

Sucrose/bovine serum albumin mediated biomimetic crystallization of calcium carbonate

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Abstract. To understand the role of the sucrose/bovine serum albumin system in the biomineralization process, we have tested the influence of different concentration of the sucrose/bovine serum albumin (BSA) on calcium carbonate (CaCO₃) precipitation. The CaCO₃ crystals were characterized by scanning electron microscope (SEM), Fourier transform infrared spectrograph (FT-IR) and powder X-ray diffraction (XRD). The possible formation mechanism of CaCO₃ in the sucrose/bovine serum albumin system was discussed.

Keywords. Bovine serum albumin; sucrose; calcium carbonate; biomineralization; mechanism.

1. Introduction

A widespread phenomenon in the biological world is biomineralization, a process by which organisms precipitate inorganic minerals. Over 60 biominerals are known, the most abundant of which are calcium carbonates, silica and iron oxides. These biominerals are organized hierarchically and ordered at many length scales that reflect remarkable physical characteristics. Mineralization in biological systems is an elegant and structurally complex process involving ionic, stereo-chemical, and structural interactions at the biomacromolecule-mineral interface.¹ Natural biomineralization products have been the focus of materials scientists because of their unique properties. A lot of experiments have been designed and carried out to search for the possible formation mechanism of natural biominerals.² The formation of calcium carbonate polymorphs, mainly calcite, aragonite and vaterite, has been reported in a number of cases such as gallstones,³ pancreatic stones in both humans and cattle,⁴ to animal phyla, algae, and in mollusk shells.⁵ It has been found that supersaturation is critical in determining the calcium carbonate polymorph precipitating. Thus, at high degrees of supersaturation, where spontaneous precipitation occurs, vaterite forms predominantly even at 25°C.⁶ The organic matrices are considered to play a principal role in biominer-

alization, but their function is still unclear. From literature it is known that carbohydrates have a minor influence,⁷ while proteins have a major effect^{8–10} on dissolution and precipitation of calcium phosphates.

BSA is widely used as matrix or soft template to induce the formation of calcium carbonate. Xue ZH¹¹ used BSA Langmuir film as a template to induce the formation of global calcite microcrystal. Zhang¹² synthesized calcite under different concentrations of bovine serum albumin. Saccharides are one group of important polymers widely present in living organisms. Saccharide was also used as organic matrix to biomimetic crystallization of calcium carbonate. A novel aragonite¹³ (CaCO₃) was synthesized with specific morphology by β -cyclodextrin. Glucan¹⁴ was used as a template to control synthesis of aragonite calcium carbonate.

This paper mainly discusses the cooperative influence of the sucrose/bovine serum albumin system which is close to biological organisms on calcium carbonate precipitation. The aim of these experiments is to find the effect on the polymorph and morphology of calcium carbonate by the sucrose/bovine serum albumin system.

2. Experimental

2.1 Materials and apparatus

The anhydrous calcium chloride (CaCl₂), sodium bicarbonate (NaHCO₃) and sucrose were analytically

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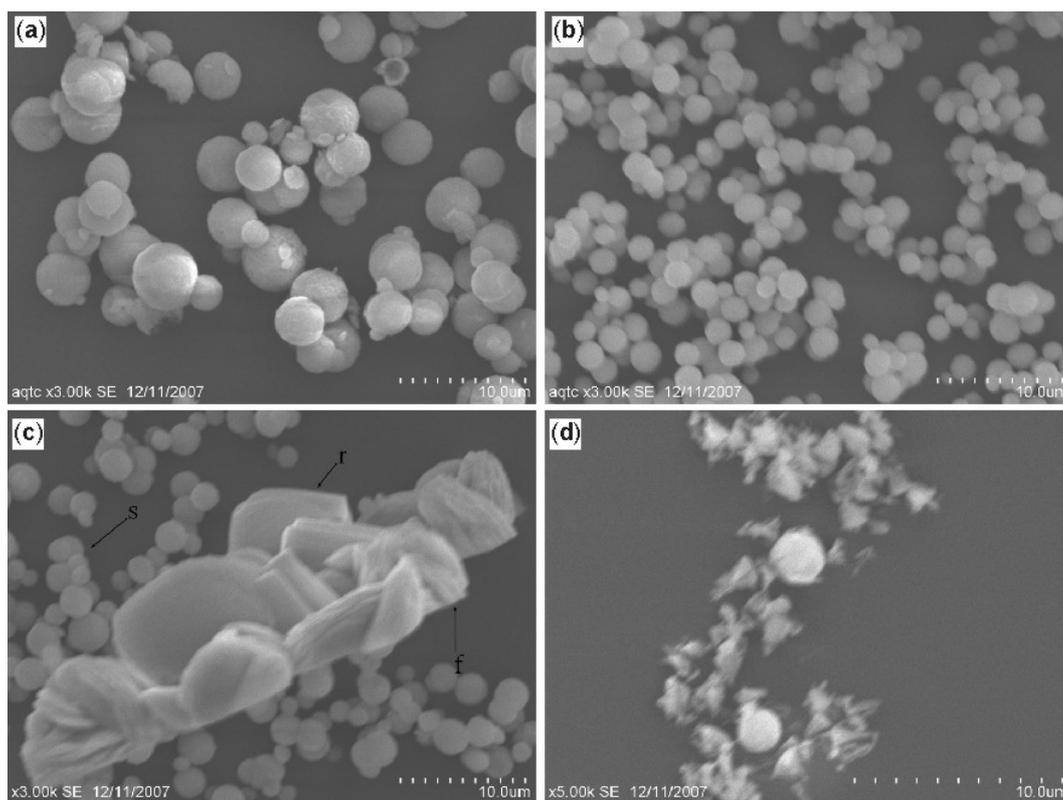


