Biomimetic Synthesis of Calcium Carbonate with the Assistance of Amino Acids

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Abstract. Two acidic amino acids (aspartic acid, glutamic acid) and four neutral amino acids (cysteine, serine, tyrosine, glycine) were used as inducers for calcium carbonate biomimetic synthesis. The results showed that aspartic acid introduced is more effective for the growth of aragonite, while glutamic acid used is more effective for the growth of vaterite. Compared to those acidic amino acids, neutral amino acids used lead to less aragonite or vaterite produced, and tyrosine and serine show stronger effects on the growth of aragonite and vaterite than cysteine and glycine. On the other hands, it was found that the increased concentration of amino acid shows positive effects on the growth of aragonite and vaterite. In addition, the enhanced concentration of calcium chloride results in the increased crystalline volume of calcite significantly, as well as the more orderly structure as shown in scanning electron microscopy investigation. Meanwhile, the high calcium chloride concentration indicates weak inhibitory effect on aragonite growth but no significant influence on the growth of vaterite.

1. Introduction

In the process of pearl mineralization, organic matter is one of the important factors that regulate the growth and nucleation of inorganic minerals[1]. Studies have shown that the organic matters secreted by cells include chitin proteins and some biomacromolecules[2], which have the functions of regulating the directional nucleation growth and provide a template for their synthesis to form nano-structures of special assembly methods[3]. Feng's Research Group extracted the water-soluble matrix (WSM), acid-soluble matrix (ASM) and acid-insoluble matrix (AIM)[4] from the pearl to carry out in vitro mineralization simulation experiment. WSM has an effect on crystal morphology and size[5]. WSM can induce aragonite in solution with different pH values, and the pH value has a significant effect on the morphology of calcite. WSM at different solution temperature has a small effect on the growth of calcium carbonate crystals, while has a significant effect on the morphology of calcium carbonate[6]. It was also found that WSM could control grains which smaller than ASM, and some organic substrates could induce the production of certain crystals[7]. Picker[8] found that specific amino acids have multiple effects on the early stage of CaCO₃ crystallization. Chuajiw[9] showed that the interaction between COO⁻ groups and Ca²⁺ cations in CaCO₃/amino acid precipitation samples is polarity or hydrophilic interaction.

Qiad[10,11] proved that acid-soluble organic matters could induce the morphology and crystal type of calcium carbonate mineralized crystals. Yang[12-15] found that aspartic and glutamic could induce the formation of calcium carbonate into vaterite crystals from studying the influence of acid amino acids on the crystal structure and morphology of calcium carbonate. Chen[16] found that the negative charge...
of amino acids after pH adjustment could attract Ca\(^{2+}\) ions, and the change of amino acid concentration would affect the number and size of crystals. Chen\[^{17}\] found that the products induced by L-phenylalanine were totally calcite, while L-phenylalanine inhibited the growth of calcite. Guo\[^{18}\] studied the regulation of L-valine, L-arginine and L-serine on CaCO\(_3\) morphology. Wang\[^{19}\] found that the concentration and temperature of L-arginine both affect the growth of calcium carbonate crystals. Xiao\[^{20}\] synthesized acicular nanometer calcium carbonate crystals using L-histidine as an organic template. Wu\[^{21}\], Ren\[^{22}\], and Liu\[^{23}\] studied the synthesis of calcium carbonate induced by L-lysine.

In our research group, we already have relevant research foundation. Yang\[^{24}\], Wu\[^{25,26}\] discovered the effect of organic matter on pearl growth, and studied the induction effect of amino acids on calcium carbonate at different deposition times. Tang\[^{27}\] studied the induction of calcium carbonate crystal morphology by different amino acid systems: various amino acids can induce the formation of calcite, but alanine, leucine, tyrosine and glutamic acid cannot induce the formation of aragonite. Under the conditions of histidine, isoleucine, valine, methionine and proline, more aragonite was found, and the interaction structure of aragonite layer and calcite was obtained under the action of mixed amino acids. Ou-yang\[^{28}\] found that different reaction temperatures and amino acid contents can regulate the morphology and composition of calcium carbonate based on the research of Tang\[^{27}\]. It is easier to prepare the nacre layer when the same volume of amino acids is mixed and the temperature is higher. This study is based on the changes in calcium chloride and amino acid concentrations that were not involved in previous studies, to study the induction effect of acidic amino acid and neutral amino acid on calcium carbonate and the influence of different calcium chloride and amino acid concentrations on the mineralization of calcium carbonate.

2. Experimental Section

2.1. Amino Acid Selection

In this experiment, two acidic amino acids and four neutral amino acids were selected, among which the acidic amino acids were aspartic and glutamic, and the neutral amino acids were glycine, tyrosine, serine and cysteine.

2.2. Experimental Reagents and Instruments

Anhydrous calcium chloride (AR), ammonia carbonate (AR) L-aspartic, L-glutamate, L-glycine, L-cysteine, L-serine, L-tyrosine (BR)

Fourier-transform infrared spectroscopy (perkin-elmer spectrum, USA, 100 resolution, better than 0.2 cm\(^{-1}\), wave number range 4000 ~ 400cm\(^{-1}\)), X-ray diffraction (Empyrean, copper target K ray, voltage 40KV, current 40 mA, emission slit 1/2°, anti-scattering slit 1°, anti-scattering slit 8.0 mm, 2\(\theta\):20°~60°, step length 0.02°, residence time 80s, scanning electron microscope (HITACHI S-4800, Japan).

