and a very weak absorption band at 1083 cm^{-1} (v_1 symmetric stretching vibration of C-O bond). Other bands at higher wavenumbers result for the coupling of $v_1+\delta_4$ and $v_1+ v_3$ signals.

In the case of the prismatic layer, δ_4 and v_3 are doubly degenerated, and appear as a single band, typically of calcite spectra. But in the case of the nacre spectra, the double degeneracy is lost, and δ_4 and v_3 appear as double bands, at 700 and 713 cm^{-1}, and 1450 and 1472 cm^{-1}, respectively (Figure 2). This is characteristic of the aragonite structure, and appear as a consequence of the hierarchical structure of calcium carbonate found in Nature, but particularly in the biogenic calcium carbonate found in marine animals. The incidence of the proteins guiding the synthesis of calcium carbonate is

reflected in the appearance of an additional distortion to the symmetry of the cell, enhancing the splitting of the δ_4 and v_3 bands.

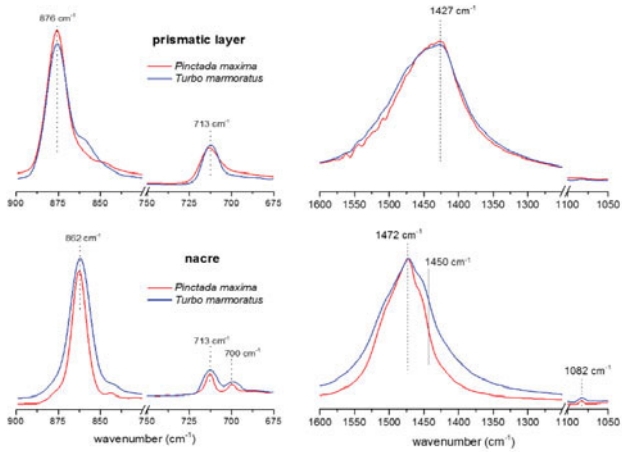

Figure 2. Comparative FTIR spectra for δ_4 bending (left panels) and v_3 stretching (right panels), showing the splitting of signals.

Hence, FTIR analysis reveals the level 2, denoting the hierarchical structure due to the splitting of some absorption bands. SEM images (Figure 3) reveals multiple aragonite plaques stacked on top of each other, perpendicular to the *c* axis of the crystal, while it can be seen that *a* and *b* axes are slightly shifted between the plaques. Some particular features, like the interlocking between plaques and the dovetail-like borders are also seen in *T. marmoratus*. The normal distribution of aragonite plaque widths yields mean values of 477 ± 64 nm (n = 375) and 487 ± 56 nm (n = 347) for *P. maxima* and *T. marmoratus*, respectively.

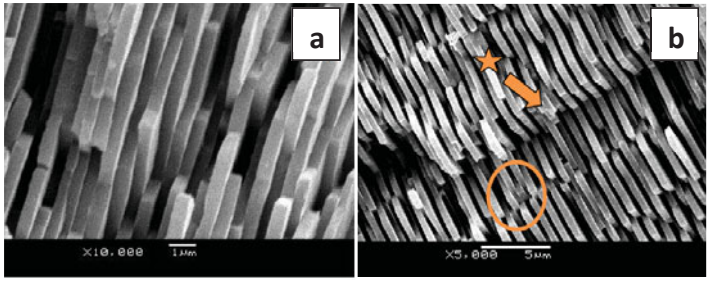

Figure 3. SEM images for (a) *P. maxima* and (b) *T. marmoratus*. In panel (b), the circle shows the interlocking between plaques and the arrow shows the dovetail-like borders.

Information from the level 3 of the hierarchical structure

From the XRD analysis, not only the chemical identification of the sample is possible. Taking as reference the most intense peaks for each diffractogram, and applying the Scherrer equation, the size of the crystallite is obtained (Table 1).

Table 1. Crystallite sizes for *P. maxima* and *T. marmoratus* nacre and prismatic layers.

Specie	Nacre layer	Prismatic layer
Pinctada maxima	39 nm	45 nm
Turbo marmoratus	47 nm	43 nm

Additional information comes from the FTIR analysis of the amide I region, which extends between 1700 – 1600 cm^{-1} in the spectra. In such spectral region, it is possible to assess the nature of the secondary structure of the proteins located between the aragonite plaques. It is well documented that most of the proteins have a β-sheet structure, and this can be assessed through complicated analytical methods involving extraction and purification of the proteins. Infrared spectroscopy provides a way to assess the presence of β-sheet, together with a rough quantification, based in the deconvolution of the 1700 – 1600 cm^{-1} spectral region, employing the Levenberg-Marquand algorithm [8]. Results (Table 2) clearly show the predominance of beta structures within the proteins for both *P. maxima* and *T. marmoratus*.

