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Regular Article

Protein-directed assembly of cobalt phosphate hybrid nanoflowers

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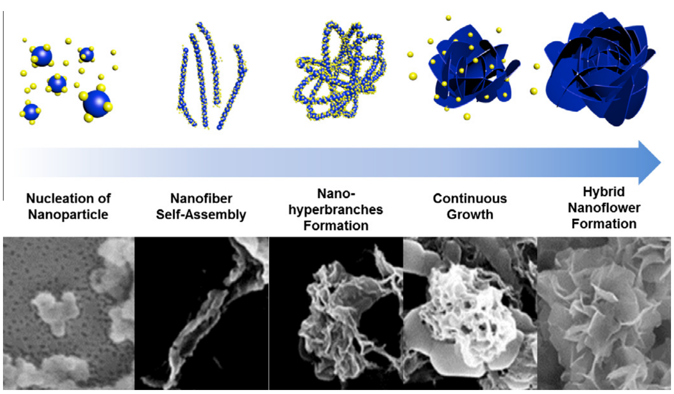
GRAPHICAL ABSTRACT

Formation mechanism of organic-inorganic hybrid nanoflowers were studied by analysis of time-dependent growth stage of protein-directed assembly method, leading to provide clues to fabricate other bio-metal hybrid materials.



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ABSTRACT

The understanding and controlling of biomimetic hybrid materials are a key objective in bio-nanotechnology, materials chemistry, and colloid science fields. Biomaterials, such as, enzyme, DNA, RNA, and proteins have become important templates for the construction of inorganic–organic hybrid nanoflowers. From this perspective, we present a simple approach to synthesize protein and metal hybrid flower-like structure using bovine serum albumin (BSA) and cobalt phosphate, and the results of our study on the formation mechanism involved. The time dependent growing stage and formation mechanism were analyzed by electron microscopes and spectroscopic techniques. The protein-directed assembly method for preparation of hybrid nanoflowers described in this work could be used to fabricate other bio-metal hybrid materials with possible applications in biosensors, bioanalytical devices, and industrial biocatalyst fields.

1. Introduction

Control of the formation of complex three dimensional (3D) functional nanohybrids using organic and inorganic building blocks has brought the attentions of researchers for diverse applications in catalysis, energy storage, drug delivery, and biosensors [1–8]. The majority of such materials have been synthesized using solution-based chemistry approaches, such as, hydrothermal, sol-gel, chemical precipitation, and self-assembly methods [9–12]. Although these methods provide great versatility in terms of providing required morphologies, eco-friendly synthesis

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approaches to achieve functionalities, and high surface to volume ratios while avoiding to use toxic solvents and heavy metals are still considered as major challenges.

Accordingly, new alternative synthetic approaches are required, especially, those based on green chemistry using biomaterials. To meet such demands, DNA [13–15], RNA [16], and peptides [17] have been popularly adopted as building blocks to construct different types of nanoscaled structures under well controlled environment. However, these biomolecules require considerable effort to design molecular sequences and they are expensive, which limit their application potentials and cause broad expansion to scale up production of nanohybrid materials and nanostructures. Recently, proteins are the most abundant materials produced by living organisms and that could be exploited as building blocks to construct diverse hybrid nanomaterials. Up to date, considerable efforts have been directly toward understanding the mechanisms underlying interactions between proteins and inorganic materials. Previously, numerous scientists have been explored ''biominerali zation'' phenomenon by reacting different inorganic ions or nanoparticles with proteins and other biomolecules [18]. Major researches have focused on calcium (Ca) based materials for teeth or bone replacement, bone repair, and bone regenerations [19]. Such studies dealt with fundamental model of protein–inorganic hybrid materials and other kinds of protein and inorganic hybrid assemblies become a turn point in those research areas. Recently, Zare and his co-workers reported protein-inorganic nanoflower structures, which were constructed using commercially available bovine serum albumin (BSA) as a building component and different amounts of copper (Cu) ions, and examined in the context of enhancing enzyme activities [20]. Tezcan and co-workers used protein and zinc (Zn) ions to construct one–dimensional (1D) nanotubes, two–dimensional (2D), and 3D crystalline arrays [21]. Our group also described hybrid structured nanoparticles using proteins and various metal ions to produce quantum dots (QDs) and iron oxide and gold nanoparticles [22]. Although previous studies have been fabricated diverse types of inorganic-organic hybrid materials, growth mechanism behind of protein-based organic-inorganic is not understood and has been rarely investigated. This is particularly true for cobalt-based materials, which are commonly used in energy storage systems, sensors, and catalysis [23–25].