2.3. Experimental Procedure

CaCl\(_2\) solution and amino acid solution at the concentration of 10 mmol/L and 20 mmol/L were respectively prepared. Group A was formed with the amino acid solution (10 mmol/L) and CaCl\(_2\) solution (20 mmol/L). Group B was formed with amino acid solution(10 mmol/L) and CaCl\(_2\) solution (10 mmol/L). Group C was formed with amino acid solution (20 mmol/L) and CaCl\(_2\) solution (10 mmol/L). Calcium carbonate mineralization was carried out under the closed condition for 30 days and the \((\text{NH}_4)_2\text{CO}_3\) was changed every 10 days during mineralization to ensure that the experiment was carried out under CO\(_2\) saturation.
3. Results and Discussion

3.1. FT-IR
In the infrared spectrogram (Figure 2), the calcium carbonate crystal of the blank group in group B was significantly absorbed at 1475 cm\(^{-1}\), 856 cm\(^{-1}\), and had a weak peak at 1083 cm\(^{-1}\), 710 cm\(^{-1}\). It was speculated that there were both aragonite and vaterite in the blank group. The absorption peak of aragonite induced by aspartic was obvious, but that of vaterite was weakened. The absorption peak of glutamate globular aragonite was obvious and that of calcite disappeared. The crystal characteristic peak of glycine and cysteine didn’t change obviously. The red shift of serine, tyrosine and aragonite absorption peak indicates that these two amino acids have certain induction effect, and the CO\(_3^{2-}\) generated is unstable.

![Figure 1. Diagram of experimental device.](image)

![Figure 2. Infrared spectrum corresponding to group A, B and C.](image)

A: 10 mmol/L of amino acid solution and 20 mmol/L of CaCl\(_2\) solution;  
B: 10 mmol/L of amino acid solution and 10 mmol/L of CaCl\(_2\) solution;  
C: 20 mmol/L of amino acid solution and 10 mmol/L of CaCl\(_2\) solution; 

The absorption peak of cysteine in the blank group disappeared and the characteristic peak of calcite appeared by comparing group A with group B. The absorption peak of globular aragonite in aspartic, glutamate, glycine, tyrosine and serine weakened and even disappeared, while the existing aragonite absorption peak appeared blue shift, indicating that the stability of aragonite was enhanced. The absorption peak corresponding to other functional groups has a certain movement, and it is presumed that the concentration of Ca\(^{2+}\) is increased, and the intermolecular forces such as hydrogen bonds are enhanced, so that the increase of the half-peak width causes the peak shift. At the same time, the absorption peak of group A in 3200~3500 cm\(^{-1}\) showed obvious weakening compared with group B. It may be that the increase of calcium chloride concentration made the mineralization of calcium carbonate more tight, and the space of -OH stretching and bending vibration are weakened.
The comparison between group C and group B showed that the intensity of characteristic peaks of each group increased after increasing the concentration of amino acids, indicating that the dipole moment in the crystal of group C changed greatly, that is, the crystal instability generated increased. The aspartic acid and glutamic acid in group C showed obvious absorption peaks of aragonite and calcite, the absorption peaks of glycine aragonite and vaterite increased, the absorption peak of serine and cysteine aragonite increased, while tyrosine was not obvious. It can be speculated that the increasing concentration of amino acids is conducive to the formation of aragonite and calcite. At the same time, the absorption peaks of other functional groups all show small fluctuations, which may be due to the amino acid groups, not involved in the reaction, affect the fluctuation of the absorption peak with the increasing concentration of amino acid.

3.2. XRD
In the XRD pattern (Figure 3), the blank group of group B has a strong peak of calcite at 29.8°. Calcite was induced by aspartic, serine and tyrosine. The crystals induced by cysteine and glutamate had strong peaks near 26.5° and weak peaks between 40°~50°, indicating the presence of a large amount of calcite and a small amount of aragonite mixed with vaterite. There was such a weak peak of glycine at 26.5°, and it is speculated that a small amount of aragonite exists.

The comparison between group A and group B showed that aragonite and vaterite absorption peak, induced by six kinds of amino acid, weakened and even disappeared while calcite absorption peak become obviously with the increasing concentration of calcium chloride. It can be speculated that increasing the concentration of calcium chloride is conducive to the formation of calcite, but reduces the induction effect of amino acids on aragonite and vaterite. The possible reason for this phenomenon is that there is a saturation ratio between calcium chloride and amino acid. When calcium chloride is supersaturated, some Ca$^{2+}$ occupying the amino acid template are removed, and Ca$^{2+}$ are still present. These Ca$^{2+}$ are free from the constraints of the template and form a crystal of calcite freely and rapidly with carbonate. The crystal has a spatial conformation with a coordination number of six, the lowest energy and stable structure.

