Dalton Transactions

Accepted Manuscript

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Structural Roles of Amphiphilic Peptide Tails on Silica Biomineralization

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ABSTRACT: De novo synthesized amphiphilic peptides are easy to be designed to form various nanostructures. Natural biomineralization creates the most intricately stunning inorganic structures, such as diatoms and shells, in which peptide plays an important role. Here, we present the biomineralization of three designed amphiphilic peptides which have different types of hydrophobic tails. By changing the hydrophobic tails from phenylalanine-serine tail to alkyl-serine tail and serine-only tail, the conformations of peptides varied from the type II β-turn to α-helix and random coil, which gave rise to the silica biomineralization nanostructures with nanoribbons to nanofibers and hollow nanospheres, respectively. Figuring out the structural roles of hydrophobic tails of amphiphilic peptides can improve strategies toward the bottom-up synthesis of nanomaterials as well as peptide scaffold engineering.

In nature, the fundamental building units of biological system are peptides, which have the largest number of distinct interactions, such as hydrogen bonds, electrostatic interaction and hydrophobic interaction[1]. These interactions carry out internally or with other molecules, and hence form the greatest variety of functional nanostructures. To generate the self-assembled nanostructures that inspired from nature, amphiphilic peptides are widely adopted because they are easy to be designed and synthesized. A typical amphiphilic peptide molecule can be designed to consist of two regions: a structural and functional hydrophilic peptide sequence and a hydrophobic tail conjugated to the hydrophilic sequence by an amide bond. The hydrophobic tails are commonly alkyl chains, or sometimes, polypeptides and polymers [2]. In general, the aggregation of amphiphilic peptides can be induced in aqueous solution, and thus self-assembled into various nanostructures. Up to now, a variety of amphiphilic peptides have been designed and self-assembled into various morphologies such as monolayers[3], nanofibers[4], nanobelts[5], spherical nanoparticles[6], and bilayer membranes[7].

The biomimetic synthesis of inorganic biomaterials is currently an active research field in material science[8], and amphiphilic peptides are an attractive class of self-assembling systems for biomimetic mineralization due to their amphiphilic nature and the ease of synthesis. Proteins are able to template biologically generated silica, such as orthosilicic acid present in ocean water[9]. Therefore, numerous strategies have been developed for the sol-gel condensation of silica using amphiphilic peptides as template[4a, 10]. However, such silica biomineralization processes were mostly conducted by positively charged peptides, whereas proteins always have different surface charges when they function as enzymes or membrane channels. This limits the understanding of the structural roles of amphiphilic peptide tails.

Herein, we present a biomimetic synthesis system using three designed amphiphilic peptides (Figure 1). These peptides were designed consisting two identical parts, a hydrophilic head group of glutamic acid, which would be negatively charged in the natural pH circumstance (6.0-8.0) because of the pK_a=2.10 and 4.07 of the carboxylic acid group, and a less hydrophilic peptide sequence consisted by five serine residues. A different hydrophobic tail (phenylalanine of P1 and palmitic acid chain of P2) that conjugated to the hydrophilic part was designed to investigate its influence on silica biomineralization. Another peptide with the same hydrophilic head group, but without hydrophobic tail (P3) was designed as a reference. In this case, the serine sequence can act as the hydrophobic tail because of its lower hydrophilicity compared with charged glutamic acid. The silica biomineralization was developed through a co-structure directing agent (CSDA) route[11], in which trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride (TMAPS) was used as CSDA to induce the proper interaction between negatively charged peptides and silica source (tetraethoxysilane, TEOS). The positively charged quaternary ammonium group of TMAPS interacts with the negatively charged head groups of the peptides through electrostatic interaction, and the silane site of TMAPS is co-condensed with TEOS for subsequent assembly of the silica framework. Thus, the peptides and silicate species were bridged to favor their self-organization into ordered assemblies. After hydrothermal treatment peptide-templated silica (PTS) materials with different nanostructures were formed (see the Supporting Information for further details).

