Multiradiate calcium phosphate patterns derived from a grading polysaccharide-acidic protein system†‡

Yi Shi,* Yangh Zhang,** Wuli Yang* and Yi Tang*

Received (in Cambridge, UK) 13th August 2008, Accepted 21st October 2008
First published as an Advance Article on the web 19th November 2008
DOI: 10.1039/b814095b

A series of multiradiate calcium phosphate patterns have been observed by a grading chitosan-polyaspartic acid (PAsp) system, and their morphology evolution reveals the effect of chitosan and PAsp on the interfacial biomineralization.

After thousands of million years’ natural selection, the morphology and structure of living organism have evolved into exquisite perfection.1 Bioinorganic minerals, as the main part of these biological hierarchies, undoubtedly take a pivotal role in the life activity.2 For example, it can work as skeleton (bone), as protection (mollusc shell), as tool (teeth), or as gravity sensor (statoliths).3,4 Generally, the two-component system with insoluble matrix of polysaccharides–hydrophobic proteins and soluble acidic proteins is believed to be of particular importance in formation and biofunctions of these minerals.5,6 The insoluble matrix such as collagen, cellulose, chitin, and chitosan are interfacially active in the organic-matrix mediated biomineralization.1,7 As an anchor, the soluble acidic proteins rich in aspartic, glutamic, and serine acid residues involve in nucleation and growth of biominerals via chelating inorganic metal ions.8 Although the interfacial biomineralization has drawn much attention due to its generalization in heterogeneous nucleation, preferential growth, pathological calcification, and repair substitution,9–12 it is still a long way to reveal the definite mechanism of their crystallization and evolution,13 especially for calcium phosphate.

Herein, a series of multiradiate crystals of calcium phosphate have been prepared firstly on the surface of chitosan film (as insoluble matrix, Fig. S1A†) in association with polyaspartic acids which are believed to be able to well simulate the polysaccharide–acidic protein system in biology. The polyaspartic acids (~10 KDa) with different ratios of hydroxyl to carboxyl (donated as PAsp-x%OH, Fig. S1B†) are employed as acidic proteins. Owing to its serial changing charge density with x value, PAsp-x%OH is expected to declare the effect of charge density on the evolution of biominalerization. Acidic PAsp-x%OH can adsorb on the surface of the positively-charged chitosan matrix, and calcium ions can bind with the adsorbed PAsp-x%OH to construct a local high concentration of the calcium ion, inducing the nucleation of calcium phosphate on the surface of chitosan matrix (Scheme S1†).14–20 Notably, the combination and interaction among chitosan matrix, PAsp-x%OH and calcium ions can be precisely regulated through changing the carboxyl content in PAsp-x%OH, and also the deposition process of biominerals.

Experimental details are given in supplementary information.† Briefly, the chitosan-coated glass substrates were prepared by dip coating method and then were transferred into the solution of PAsp-x%OH and calcium acetate. Afterwards, the sodium dihydrogen phosphate solution was added and the resulting mixture was kept at 18 °C for a period from a few minutes to several hours. Finally, the substrates were taken out, washed with deionized water and dried at room temperature.

Fig. 1 shows scanning electron microscopy (SEM) images of all the products obtained for a reaction of 4 h. Their XRD patterns are indexed to typical brushite (CaHPO₄·2H₂O, DCPD, data not shown). It is obvious that a series of multiradiate calcium phosphate patterns were formed on the surface of substrates. However, with the increase of x value in PAsp-x%OH from 0 to 100%, and even in the absence of PAsp, the radial chines of these multiradiate structures gradually transfer from “ice crystal-like” 2-dimensional minerals elongating along on the surface of chitosan matrix to “urchin-like” 3-dimensional crystals. More detailed, the chines extended along the substrate are undergoing from thin to wide and from slight lean to complete vertical on chitosan matrix with increasing x of PAsp-x%OH from 0 to 50% (insets of Fig. 1A–E). Furthermore, the Ca/P ratios of the products also decrease from 1.44 to 1.06 with the increase of x from 0 to 50 (Fig. S2†). The evolutions of the product morphology and composition imply that the interaction between chitosan and PAsp-x%OH changes with changing the x of PAsp-x%OH, and so modulate their interaction with calcium ions as well as the crystallization behaviour of the biominerals.