Table 2. Percentage of the secondary structure of the proteins present in the nacre layer of *P. maxima* and *T. marmoratus*.

Secondary structure	Percentage of total protein	
	Pinctada maxima	*Turbo marmoratus*
β-sheet	38 ± 2	31 ± 4
β-turn	44 ± 1	41 ± 2
α-helix	12 ± 2	8 ± 1
3_{10}-helix	20.9 ± 0.2	8 ± 1
Random coil	3 ± 1	9 ± 2

Percentages as mean ± SD of three independent data analyses.

The synergic role of the components in the stiffness of the biomineral

The isolated components of the biomineral, i.e. $CaCO_3$ and the protein, do not explain by themselves the stiffness observed in the composite material found in the Nature. Hence, is the structure, and the particular way they are assembled that constitutes the grounds to understand the enhanced properties observed. There is a general consensus that such properties rely on the structural stability of the interface between the protein and the mineral [9], and particularly, the uniformity of the shear stress at this interface is fulfilled by the structure deduced from the level 2 of the hierarchical structure [10]. Such strong interaction at the interface is caused by multiple contact points provided by the surface roughness of the plaques [11], as deduced from Atomic Force Microscope measurements [12]. Hence, according to our results, FTIR and SEM analyses provide a first clue to assess the structural details related to the novel properties of the biominerals. In addition, FTIR offers a simple mean to analyse the organic face without complicated extraction procedures.

CONCLUSIONS

In both marine animals, the nacre layer is composed by aragonite, while the prismatic layer corresponds to calcite. At the level 3 of the hierarchical structure, minute crystallite grains around 30 – 50 nm are deduced from XRD analyses, and form part of plaques, that are accommodated perpendicularly to the *c* axis, providing extra strength to the nacre layer. FTIR analyses of the organic phase within the nacre layer show the dominance of proteins (mainly with a β structure), which are related to the nucleation and growth process of the calcium carbonate crystal, and also strongly interact with the plaques. The aragonite plaques have a mean width at ca. 450 – 500 nm, and constitute the level 2 of the hierarchical structure, as revealed through SEM images. The interface between the plaques and the proteins explain the high stiffness observed in the shells of these animals. Summing up, FTIR and SEM analyses are complementary techniques that reveals the secondary level of the hierarchical structure, which is closely related to the enhanced properties of the biominerals. We envisage that these methods will be of importance in the characterization of designed biomimetic materials.

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REFERENCES

[1] J. Sun and B. Bhushan, RSC Adv. **2**, 7617 (2012).
[2] C. Liu, L. Xie and R. Zhang, Sci. Rep. **5**, 1 (2015).
[3] H. Kakisawa and T. Sumitomo, Sci. Technol. Adv. Mater. **12**, 64710 (2011).
[4] F. Nudelman, Cell Dev. Biol. **46**, 2 (2015).
[5] D. Volkmer, in *Handbook of Biominerization,* edited by E. Baeuerlein (Wiley-VCH, Weinheim, 2007), p. 65.
[6] H. Imai and Y. Oaki, in *Handbook of Biominerization,* edited by E. Baeuerlein (Wiley-VCH, Weinheim, 2007), p. 89.
[7] E.M. Gerhard, W. Wang, C. Li, J. Guo, I.T. Ozbolat, K.M. Rahn, A.D. Armstrong, J. Xia, G. Qian, J. Yang, Acta Biomater. **54**, 21 (2017).
[8] H. Yang, S. Yang, J. Kong, A. Dong, and S. Yu. Nat. Protoc. **10**, 382 (2015).
[9] S. Askarinejad and N. Rahbar, J. R. Soc. Interface. **12**, 20140855 (2015).
[10] G. Liu, B. Ji, K.-C. Hwang, D.C. Khoo, Comp. Sci. Technol. **71**, 1190 (2011).
[11] P. Ghosh, D.R. Katti, K.S. Katti, Biomacromolecules **8**, 851 (2007).
[12] B. Mohannty, K.S. Katti, D.R. Katti, Mech. Res. Commun. **35**, 17 (2008).