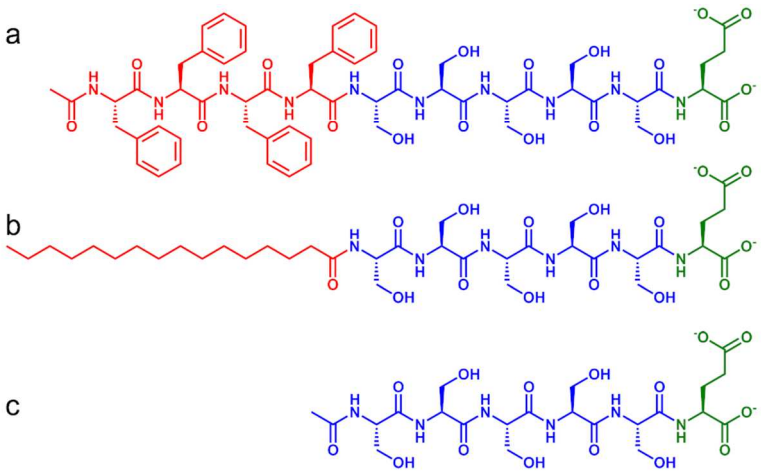

Figure 1. Structural formula of P1: Ac-FFFFSSSSSE-COOH (a), P2: C_{16}-SSSSSE-COOH (b) and P3: Ac-SSSSSE-COOH. Ac: acetyl group; F: phenylalanine; S: serine; E: glutamic acid.

The scanning electron microscope (SEM) image of PTS1 synthesized with P1 presented a twisted nanoribbon morphology with the width of 20-25 nm and the length of 2-10 μm. (Figure $2a_1$; see also the Supporting Information Figure S3a for a low-magnification image). The transmission electron microscopy (TEM) images (Figure $2a_2$) confirmed that these nanoribbons had a twisted morphology (indicated by arrows), and the nanoribbons were composed by a bilayer structure (inserted image in Figure $2a_2$). By changing the hydrophobic tails from phenylalanine sequence to alkyl chain, the nanostructure of PTS2 synthesized with P2 changed from twisted nanoribbons to twisted nanofibers (Figure $2b_1$; see also the Supporting Information Figure S3b for a low-magnification image) with ~180 nm in diameter and 1-4 μm in length. Between the two arrows in Figure $2b_1$, the nanofiber is twisted by 180°, which means that the distance between the two arrows is half of the pitch length of the nanofiber. The pitch length of the nanofibers was estimated as ~200 nm. Figure $2b_2$ revealed that these particles were twisted (indicated by arrows), and contained cylindrical pores along the long axis (inserted image in Figure $2b_2$). Combining Figures $2c_1$ and c_2, it was revealed that PTS3 synthesized with P3 had a

hollow nanosphere morphology with diameter of 0.8-2 μm. There were some small particles existed, but the hollow nanospheres were the dominate product.

Thermogravimetric analysis (TGA, Figure S4) showed that the peptide weight contents of PTS1, 2 and 3 were 48, 57, and 48%, respectively. After calcination under 650 ^{o}C, the chiral nanostructures of PTS2 was remained (Figure S5). However, the nanostructures of PCMS1 and 3 were collapsed because the peptides between silica layers were removed after calcination, and thus the silica layers loss the supports to keep their structures.

