Facile Fabrication of Hierarchical Flower-Like BSA/Layered Double Hydroxide Hybrids

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This work presents the synthesis of hierarchical flower-like bovine serum albumin/layered double hydroxides (BSA/LDH) hybrids based on the assembly of biological proteins and LDH nanosheets. The BSA/alumina sols are first obtained by a sol-gel process, followed by self-assembling into spherical aggregates. These preformed hybrid sols are then used as biohybrid precursors and aluminum sources to fabricate the hierarchical architectures based on in situ growth of LDH nanoplatelets around biomolecules. The facile method is expected to be used for fabricating other hierarchical bioinorganic hybrids for potential application in the areas of biocatalysts and drug delivery.

Keywords: biomaterials, hierarchical structures, crystal growth, biomolecules, assembly

Introduction

Bioinorganic hybrid materials derived from biomolecules and nanomaterials are a new type of functional organic-inorganic hybrids that involve an organic counterpart of biological origin, for instance a biopolymer, assembled to a nanosized inorganic solid.[1] In recent years, intensive efforts have been made to synthesize bioinorganic hybrids with various compositions and functions, which can serve as bio-/chemosensors,[2] biocatalysts,[3] diagnostic agents,[4] and drug carriers.[5] Moreover, bioinorganic hybrids may exhibit not only the functionalities as complex as that of natural systems but also combined properties that arise from the disparate components or the interactions between the biological molecules and inorganic materials. Special interest has been devoted to fabricate bioinorganic hybrids due to their high composition flexibility and good biocompatibility.[6] In addition, new biological, physical, and chemical properties can be commonly observed from the synergy of their constituents, which are essentially different from those of the components taken separately or physically combined properties of each component.[6]

Layered double hydroxides (LDH) are one of the most useful classes of inorganic layered compounds that have attracted great attention in the last decades due to their potential applications in catalysts,[8] separations,[9,10] and optics.[11] Layered host structures with swelling properties are favorable for the intercalation or immobilization of biomolecules such as enzymes, DNA, amino acids, and peptides. Significant efforts have been directed toward the preparation of biomolecules/LDH hybrid materials. Utilizing the electrostatic interactions between LDH nanoplatelets and biomolecules, various bioinorganic hybrids with desired biocompatibility have been fabricated by self-assembly between oppositely charged LDH nanosheets and biomolecules, including DNA/LDH,[12,13] collagen/LDH,[14] and DNA/LDH/SiO2.[11] Desigaux et al.[15] prepared the DNA/LDH hybrids synthesized by a coprecipitation route involving the in situ formation of LDH around DNA molecules, forming the sandwich structure with DNA molecules between hydroxide layers. Sun et al.[14] prepared the collagen/LDH hybrids through the immobilization of collagen onto exfoliated LDH nanosheets. However, it is hard to control the structure and morphology of the hybrids. Utilizing the exfoliated LDH nanosheets and DNA as building blocks, our group very recently synthesized core–shell structural SiO2@DNA–LDH hybrid materials[11] via layer-by-layer assembly of exfoliated LDH nanosheets with DNA biomolecules over the surface of SiO2 particles. However, the fabrication processes of LDH-based hybrids are somewhat more complex and uncontrollable, involving the preparation, exfoliation, and assembly of LDH, which are time consuming and involves toxic chemicals. Hence, it should be interesting to fabricate LDH based hybrids with controlled
morphological and tunable structure by a simple green synthetic route.

In this article, we report a controlled synthesis of hierarchical flower-like BSA/LDH hybrids by a self-assembly process under mild conditions. A possible formation mechanism for the controlled organization of primary building units into special three-dimensional hierarchical structures has also been proposed on the basis of the interaction between BSA and LDH nanocrystals.