Figure 1. SEM of CaCO_3 crystal obtained from the sucrose/bovine serum albumin system sucrose/BSA: (a) 5 wt%/0, (b) 5 wt%/1 $\times 10^{-6}$ mol L^{-1} , (c) 5 wt%/4 $\times 10^{-6}$ mol L^{-1} , (d) 20 wt%/0; in figure 1c: s (spherical), r (rhomboidal), f (filamentous).

pure. The bovine serum albumin were extra pure (more than 99%), and purchased from Sigma Chemicals. All solutions were prepared with doubly deionized water. The sizes and morphologies of CaCO_3 precipitates were characterized by using SEM on a DSM 940 A (Carl Zeiss, Jena) microscope. The specimen powders were prepared for SEM analysis by coating each with a thin gold/palladium layer to prevent specimen charging under the electron beam. Calibration of CaCO_3 pellets (in a proportion of 1% in KBr powder) was performed and recorded with a Niolet 870 Fourier transform infrared spectrometer between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} . The XRD measurements were made by an MAP18XAHF X-ray diffractometry at a scanning rate of 1°/min, using a monochromatized $\text{CuK}\alpha$ radiation ($\lambda = 0.154$ nm).

2.2 Method of synthesizing CaCO_3 crystals

Calcium Carbonate was prepared according to the previously described methods.¹⁵ 0.84 g of anhydrous sodium carbonate (Na_2CO_3) was placed at the bottom of a large beaker (250 mL), and 1.11 g of anhydrous calcium chloride (CaCl_2) was placed at the

bottom of a small beaker (100 mL). The small beaker was placed in the large beaker. A series of the sucrose/bovine serum albumin solutions at different concentrations were added to the two beakers. The aqueous solution of the sucrose/bovine serum albumin was not added to the beaker until the solution surface exceeded the inner small beaker wall by 5–6 mm. The solution was kept at 25°C for 7 days without stirring. The sucrose/the bovine serum albumin concentration was 5 wt%/0, 5 wt%/1 $\times 10^{-6}$ mol L^{-1} , 5 wt%/4 $\times 10^{-6}$ mol L^{-1} and 20 wt%/0, respectively. When a large number of crystals were formed in the reaction vessel, the crystalline calcium carbonate was collected. The obtained crystalline CaCO_3 was filtered through the solution and rinsed with distilled water and anhydrous alcohol at least five times, then centrifugalized, dried and collected.

2.3 Powder X-ray diffraction, SEM and FT-IR analysis

The obtained crystalline CaCO_3 was collected for the determination of SEM, FT-IR and XRD.

3. Results and discussion

3.1 Characterization of CaCO₃ crystals produced in different concentrations of the sucrose/bovine serum albumin aqueous solutions

3.1a SEM images: Figure 1a–c showed SEM pictures of CaCO₃ particles precipitated in sucrose solutions concentration 5 wt% and different concentrations bovine serum albumin. Well-dispersed spherical particles with smooth spherical surfaces were gained (figure 1a) in the system without bovine serum albumin. The particles size was 2–4 μm. When bovine serum albumin concentration [BSA] = 1 × 10⁻⁶ mol L⁻¹, the well-dispersed spherical particles were still gained, which became smaller with size of ~2 μm (figure 1b). Surprisingly, when [BSA] was increased to 4 × 10⁻⁶ mol L⁻¹, some of the crystallites were spherical and some rhomboidal. Also seen are bundles of filamentous particles (figure 1c). Figure 1d showed the CaCO₃ particles like catkin which formed in sucrose solution with concentration of 20 wt%.