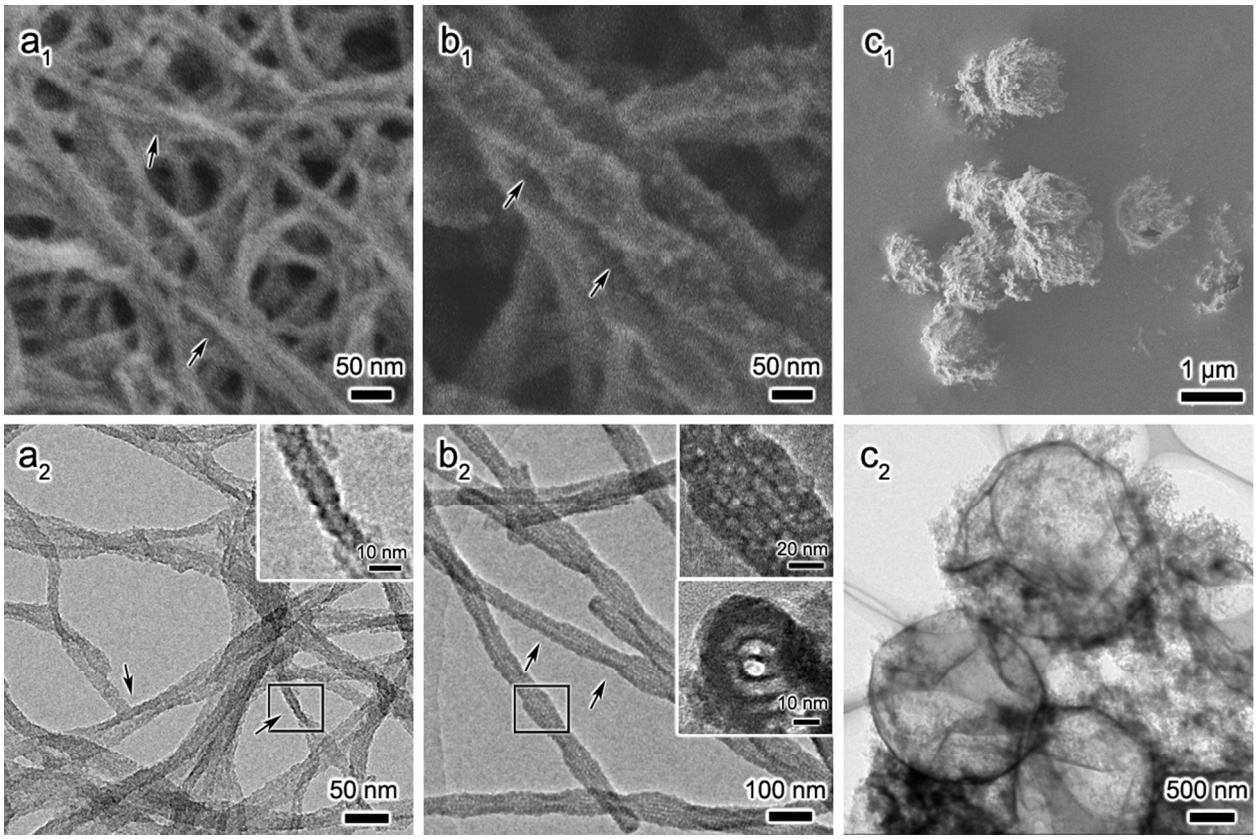

Figure 2. SEM and TEM images of PTS1 nanoribbons (a_1 and a_2), PTS2 nanofibers (b_1 and b_2) and PTS3 hollow nanospheres (c_1 and c_2).

Figure 3 presented the circular dichroism (CD) and UV/Vis (UV/Vis) spectra of the sol of P1-3 with TMAPS and TEOS. It showed that P1-3 had different conformations. The CD spectrum of

