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Effect of the self-assembly of collagen on crystallisation of calcium carbonate in aqueous solution

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Effect of the self-assembly of collagen on crystallisation of calcium carbonate in aqueous solution

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In order to investigate how the self-assembly of organic matrix influences crystallisation and growth of inorganic minerals, we selected collagen as the matrix and conducted three experiments of crystallisation of $CaCO_3$ in different reaction systems: H_2O system, as-assembled collagen fibrils system and self-assembling of collagen system. It is found that (i) the self-assembly process of organic matrix had a remarkable effect on the morphology of inorganic minerals: $CaCO_3$ crystals formed in the as-assembled collagen fibrils system were global clusters and those formed in the self-assembling of collagen system appeared as interlaced networks and (ii) the organic matrix decided the polymorph of crystals: $CaCO_3$ crystals were calcite in the H_2O system and appeared vaterite in the collagen system. From this study, we can conclude that the self-assembly of collagen fibrils greatly affect the crystallisation and growth of $CaCO_3$. Such results are significant in understanding the mechanism of biomineralisation in calcified tissues in general, and useful in the synthesis of biominerals.

Keywords: collagen fibrils; self-assembly; crystallisation; $CaCO_3$

Introduction

To this day, more than 60 different inorganic minerals in biological organisms are known. Some of them form mineralised tissues with extremely sophisticated shapes and highly organised microstructures (*1*, *2*). From the studies of those minerals, it is found that crystal morphology, size, polymorph and orientation are affected by certain environmental conditions, in particular, the presence of a matrix (*3*–*5*). Therefore, some researchers have been intensively interested in the interactions between minerals and the matrix. Mann (*6*) proposed that the crystallisation of biominerals was a process similar to casting an inorganic replica in a static organic mould. Chang et al. (*7*) found that the self-assembled amelogenin protein microribbon structures controlled the oriented growth of apatite crystals during enamel mineralisation. However, the real biological organisms are sophisticated. The formation of inorganic minerals and the assembly of matrix are not two absolute independent processes (*8*). Generally, biomineralisation is concomitant with the self-assembly of matrix, and the latter controls the crystallisation tendency of the former. We studied the effect of self-assembling of sodium acrylate on the crystallisation of $CaCO_3$ (*9*), indicating that the self-assembly of sodium acrylate affected the shape of $CaCO_3$.

Experiment

In the present work, we studied the effect of self-assembling of collagen on the crystallisation of $CaCO_3$, which is more significant in understanding the mechanism of biomineralisation. $CaCO_3$, one of the most important building materials in biomineralising systems such as alga, trilobites, fish, mollusca, sea squirt, etc. makes up an attractive model mineral for studies in the laboratory (*10*, *11*). Collagen is the most important and abundant structural protein for the extracellular matrix (*12*). Generally, there are several different types of collagen. We selected type I collagen as the matrix. Type I collagen monomer is stable below 4°C and $pH \leq 2$. When the temperature is 30°C and the pH value also reaches 5, the network fibrils can be formed by the self-assembly of the collagen monomer (*13*).

A set of equipments were specially designed. During the reaction process, the pH of the reaction solution (collagen + $CaCl_2$) was gradually increased by slow diffusion of NH_3 from the NH_4HCO_3 solution, as well as providing CO_3^{2-} for the reaction solution. The increase in the pH value induced the self-assembly of collagen monomer to network fibrils. The process of the self-assembly of collagen and crystallisation of calcium carbonate took place simultaneously.

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In a typical synthesis, three sets of experiments were carried out at 30°C in isolated environmental chambers. The first set was the experiment with the self-assembly of collagen monomer. The chamber contained two beakers – one with 0.25 M NH_4HCO_3 solution and the other with a solution of pure collagen monomer (1.0 mg/ml). The second set was the control experiment, i.e. crystallisation of calcium carbonate in H_2O and as-assembled fibrils systems, respectively. The chamber contained three beakers – one with 0.25 M NH_4HCO_3 solution, another with $CaCl_2$ solution ($[CaCl_2] = 1.0$ M, pH = 2) and the third one with $CaCl_2$ solution and as-assembled fibrils (1.0 M $CaCl_2$, 1.0 mg/ml collagen monomer). The third set was the master experiment with crystallisation of calcium carbonate during the process of self-assembling collagen monomer. The chamber contained two beakers – one with the 0.25 M NH_4HCO_3 solution and the other with a solution of $CaCl_2$ and collagen monomer (1.0 M $CaCl_2$, 1.0 mg/ml collagen monomer, pH = 2).

In order to comprehensively understand the self-assembly process of collagen monomer and the influence of the self-assembly collagen monomer to crystallisation of calcium carbonate, a set of time-series experiments in the third set were run (12, 24 and 72 h after sealing the system), respectively. The reaction solutions were withdrawn for transmission electron microscopy (TEM) observation, and the obtained solid products were characterised by powder X-ray diffraction (XRD) on a Rigaku Dmax-2000 diffractometer with Cu Kα radiation.