Experimental

Materials

The bovine serum albumin (BSA) was purchased from Shanghai Huixing biochemical reagents Co., Ltd. Aluminum nitrate nonahydrate (Al(NO₃)₃•9H₂O) and zinc nitrate hexahydrate (Zn(NO₃)₂•6H₂O) were obtained from Sinopharm Chemical Reagent Co., Ltd. Hexamethylenetetramine (HMT, C₆H₁₂N₄) and NH₃•H₂O were purchased from Jiangsu Yonghua Fine Chemical Co., Ltd and Shanghai Chemical Reagent Co., Ltd, respectively. All the reagents used in this synthesis were commercially available and double-distilled water was employed in all experiments.

Preparation of Hierarchical BSA/LDH Hybrids

In the present work, BSA/alumina sol was employed as precursors for the synthesis of BSA/LDH hybrids. The synthesis of BSA/alumina sol was similar to our previous reports of the synthesis of the BSA/ZnO hybrids with a modification.[6] Briefly, 30 mL of salt solution containing 0.01 mol Al (NO₃)₃•9H₂O was added quickly into 50 mL of 2 g/L BSA solution under vigorous agitation. Then, an aqueous ammonia solution was slowly added until the pH reached 9.0 and the mixture was stirred for about 30 min to form a homogeneous colloid. After that, the precipitates were collected and rinsed thoroughly with distilled water to obtain BSA/alumina sol.

By utilizing the BSA/alumina sol as biohybrid precursors and aluminum sources, the hierarchical BSA/LDH hybrids were fabricated by in situ growth of LDH nanoplatelets around biomolecules. In a typical procedure, 0.02 mol of Zn(NO₃)₂•6H₂O and 0.02 mol of HMT were dissolved in 80 mL deionized water to form a clear solution. Then, the prepared BSA/alumina sol was added into the mixed solution and well stirred at room temperature for 30 min. The mixture was put into 100 mL Teflon-lined stainless steel autoclave. Subsequently, the autoclave was sealed and transferred to an electric oven and maintained at 115 °C for 10 h. Lower cooling down to room temperature naturally, the products were obtained and thoroughly washed with distilled water for several times.

Sample Characterization

Structural characterization of the BSA/LDH hybrids was performed using X-ray diffraction (XRD) on Bruker-AXS D8 X-ray diffractometer system with Cu Kα radiation. The morphology of the as-prepared products was characterized by scanning electron microscopy (SEM; Hitachi S-3400N).

Results and Discussion

The reactive functional groups on the surfaces of BSA consist of carboxyls, amines, hydroxyls, and phosphoryls that can bind metal anions through coordination or electrostatic interactions. In the preparation of the BSA/alumina sol, the aluminum ions were adsorbed on the surfaces of biomolecules via electrostatic attraction between the organic functional groups of BSA and metal ions. Hence, the alumina sol was coated on the surface of BSA in the sol-gel process. Figure 1A shows the SEM image of BSA/alumina sol, demonstrating that the spherical morphology can be observed by assembly of biomolecules and alumina sols. From the SEM images it can be seen that the typical diameter of the BSA/alumina micrometre spheres in the range of 1.0 – 3.5 μm. However, it should be noticed that the diameters of hybrid microspheres are not uniform. The formation of spherical morphology may due to the cross-linking of alumina sol-coated biomolecules during the sol-gel process.

BSA, which is a globular protein comprising 607 amino acid residues, can be assembled with inorganic nanomaterials to form bioinorganic hybrids. In the present system, the alumina sols are coated on the surface of the biomolecules via a sol-gel process, and then the LDH nanosheets are grown on surfaces of BSA/alumina sols, forming the hierarchical BSA/LDH hybrids. Figures 1B–D show the SEM images of BSA/LDH hybrids. It is seen in Figure 1B and 1C that the hybrids display a hierarchical architecture with flower-like morphology. It should be noted that the sizes of hybrid microspheres are larger than that of the BSA/alumina microspheres. The LDH platelets are intercrossed with each other and self-assemble into hierarchically flower-like superstructures. The flower-like morphology can provide the dominant contribution to the surface areas and pore volume of hybrids. An enlarged image presented in Figure 1D shows that numerous LDH nanoplatelets are grown in all directions with many voids between them. It can be seen that the size distribution of the LDH nanoplatelets obtained by this process was not uniform, with the majority crystallites ranging from several micrometers to several tens of micrometers in size.