3.1b FT-IR spectra and XRD: Figure 2a–d was the FT-IR spectra of CaCO₃ crystals got from sucrose/the bovine serum albumin system solutions with the concentrations of 5 wt%/0, 5 wt%/1 × 10⁻⁶ mol L⁻¹, 5 wt%/4 × 10⁻⁶ mol L⁻¹ and 20 wt%/0, respectively. Bands at 874 and 744 cm⁻¹ indicated the formation of vaterite (figure 2b–d). While in figure 2a, simulta-

neous occurrence of absorption peaks at 874, 710 and 744 cm⁻¹ indicated the presence of crystalline calcite and vaterite.¹⁶ There was little difference among figure 2b–d curves. The slight discrepancy between experimental and published IR values could be attributed to the required grinding of a CaCO₃ sample with KBr to produce pellets.¹⁷ Figures 3a–d showed the XRD patterns of CaCO₃ particles obtained in sucrose/BSA system. Figure 3a showed the XRD of CaCO₃ particles formed in the system only with 5 wt% sucrose. Using the reflection peaks at (104) plane for calcite and (110) plane for vaterite, we can find that there are not only sharp calcite but vaterite reflections in figure 3a, confirming that low concentration (5 wt% sucrose) led to the formation of calcite and vaterite. While high concentration (20 wt% sucrose, figure 3d) led to the formation of vaterite. As figures 3b–c had shown, only the diffraction peaks from vaterite appeared, this provides further evidence for only the existence of vaterite in the products formed in sucrose/BSA solution. The XRD patterns of CaCO₃ induced by 5 wt%/1 × 10⁻⁶ mol L⁻¹, 5 wt%/4 × 10⁻⁶ mol L⁻¹ sucrose/BSA were similar to each other. The assignment of polymorphs to calcite or vaterite was carried out by comparison of literature data with their XRD patterns.¹⁸ Assuming that the specimen is a uniform mixture of two components and micro-absorption effects are neglected, it can be shown as equation (1)¹⁹

$$\frac{I_A}{I_B} = \Lambda \times \frac{X_A}{X_B}, \quad (1)$$

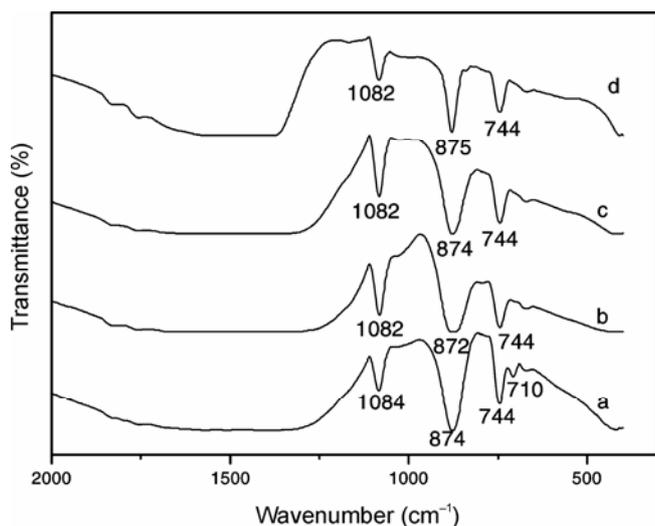


Figure 2. FT-IR spectra of CaCO₃ crystal obtained from sucrose/BSA system, sucrose/BSA. (a) 5 wt%/0, (b) 5 wt%/1 × 10⁻⁶ mol L⁻¹, (c) 5 wt%/4 × 10⁻⁶ mol L⁻¹, (d) 20 wt%/0.