P1 (Figure 3a) exhibited two positive bands near 272 nm and 219 nm, and a negative band near 192 nm, which indicated the conformation of type II *β*-turn. The 272 nm band is due to the aromatic side chains of phenylalanine,[12] the 219 nm band can be assigned to the $n \rightarrow \pi^*$ transition, and the 192 nm band is attributable to the $\pi \rightarrow \pi^*$ transition. Unlike the α-helix, there is no unique CD signature of *β*-turns because of the range of conformers included in this structural category. The predominate CD spectrum of type II *β*-turn was predicted with a positive band near 210 nm and a negtive band near 190 nm[13]. Here, the positive band was red-shifted by ~9 nm due to the steric effects of the side chain of phenylalanine. The CD spectrum of P2 (Figure 3b) exhibited two negative bands near 216 nm and 200 nm, which can be assigned to the $n \rightarrow \pi^*$ transition and the $\pi \rightarrow \pi^*$ transition of the α-helix conformation, respectively. The characteristic CD spectrum of α-helix shows a negative band at ~222 nm of the $n \rightarrow \pi^*$ transition, a negative band at ~208 nm and a positive band at ~190 nm attributed to the exciton splitting of the $\pi \rightarrow \pi^*$ transition[14]. The CD spectrum of P2 was blue-shifted by ~6 nm compared to the characteristic one due to the less number of the amino acid residues that formed α-helix conformation. The CD spectra of α-helix consisted by short peptide sequences have been already studied, and the CD spectra blue-shifted with the decreasing of the number of amino acid residues[15]. Figure 3c presented a negative band near 197 nm, which is the characteristic CD spectrum of random coiled unordered conformation formed by the most small peptides[16].

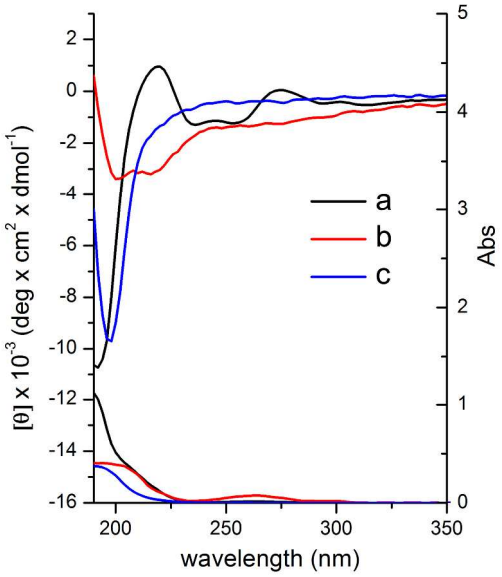

Figure 3. CD, UV/Vis spectra of P1-3 (a, b and c), showing the conformations of type II *β*-turn, α-helix and random coil, respectively.

A schematic illustration of the helical nanoribbon, nanofiber, and hollow nanosphere is presented in Figure 4. The different tails of the amphiphilic peptides resulted in the different conformations, and thus the different nanostructures after biomineralization. In the case of PTS1 (Figure 4a), because of the hydrophobicity and the steric effects of phenylalanine, P1 prefers type II *β*-turn conformation which is stablized through intermoluclar hydrogen bonds in the hydrophobic tail. Meanwhile, the hydrophilic region remains natural stretch in aqueous solution. Thus, P1 molcules have a small curvature that is sutiable for forming the bilayer structure (Figure 4a_2). Among the P1 molucles, intramoluclar hydrophobic interaction is the driving force for their self-assembly, and the twisty comes from the chiral nature of the peptide (Figure 4a_3). For PTS2 (Figure 4b), because the alkyl chains are unable to be hydrogen bond donators or acceptors to be involved in the secondary structure, P2 adopts the α-helix conformation in the hydrophilic serine region, and results in a larger volume of the hydrophilic head. Therefore, P2

has a relatively larger curvature compared to P1, and is favourable for self-organizing into rod-like assemblies and finally forms nanofibers (Figures 4b_1 and b_2). P3 was designed without hydrophobic tail, which differs from P1 and P2. Although serine is more hydrophobic than charged glutamic acid and it can act as the hydrophobic part in P3 when self-assembling, P3 is unfavorable to form the long range ordered assemblies due to the random coil conformation. Hence, PTS3 resulted in a hollow sphere structure (Figure 4c_1). P3 self-assembled into vesicles because of the greater extent of its total hydrophilicity (Figures 4c_2 and c_3).