After increasing the concentration of amino acid in group C, the XRD peaks were significantly changed, aspartic showed the absorption peak of aragonite, the absorption peak of glutamic was weakened, and the absorption peak of aragonite was enhanced. The absorption peak of aragonite appeared in serine, the absorption peak of vaterite appeared in tyrosine, and the three absorption peaks of cysteine were enhanced. The comparison of group B and group C can be found that glutamate has strong ability to induce aragonite, aspartic acid has strong ability to induce aragonite, glycine and cysteine have weak induction ability, tyrosine and serine have the weakest induction ability. After the concentration of amino acids increased, the ability of six amino acids to induce aragonite and vaterite was significantly enhanced.
A: 10 mmol/L of amino acid solution and 20 mmol/L of CaCl₂ solution;

B: 10 mmol/L of amino acid solution and 10 mmol/L of CaCl₂ solution;

C: 20 mmol/L of amino acid solution and 10 mmol/L of CaCl₂ solution;

Figure 3. XRD pattern corresponding to group A, B and C

1: Blank; 2: L-aspartic; 3: L-glutamic; 4: L-glycine; 5: L-cysteine; 6: L-serine; 7: L-tyrosine

3.3. SEM
In group B, a large number of regularly structural hexagonal and quadrilateral calcites (2~3 μm) were observed in the blank group, but the arrangement was disordered. After the addition of amino acids, the crystals induced by glutamate and aspartic acid were observed to be scale-like, closely arranged and in the same direction, mostly pentagonal and quadrilateral aragonite (1~2 μm), and there was
vaterite in glutamate. The crystals induced by serine became smaller and disordered, and there were vaterite particles (100–200 nm) and quadrilateral aragonite (1 μm). Cysteine induces the synthesis of aligned aragonite (100 nm) and a small amount of calcite (2 μm). Glycine induced flaky calcite (2–3 μm) with large size and different shapes. There was a small amount of columnar aragonite in the calcite gap, and the overall arrangement of the calcite was orderly compared with the blank group. Tyrosine crystal induced by separate, mineralization in the same direction, there is irregular calcite needle with small particles aragonite and small vaterite.. By comparing the six amino acids with the blank group, it can be found that glutamate crystals induced by aspartic are ordered and regular in shape, and the aspartic acid induces the best effect of aragonite, and the glutamic acid induces the the best effect of vaterite, followed by tyrosine and cysteine, and serine and glycine are the weakest.

With the increasing of calcium chloride concentration in group A, it was observed that the amount of calcite in blank group A1 increased, the crystals were more regular, and the arrangement was tight. The number of A7 calcite quantity increased, but there were still obvious spherolite and a small amount of aragonite. A3 calcite has irregular shape, increased size and more regular in arrangement than B3. A4 has a large amount of calcite arranged in a scale-like order and a small amount of vaterite. The calcite of A5 was obviously increased by twice as much as that of B5, and aragonite was found in disordered arrangement. A6 calcite is highly ordered in arrangement and its size is twice as large as that of B6. The presence of acicular aragonite indicates that the increasing concentration of calcium chloride leads to the increase in size, quantity and order of calcite, which is conducive to the mineralization and crystallization of calcite, but has little effect on aragonite and vaterite.

After increasing the concentration of amino acid in group C, the comparison with group B showed that the size of group C was significantly reduced and the structure was more compact and neat. In group C2, there were a large number of acicular clusters and particle aggregation of nano-aragonite, and the number of C7 vaterite was significantly increased. C3 crystals become smaller and arranged in order. C4 calcite becomes larger, and spherolite occurs at the same time. Vertical aragonite appeared in C5 and C6, with a small amount of spherolite. Therefore, it can be concluded that the size of the crystals induced by the increase of the concentration of amino acids is smaller and more orderly, which is conducive to the production of aragonite and vaterite.
A: 10 mmol/L of amino acid solution and 20 mmol/L of CaCl₂ solution;

B: 10 mmol/L of amino acid solution and 10 mmol/L of CaCl₂ solution;

C: 20 mmol/L of amino acid solution and 10 mmol/L of CaCl₂ solution;

Figure 4. SEM images corresponding to group A, B and C

1: Blank; 2: L-aspartic; 3: L-glutamic; 4: L-glycine; 5: L-cysteine; 6: L-serine; 7: L-tyrosine

4. Summary
Based on the analysis of FT-IR, XRD and SEM, there are three main conclusions. Firstly, aragonite and vaterite can be induced by adding acid amino acid during the mineralization of calcium carbonate. And aspartic acid introduced is more effective for the growth of aragonite, while glutamic acid used is more effective for the growth of vaterite. Compared to those acidic amino acids, the neutral amino acids...
used lead to less aragonite or vaterite produced. The induced effects of four neutral amino acids from strong to weak were tyrosine, serine, cysteine and glycine. Secondly, the increased amino acid concentration leads to more aragonite and vaterite produced under the experimental conditions. Last but not least, it is found that the calcium chloride concentration investigated is highly positively correlated with calcite growth, but negatively correlated with aragonite growth. As for vaterite growth, there is no significant influence when calcium chloride concentration was changed under the experimental investigation.

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