Herein, we present a straightforward and effective method for synthesizing protein/cobalt hybrid flowers through BSA-assisted self-assembly with cobalt ions. We also emphasize that the phosphate in phosphate buffer saline (PBS) buffer solution, metal ions, and protein are the essential components for direct self-assembly and construction of hierarchical nano- and microstructures of artificial hybrid flowers. The protein was particularly employed as nucleation and growth sites for deposition of cobalt phosphate and as structural guiding supporters to form the flower morphology. Different growth stages were intensively identified and studied. During growth stages, zero dimensional (0D) nanoparticles, 1D nanowires or nanofibers, and 2D nanopetals, and 3D nanoflower were sequentially observed. Moreover, intensive analysis using electron microscopic and spectroscopic techniques observed time-dependent growth process of hybrid nanoflowers. As prepared nanoflower had porous architectures comprised of assembled protein–cobalt hybrid particles.

2. Experimental

2.1. Chemicals and synthesis of BSA/cobalt nanoflowers

Cobalt sulfate hydrate (Sigma Aldrich, USA), phosphate buffered saline (PBS) solution (Gibsco, USA), bovine serum albumin (BSA, Sigma Aldrich, USA) were purchased and used without further purification. Initially, BSA (0.2 mg/mL) and cobalt (10 mM) were dissolved in PBS. The protein (1 mL) was then injected into tube type reactor and cobalt solution (1 mL) was added. The mixture was then left at room temperature for 24 h, centrifuged at 5000 rpm for 1 min, and washed with PBS several times to remove remaining proteins and metal ions. To probe the growth mechanism, different reaction times (1, 2, 3, and 24 h) were performed, and each sample was carefully collected. The products were placed on either a Si wafer or a transmission electron microscopy (TEM) grid and dried at room temperature.

2.2. Characterization

The morphologies of the resultants were analyzed by a scanning electron microscopy (SEM, FEI Nova 200 Nanolab) and TEM (JEOL 3011 HREM, 300 kV) equipped with an energy dispersive X-ray spectroscopy (EDAX) detector. High–angle annular dark–field (HAADF) images were collected using an energy filtering TEM (EM 912 Ω, Carl Zeiss) operating at 120 kV. Powder X-ray diffraction (XRD) was used to analyze the crystal structures of nanoflowers using a Rigaku X-ray diffractometer with a Cu Kα radiation sources. X-ray photoelectron spectroscopy (XPS, Kratos Analyticals, UK) was performed using a monochromatic Al-K_{α} X-ray source. Circular dichroism (CD) spectroscopy was carried out on JASCO J-815 unit. FT-IR analysis was performed using Nicolet 6700 spectrometer equipped with the MCT-A detector. Ultraviolet–visible (UV/Vis) spectroscopy (OPTIZEN POP, Mecasys, Korea) was conducted at room temperature.

3. Results and discussion

3.1. The morphologies of BSA/cobalt nanoflowers

Hybrid nanoflowers were initially produced by mixing of BSA in PBS solution and cobalt sulfate hydrate, and allowing this mixture to stand at room temperature for 24 h. Finial product showed pink precipitate composed of BSA and cobalt. The morphologies of BSA/-cobalt nanoflower were observed using SEM and TEM (Fig. 1). When observing top and side sections of SEM images (Fig. 1a–d), strong interactions between BSA and cobalt phosphate toward anisotropic growth induced to form 3D hierarchical porous microspheres. Sphere diameters were ~3 μm and microspheres were primarily composed of interconnected nano-petals of thickness ~6 nm. TEM images revealed a hierarchical interconnected network of thin nano-petals with randomly stacked multilayers (Fig. 1e–h). No significant crystal lattice was found in a single nano-petal and thicker nano-petals also found from the edge of nanoflowers due to occasional edge folding. To confirm the major components of hybrid nanoflowers, we turned to EDAX analysis. Prominent EDAX peaks were assigned to cobalt, phosphorous, oxygen, carbon, and copper (attributed to the TEM grid), indicating the hybrid nano–flowers were mainly composed of cobalt phosphate and BSA (Fig. 1i). TEM tomography displayed the geometrical features of BSA/cobalt nanoflower more vividly in HAADF scanning TEM (Fig. 2a–e). Folded areas were of greater intensity due to the presence higher cobalt oxide concentrations. Elemental EDX maps were conducted (Fig. 2f–h); O, C, and Co elements clearly overlapped over the entire hybrid nanoflowers, indicating the uniform and homogeneous distribution of cobalt and BSA.

Examination by XPS was further performed to understand of the surface electronic state of BSA/cobalt nanoflowers (Fig. 3). The survey spectrum revealed that nanoflowers were mainly composed of C, O, Co, and P elements (Fig. 3a). The high-resolution of Co $2p_{2/3}$ showed two peaks at 778.7 and 794.8 eV for $2p_{2/3}$, which