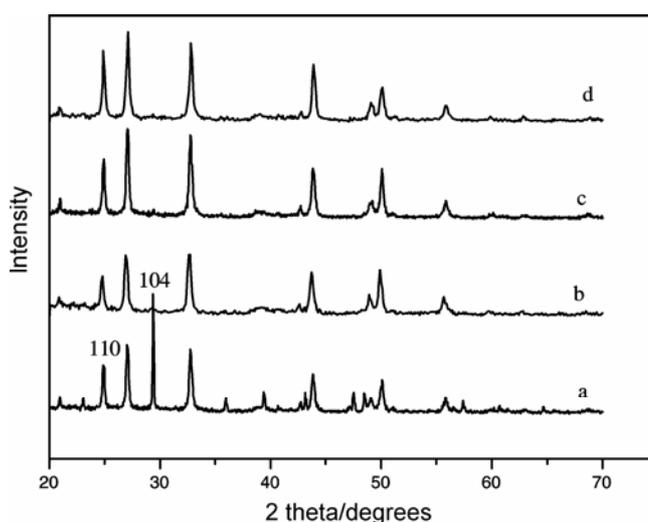


Figure 3. XRD patterns of CaCO₃ crystal obtained from sucrose/BSA system, sucrose/BSA. (a) 5 wt%/0, (b) 5 wt%/1 × 10⁻⁶ mol L⁻¹, (c) 5 wt%/4 × 10⁻⁶ mol L⁻¹, (d) 20 wt%/0.

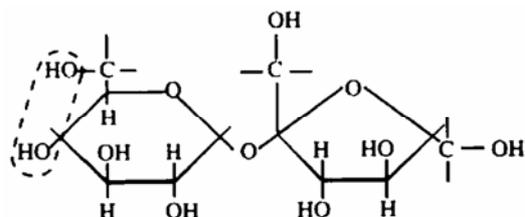
where Λ is a proportionality constant, which depends on the component, the diffraction line and the mass absorption coefficient of the species present. I_A/I_B represents the ratio of the intensities of two selected diffraction lines in a mixture of two substances, and X_A/X_B is the molar diffraction of the two substances. A plot of I_A/I_B should yield a straight line with an intercept of zero. The calibration line for calcite–vaterite mixture was constructed. For the vaterite–calcite mixture the equation for the calibration line was obtained by linear regression of the experimental data, it can be described by the following relationship (2):

$$\frac{I_C^{104}}{I_V^{110}} = 7.691 \times \frac{X_C}{X_V} \quad (2)$$

From figure 3a the intensity ratio $I_{(104)}/I_{(110)}$ were about 2.4, according to (2), we can calculate that the absolute contents of calcite as 23.5%.

3.2 The formation mechanism of CaCO_3 crystal in the sucrose/ bovine serum albumin system

The sucrose ring molecules (scheme 1) may be helpful to induce spherical calcium carbonate particles. With the addition of the BSA, protein–sucrose complexes were formed. At the same time, the process weakened the role of the sucrose; on the other hand, there was a strong coordination between BSA and Ca^{2+} . Ca^{2+} ions were attracted by oxygen atom of C=O bond and the oxygen of hydroxyl from all directions in the system. Therefore the spherical morphology of CaCO_3 could exist stably. With steric hindrance of the ring sucrose structure, more and more Ca^{2+} became free which made the formation of calcium carbonate toward the easy and stable direction. The special shape of calcium carbonate (figure 1c) might explain the possibility of the assumption. However, high concentrations of sucrose were not helpful to induce calcium carbonate particles with



Scheme 1. Structure of sucrose.

regular morphology. The sucrose's viscosity might have limited the movement of Ca^{2+} in the system. The spherical calcium carbonate particles (figure 1d) would have disappeared when the concentration of sucrose was up to a certain amount.

During the formation of calcium carbonate, the nucleation and growth of the crystals might be affected by the sucrose/bovine serum albumin through electrostatic matching, structural and interfacial molecular recognition. The stereo-structure of the crystal nucleus was affected by this interfacial molecular recognition. And the activation energy of nucleation (ΔG^*) was decreased by interfacial molecular recognition. With the decrease of ΔG^* , the vaterite has more easy formed and existed under this condition.²⁰

4. Conclusions

In our investigation, an easy route nucleation model is employed in the nucleation of CaCO_3 under the influence of sucrose/bovine serum albumin. The experimental results indicated that sucrose and sucrose/bovine serum albumin could direct the nucleation and crystallization of CaCO_3 . Sucrose tended to induce vaterite and calcite formation in the low concentration of sucrose, while the addition of bovine serum albumin led to the stable formation of vaterite. This research may provide new insights into the control of morphologies of CaCO_3 and the controllable synthesis of novel inorganic materials.

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