

Crystal Structure Analysis of Biominerals

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Biominerals are nanocomposite materials containing minerals. They are synthesized in biological systems at ambient temperature and pressure with limited elements such as Ca^{2+} , PO_4^{3-} , CO_3^{2-} , etc. Most biominerals, shells, egg shells, pearls, etc., are not merely simple mixtures of organic and inorganic materials. They are nanocomposite assemblies with complicated structures and novel function. Many material scientists have been attracted to develop biomimetic materials. We propose that controlling the crystal structure in biomineralization should be also taken accounts for adapting the materials to function. In order to clarify the difference between biominerals and usual synthetic inorganic materials, time-of-flight neutron powder diffraction, X-ray diffraction, fluorescent X-ray analysis, atomic absorption spectrophotometry and ICP emission spectrochemical analysis were carried out. Results indicated that the crystal structure parameters of aragonite and calcite in biominerals cannot be explained by a simple elemental substitution.

KEYWORDS: biomineral, biomineralization, X-ray powder diffraction, neutron powder diffraction

§1. Introduction

Biominerals, such as shells, eggshells, pearls, corals, etc., are nanocomposite materials with organic and inorganic substances.¹⁾ They are characterized by having nacreous, prismatic, foliated, crossed lamellar or composite prismatic structure, etc., which are relevant to novel function of the materials. For example, the mechanical strength and toughness of some shells are thousands times larger than conventional synthetic inorganic materials. It is proposed that the hierarchical microarchitecture of the crossed lamellar structure in the shells is associated with increase in their mechanical strengths and toughness. Many material scientists have been attracted to develop biomimetic materials. They consider biominerals as a model for new synthetic nanocomposite material. It is also noted that biominerals are synthesized in biological system at ambient temperature and pressure. This is quite important in view of applications as well as basic synthetic chemistry. The process of forming biominerals, which is called biomineralization, are intensively studied recently. We speculate that the crystal structures of biominerals are controlled in biomineralization process for optimizing the materials to function. Here we report on the crystal structural studies on several kinds of shells and eggshells in order to show the structural difference between those biominerals and usual synthetic inorganic materials.

Shells and eggshells consist of CaCO_3 as their main inorganic components. CaCO_3 have three polymorphs: aragonite, calcite and vaterite phases.²⁾ The aragonite phase is orthorhombic with the space group of Pnma while the calcite phase is trigonal with the space group of $R\bar{3}c$. The vaterite phase, which is seldom obtained in the usual biological systems, has the $P6_3/mmc$ space group. Among shells, Japanese littleneck, orient clam, corbicula and periwinkle are composed of the aragonite phase

while oyster, scallop shell are of the calcite one. Eggshells are also composed of the calcite. Abalone and Turbuan shells contain both phases. In order to obtain reliable structure analysis, we selected shells and eggshells without a mixture of aragonite and calcite phases.

§2. Experiment

X-ray powder diffraction data were taken with a Bragg-Bretano type diffractometer with $\text{CuK}\alpha$ radiation monochromatized with a curved graphite ($\lambda = 1.5418\text{\AA}$) at room temperature (RT). High resolution neutron powder diffraction data were collected at RT on a time-of-flight (TOF) neutron diffractometer *Sirius*³⁾ at Neutron Science Laboratory (KENS) of High Energy Accelerator Research Organization (KEK). Both X-ray and neutron diffraction data were analyzed by the Rietveld method.

The elemental analyses were also carried out to search for minor constituent phases qualitatively by the fluorescent X-ray analysis and quantitatively by the atomic absorption spectrophotometry (ABS) and ICP emission spectrochemical analysis.

§3. Results and Discussion

In order to evaluate accurate lattice parameters from X-ray diffraction data, we combined the Rietveld method with an internal standard method using Si as an internal standard. In order to examine the combined method, we selected two kinds of shells, Japanese littlenecks and corbiculas with two different habitats, and compared the lattice parameters obtained from the Rietveld analysis with and without internal standard. In the case of the combined method, better values of lattice parameters with five times smaller standard deviations are attained. Even the difference in the habitats of corbiculas can be extracted whereas it was impossible with the conventional Rietveld method without an internal standard. X-ray results given below are all based on the Rietveld