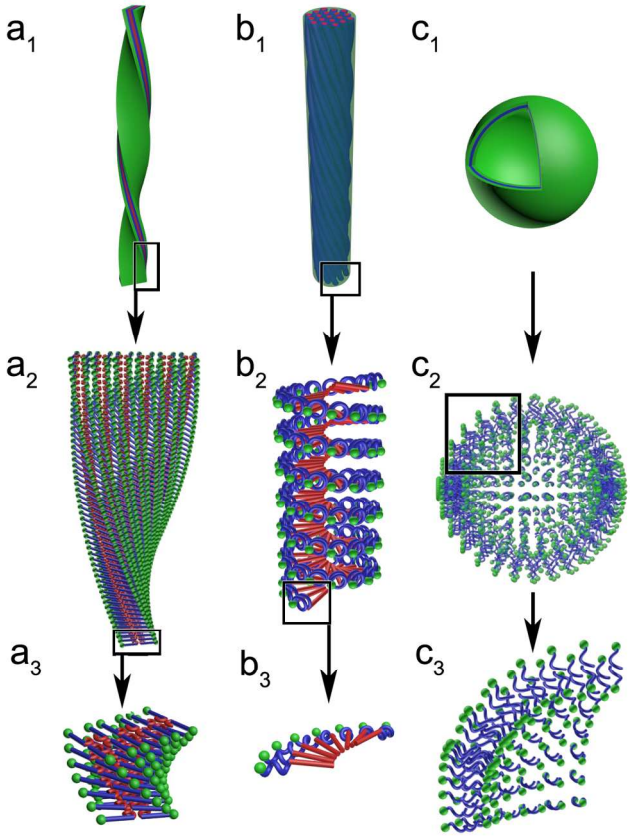

Figure 4. Schematic illustration of PTS1 nanoribbons (a_1-a_3), PTS2 nanofibers (b_1-b_3) and PTS3 hollow nanospheres (c_1-c_3) prepared from P1-3, respectively. PTS1 with a bilayer lamellar structure (a_1) was formed by the arrangement of P1 which has the type II β-turn conformation (a_2) through intermolecular hydrophobic interactions of the side chains of P1 (a_3). PTS2 with

cylinder pores (b_1) was formed by the arrangement of P2 which has the α-helix conformation (b_2) through hydrophobic interactions of the alkyl tails of P2 (b_3). PTS3 with a bilayer vesicle structure (c_1) was formed by the arrangement of P3 that is randomly coiled (c_2). The bilayer was formed by the hydrophobic interactions of the randomly coiled serine part (c_3).

In conclusion, PTSs with different nanostructures, including nanoribbons, nanofibers and hollow nanospheres were prepared by the biomineralization of amphiphilic peptides which have different hydrophobic tails. The amphiphilic peptides, with negative surface charge, co-assembled with TMAPS and TEOS through noncovalent interactions, including electrostatic interactions, hydrogen bonds and hydrophobic interactions, and formed different nanostructures. These results indicated that the hydrophobic tails of amphiphilic peptides play important roles on self-assembly and silica biomineralization. The resulted different nanostructures could improve the understanding of the mechanism of peptide self-assembly and help expand the strategies toward the bottom-up synthesis of nanomaterials.

ASSOCIATED CONTENT

Supporting Information.

Electronic supplementary information (ESI) available. See DOI: 10.1039/c000000x/

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ACKNOWLEDGMENTS

We acknowledge the support of the National Natural Science Foundation (Grant Nos. 21101105) of China and Evonik Industries. We wish to thank the Instrumental Analysis Centre of Shanghai Jiaotong University for their collaboration on the CD measurements.

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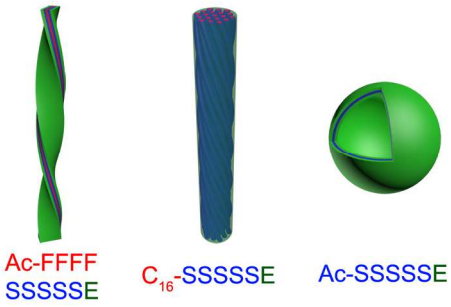

By changing the tails of amphiphilic peptides, the nanostructures after silica biomineralization were varied due to the conformation changes.