The formation process of the multiradiate patterns can be deduced from the morphology transition in the initial reaction stage (0–20 min, Fig. S3†). When the x value in PAsp-x%OH is low enough, e.g., 15% as shown in Fig. 2, a ‘seed’ appear firstly on the chitosan matrix to supply a mineralized centre for the growth of mineral. Then many small chines emerge radially on the substrate, and gradually branch off and extend along the surface of the chitosan matrix, and finally form the...
crystals with 2-dimensional radial morphology. When the hydroxyl content in PAsp-x%OH become higher, such as x% = 100% (Fig. S4w), the DCPD slices begin to precipitate, and then gradually evolve into 3-dimensional radial crystals on the chitosan matrix. Obviously, the chitosan matrix provides a platform for the interfacial biomineralization of the multiradiate patterns.14,17 Furthermore, when the chitosan matrix is absent, the product displays a very different morphology (Fig. 3A, B and C) even if the same PAsp-x%OH is employed in the reaction system. Also, when neither chitosan nor PAsp is involved in the reaction solution, only typical DCPD slices (Fig. 3D) can be obtained. It is worth recalling that the radiate patterns rather than brushite slices are formed on the chitosan matrix even in the absence of PAsp (Fig. 1H). Obviously, the chitosan matrix templates the radial crystallization of calcium phosphate on the interface. Chitosan can enrich inorganic ions on the surface by its functional groups to induce interfacial biomineralization even if the PAsp-x%OH is absent.17 This also means the insoluble polysaccharide matrix plays an important role in interfacial biomineralization.18–20 However, the insoluble matrix (chitosan) does not solely dominate this process, the soluble proteins (PAsp-x%OH) can interact with calcium ions,21 and facilitate their deposition on the surface of chitosan, and so precisely regulates the morphology evolution of the products.

The interaction among chitosan matrix, calcium ions and PAsp-x%OH changes with the variation of hydroxyl content in the PAsp-x%OH. Therefore, according to the evolution process of multiradiate calcium phosphate patterns, the effect of PAsp-x%OH on the morphology evolution of calcium phosphate patterns could be summed up to follow its interaction with chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4). When the hydroxyl content is low, there is a strong interaction between negative-charged PAsp-x%OH (x < 50) and positive-charged chitosan matrix and calcium ions (Fig. 4).

This journal is © The Royal Society of Chemistry 2009


Fig. 1  SEM images of the as-synthesized calcium phosphate materials for a reaction of 4 h in the presence of (A) PAsp-0%OH, (B) PAsp-8%OH, (C) PAsp-15%OH, (D) PAsp-30%OH, (E) PAsp-50%OH, (F) PAsp-80%OH, (G) PAsp-100%OH and (H) in the absence of PAsp-x%OH. Scale bars of A–H are 200 μm and those of the insets are 20 μm.

Fig. 2  Morphology transformation of samples within the initial reaction time of (A) 5 min, (B) 10 min, (C) 15 min, (D) 20 min in presence of PAsp-15%OH.

Fig. 3  SEM images of samples for the reaction of 4 h in presence of PAsp-0 (A), 80 (B), 100 (C) %OH without chitosan and typical brushite slices (D). Scale bars A: 5 μm B, C, D: 50 μm.
decreased, and the nucleation site on the surface of chitosan is sharply reduced. The biomineralization of calcium phosphate thereby becomes more and more independent on the surface of chitosan. However, the radial morphology still is retained due to the confining effect of chitosan matrix for calcium ions via PAsp-\(x\)\%OH or its functional group, which is very different from that occurred in the solution.

In summary, we have successfully prepared diversified multiradiate calcium phosphate patterns firstly via a system of polysaccharide–acidic protein. The insoluble chitosan matrix confines the biomineralization to occur on interface with the aid of PAsp-\(x\)\%OH, and induces the multiradiate morphology of minerals; the morphology evolution of various multiradiate calcium phosphate patterns is derived from the participation of the soluble PAsp-\(x\)\%OH with different \(x\) values. These results could offer some views to mechanism of interfacial biomineralization. Further insight towards detailed mechanism of crystal orientation is under investigation.

Notes and references