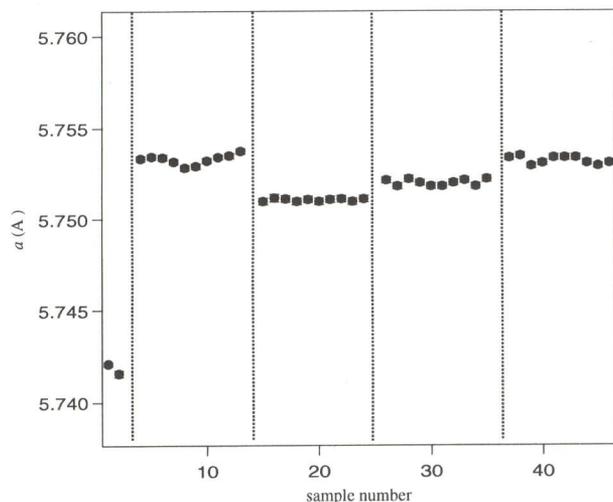


Fig. 1. a of the aragonite phases in various kinds of shells as well as synthesized and mineral aragonites. Abscissa shows the sample's number: 1 synthesized aragonite, 2 mineral aragonite, 3-12 Japanese littlenecks, 13-22 corbiculas from Ibaraki, 23-32 corbiculas from Tottori, 33-42 clams.

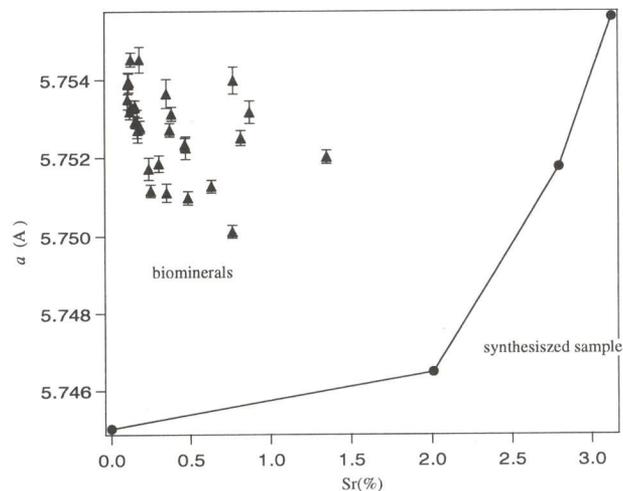


Fig. 2. a of aragonite phases *vs.* Sr content

method with an internal standard. In the case of neutron diffraction, it is not necessary to use internal standard. Crystal structures of about 40 biominerals, half of which consist mainly of aragonite phases and the rest half calcite ones, were refined on the basis of neutron diffraction data.

Figure 1 shows the lattice parameters, a , of the aragonite phases in various kinds of shells as well as synthesized and mineral aragonites obtained from X-ray data. It is obvious from the figure that a in shells is generally much larger than that of synthesized and mineral aragonites. The same tendencies are also seen in b . Since the fluorescent X-ray analysis showed the existence of small amount of Sr^{2+} , it is likely that Sr^{2+} ions partially substituted for Ca^{2+} . In fact, it was suggested the variations in the lattice parameters of aragonite phases in various biominerals were caused by the elemental substitution for Ca site.⁴⁾ In the present system, Sr^{2+} with the larger ionic size was initially thought to substitute for Ca^{2+} and be responsible for the variation of lattice parameters. However, the quantitative measurements of Sr by ABS denied this hypothesis as shown in Fig. 2, where a

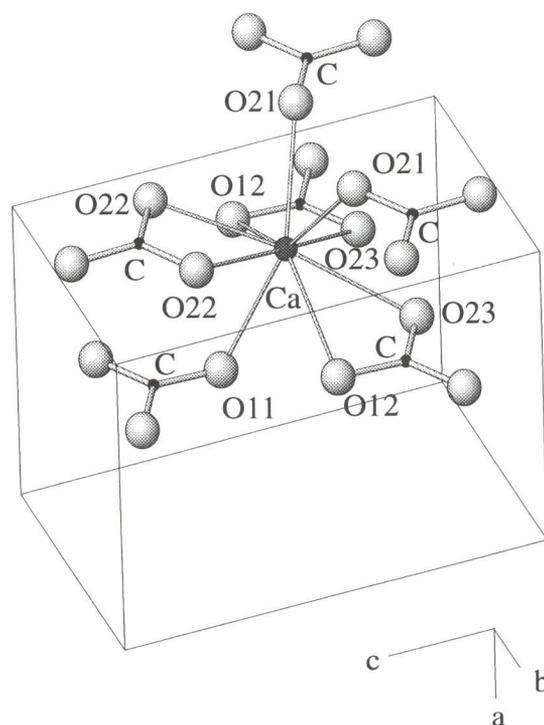


Fig. 3. Layout of a $[\text{CaO}_9]$ polyhedron in aragonite.