Discussion

To investigate the conditions under which collagen monomers self-assemble into fibrils, the TEM images of samples obtained at different reaction time of 12 h (pH 4.22) and 72 h (pH 6.78), are presented in Figure 1(A) and (B), respectively. Figure 1(A) shows that there is a 1D strand-like structure, in agreement with the original observation of TEM images of collagen monomer, indicating that the self--assembly process still did not happen when the pH value was 4.22. Figure 1(B) shows an infinite 3D network structure of collagen fibrils, indicating that network fibrils had been formed by the self-assembly of collagen monomer by increasing the pH value to 6.78 through diffusion of NH_3, when the self-assembly of collagen monomer proceeded for 72 h.

To contrast the differences of crystallisation of $CaCO_3$ in the pure H_2O and as-assembled fibrils systems, the TEM images and XRD patterns of samples were observed and are shown in Figure 2. The morphology of $CaCO_3$ (Figure 2(A)) formed in the pure H_2O system appeared a typical rhombohedral, and its XRD pattern (Figure 2(B)) proved that the crystal was calcite. The morphology of $CaCO_3$ formed in the as-assembled fibrils solution system (Figure 2(C)) were global clusters with a broader range in size, and its XRD pattern (Figure 2(D)) proved that the crystals were pure vaterite. It was obvious that the polymorph and morphology of calcium carbonate were relevant to the presence of a matrix.

Comparing Figure 2(C) with Figure 1(B), it is found that morphologies of vaterite $CaCO_3$ are spherical (Figure 2(C)), which are similar to the vacancies in the collagen fibrils structure (Figure 1(B)). It could be inferred that organic matrix as a scaffold-like template greatly influences the morphologies of $CaCO_3$.

In order to investigate the interaction between the self-assembly process of collagen monomer and crystallisation of $CaCO_3$, TEM analyses were performed after proceeding for different periods. Figure 3(A)–(C) displays the typical morphology of the samples for 12 h (pH 4.42), 24 h (pH 6.87) and 72 h (pH 6.78), respectively. After proceeding for 12 h (pH 4.42), the TEM image (Figure 3(A)) shows that the aggregation of crystals along the 1D direction was in good agreement with the structure of collagen monomer (Figure 1(A)), which could be attributed to the strand-like structure of monomer regulating the crystal growth. After 24 h, the one-dimensional arrays of the vaterite crystals had began to

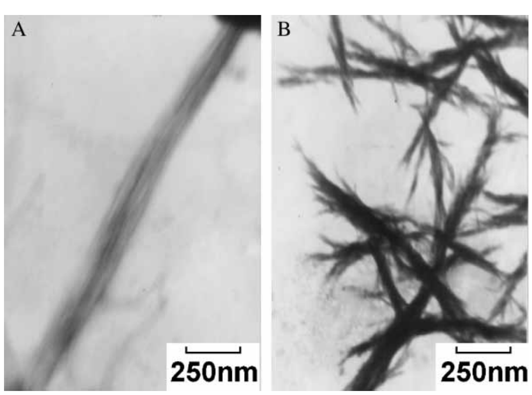

Figure 1. TEM images of the self-assembly of pure collagen monomer to fibrils at different time: (A) 12 h and (B) 72 h, respectively.

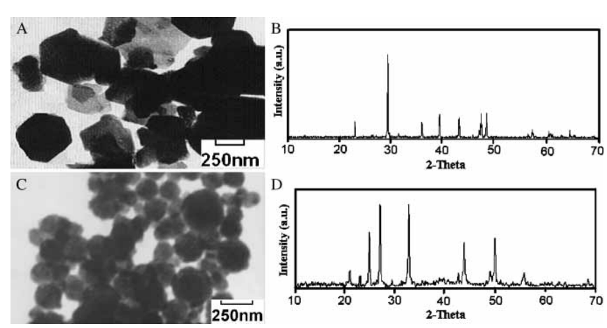

Figure 2. (A) TEM image and (B) XRD pattern of the crystals formed in the pure H_2O system; (C) TEM image and (D) XRD pattern of the crystals formed in as-assembled fibrils system.

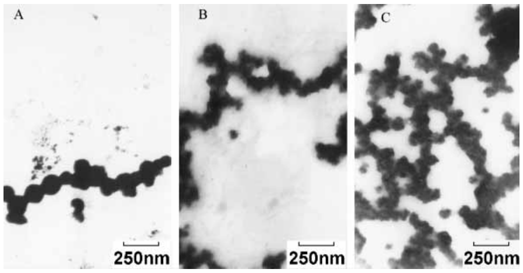

Figure 3. TEM images of crystals of calcium carbonate in the self-assembling of collagen monomer at different time: (A) 12 h, (B) 24 h and (C) 72 h, respectively.

intercross (Figure 3(B)), responding to the cross-linked fibrils. If the reaction proceeds to 72 h (pH 6.78), the morphology of crystals completely respond to the network structure of fibrils. Meanwhile, the result of its XRD pattern indicated that the crystals were vaterite.

In summary, in this work, the crystallisations of $CaCO_3$ were investigated in the pure H_2O system, the as-assembled collagen fibrils system and the process of self-assembling of collagen monomer, respectively. It is found that the process of assembly of organic matrix, collagen monomer, controlled the morphology of inorganic minerals $CaCO_3$, and the organic matrix influences the polymorph produced. Such experimental results are significant to understand the mechanism of biomineralisation and provide an illuminating synthetic approach to inorganic minerals with the complex form.

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