The XRD was used to determine the crystalline structure of as-prepared samples. The XRD pattern of BSA and BSA/LDH hybrids are shown in Figure 2. In XRD patterns, the broad hump centered at 23.8° corresponds to the diffraction of amorphous biomolecules. Owing to the amorphous nature of BSA, the XRD patterns of BSA/alumina sol (Figure S1) only show the characteristic peaks of Al(OH)₃. In order to compare the structural changes, the bare Zn-Al LDH particles are synthesized by coprecipitation methods as described previously, and the corresponding XRD pattern is shown in inset of Figure 2. The reflections of synthesized LDH particles show sharp and symmetric peaks, even at a high angle range, indicating the highly crystalline nature of the samples. However, the phase of LDH in BSA/LDH hybrids shows the relatively broad and asymmetric reflections, implying the
random and disordered structures of LDH. As compared to the bare LDH, the characteristic reflections of LDH are shifted to lower angle, indicating that the biomolecules are incorporated in the interlayer of LDH nanosheet. Similar results are observed in the literature of other biomolecules-intercalated LDH.

The preparation process of BSA/LDH hybrids involves the in situ assembly of LDH nanoplatelets with biomolecules, which can be divided into three steps, including adsorption, assembly, and crystal growth.

As we know, the surfaces of the biomolecules are covered in charged amino acid residues, which make the biomolecules have excellent metal ions adsorption capabilities. Hence, in the sol-gel process, aluminum ions were first adsorbed on the surface of BSA based on the interaction between the functional groups of the amino acid residues and the metal ions, resulting in the formation of BSA-Al\(^{3+}\) complexes. Then, the BSA/alumina sol were formed via the hydrolysis of aluminum ions under alkaline conditions. And alumina sol-coated biomolecules were self-assembled into spherical aggregates as shown in Figure 2A. During this process, these amino acid residues are critical because they create cross-linked structure (spherical aggregates) by the interaction of alumina sol-coated BSA. In the last step, the hierarchical BSA/LDH hybrids were fabricated by in situ growth of LDH nanoplatelets around biomolecules by utilizing the BSA/alumina sol as biohybrid precursors and aluminum sources. During the hydrothermal process, the HMT decomposes to formaldehyde and ammonia as primary products and the decomposition is dependent on the solution pH.

\[
\text{C}_6\text{H}_{12}\text{N}_4 + 6\text{H}_2\text{O} \rightarrow 6\text{HCHO} + 4\text{NH}_3 \tag{1}
\]

\[
\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^- \tag{2}
\]

Then, the Zn\(^{2+}\) ions reacted with the alumina sols present on the BSA molecular skeleton to form BSA/LDH hybrid nanoparticles.

\[
\text{Zn}^{2+} + \text{BSA/Al(OH)}_3 + \text{OH}^- \rightarrow \text{BSA/LDH} \tag{3}
\]

The crosslinked hybrid nanoparticles further form 3D flower-like BSA/LDH hybrids by driving force of crystal growth.
Conclusions

In summary, we present a facile and efficient method to synthesize hierarchical flower-like BSA/LDH hybrids through a sol-gel process and an \textit{in situ} growth technique. The SEM images show that the as-prepared hybrids display a hierarchical architecture with flowerlike morphology. The XRD results show that the characteristic reflections of LDH are shifted to lower angle, indicating that the biomolecules are incorporated in the interlayer of LDH nanosheet. A possible formation mechanism of BSA/LDH hybrids is proposed and discussed based on the \textit{in situ} assembly of LDH nanoplatelets with biomolecules. The as-prepared bioinorganic hybrids with hierarchical architectures have potential applications in the areas of biocatalysts and drug delivery.

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Supplementary Material

Supplemental data for this article can be accessed at the publisher’s website.

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