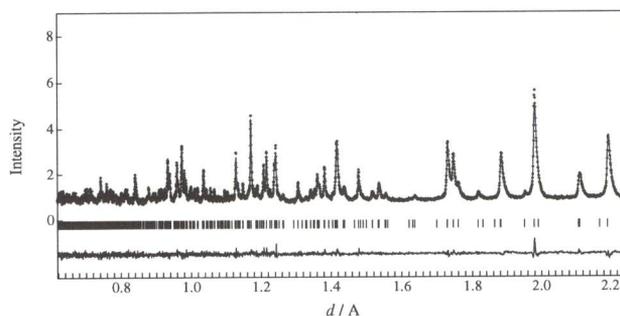


Fig. 4. Neutron diffraction pattern of Japanese littlenecks (aragonite).

of aragonite phases in shells are independent of Sr contents while that in synthesized aragonites with various Sr contents increases with increasing Sr concentrations.

The experimental result indicates the large a values in biominerals should be ascribed to other origins. Contrary to large values of a and b in shells, the values of c differ in different species. This phenomenon is unexpected because bc plane is parallel to CO_3 molecules and considered as structurally isotropic within the plane (see Fig. 3). Therefore, crystal structures of biominerals were studied with high resolution neutron powder diffraction. An example of Rietveld patterns are given in Fig. 4. Ca-O bond lengths in several biominerals are listed in Table I. In Fig. 5, two shortest Ca-O11 and Ca-O21 bonds of a $[\text{CaO}_9]$ polyhedron in several biominerals are shown. It is noted the shortest Ca-O11 bond is longer in biominerals, while the shortest Ca-O21 bond is almost invariant. This implies that a $[\text{CaO}_9]$ polyhedron is slightly deformed in biominerals.

The polyhedral volume, $V[\text{CaO}_9]$, in aragonite phases are plotted in Figs. 6 and 7 as a function of a and c , respectively. It is seen from the figures that corals

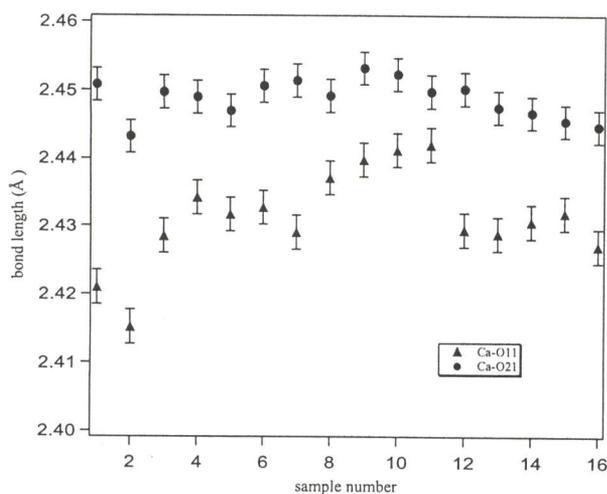


Fig. 5. Two shortest Ca-O11 and Ca-O21 bond lengths in a $[\text{CaO}_9]$ polyhedron. Abscissa shows the sample's number: 1 mineral aragonite, 2 synthesized aragonite, 3-7 Japanese littlenecks, 8 corbiculas from Tottori, 9-11 corbiculas from Ibaraki, 12-13 *Periglypta clathratas*, 14-16 corals.

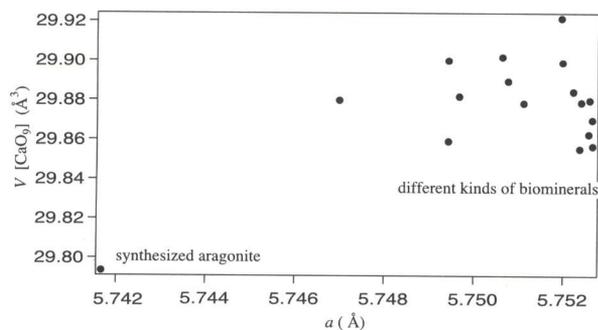


Fig. 6. a of aragonite phases and its relation to $V[\text{CaO}_9]$.

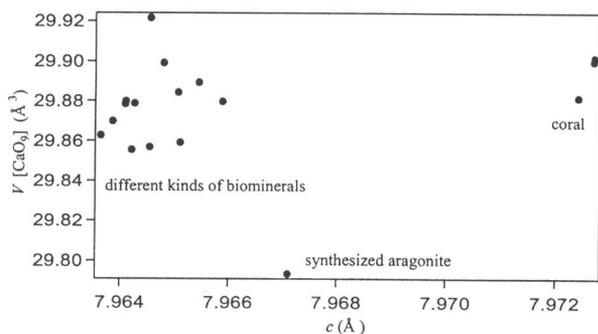


Fig. 7. The polyhedral volume $V[\text{CaO}_9]$ vs. c of aragonite phases.

have larger a , c and $V[\text{CaO}_9]$ values than synthesized aragonites, but shells have larger a and $V[\text{CaO}_9]$, but smaller c . Since C-O bonds in CO_3 molecule is rigid, this anisotropic tendency could be accommodated by the deformation of $V[\text{CaO}_9]$. Taking the difference in formation processes of shells and corals into consideration, the different tendency in c and the deformation of $[\text{CaO}_9]$

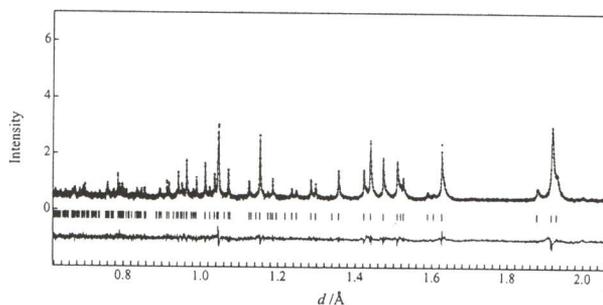


Fig. 8. Neutron diffraction pattern of oyster (calcite).

Table I. Example of bond lengths, $[\text{CaO}_9]$ volume, lattice parameters and unit cell volume, V . Sample number is the same as in Fig. 5.

Sample number	2	3	9	14
Ca-O11(Å)	2.4152(2)	2.4285(2)	2.4398(3)	2.4306(3)
Ca-O12(Å)	2.6620(12)	2.6655(13)	2.6626(16)	2.6669(15)
Ca-O21(Å)	2.4432(2)	2.4497(2)	2.4532(3)	2.4467(3)
Ca-O22(Å)	2.5306(2)	2.5251(2)	2.5207(3)	2.5284(2)
Ca-O23(Å)	2.5404(2)	2.5432(2)	2.5433(3)	2.5418(3)
$V[\text{CaO}_9](\text{Å}^3)$	29.79	29.85	29.87	29.88
a (Å)	5.74240(12)	5.75436(15)	5.75322(2)	5.75051(15)
b (Å)	4.96196(10)	4.96821(13)	4.96696(19)	4.96614(13)
c (Å)	7.96813(16)	7.96661(2)	7.96583(3)	7.97299(2)
V (Å ³)	227.100(7)	226.942(10)	227.547(15)	227.597(10)

might be ascribable to the difference in microarchitectures of both biominerals.

In the calcite phases of eggshells, both a and c are much smaller than those in minerals, synthesized ones and shells. The small lattice parameters in eggshells are likely to be explained by substitution of smaller Mg^{2+} for Ca^{2+} . In contrast, the lattice parameters of shells are quite different; a is smaller while c is larger than those in minerals and synthesized calcites. Although it has been reported that Mg^{2+} may substitute for Ca^{2+} in shells,⁴⁾ we found the values of lattice parameters could not be explained by simple Mg^{2+} substitution for Ca^{2+} . Since c plane is parallel to rigid CO_3 plane, the anisotropic change in the unit cell, *i.e.*, smaller a and larger c values in shells can only be accommodated by the deformation of $V[\text{CaO}_6]$, which might also result from formation process and microarchitectures of biominerals. An example of Rietveld patterns are given in Fig. 8.

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