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Nickel hydroxide/hydroxyapatite nanorods as affinity adsorbents for separation histidine-tagged proteins

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ABSTRACT

Nickel hydroxide/hydroxyapatite (designated as $Ni(OH)_2$/HAP) nanorods have been designed and synthesized by hydrothermal method. The size and morphology of $Ni(OH)_2$/HAP sample depend strongly on the reaction condition, and introduction of small amount of Ni^{2+} ions would be propitious to get the smaller size nanorods. By taking advantage of the high affinity of Ni^{2+} ions toward histidine-tagged (His-tagged) proteins, the $Ni(OH)_2$/HAP nanorods can be used to enrich and separate His-tagged proteins directly from a mixture of lysed cells, and their universality was evaluated by separating three kinds of His-tagged proteins. The $Ni(OH)_2$/HAP nanorods present negligible nonspecific protein adsorption and high protein binding ability, and their specificity and affinity toward His-tagged proteins can be remained after 4 times recycling. The $Ni(OH)_2$/HAP nanorods may provide many potential applications for the detection or separation of different biomolecules.

1. Introduction

Proteomics has been developed for disease diagnosis and treatment with the advent of the post genome era. The critical process of proteomics research is the separation and eTnrichment of proteins from biosamples [1–4]. The biotechnologies nowadays enable proteins to easily express with a tag, and many protein-purification methods are based on the specific interactions between immobilized ligands and affinity tags on the protein [5,6]. Among affinity tags, histidine tags are preferably considered in protein preparation, which can specifically interact with the immobilized metal ions to create strong, yet reversible binding [7–9]. Isolation of His-tagged proteins typically involves immobilizing metal ions, selectively binding proteins and effectively releasing recombinant proteins from the support surface. Generally, nitrolotriacetic acid (NTA), iminodiacetic acid (IDA) and L-cysteine are used as chelating ligands to immobilize metal ions [10–12]. More recently, nanomaterials have emerged as a promising separation nano-tool in the proteome research [13–15]. For example, Au/Fe_3O_4 nanoparticles modified with Ni^{2+}-NTA are applied to the separation of His-tagged proteins [16]. Fe_3O_4/Cys nanospheres have been used to purify His-tagged proteins directly from the mixture of lysed cells [12]. A major limitation of current nanoparticle-separation system is low surface metal ion density, leading to low purification efficiency. To overcome this limitation, some alternative adsorbents have been suggested, including microgels [17], polymer brush-modified nanoparticles [18], nanostructured adsorbent [19–21], and NiO based nanoparticles [22,23]. However, the efficiency of these systems has not been clearly to be proven to be superior to others, so it is necessary to develop novel particle adsorbent.

Hydroxyapatite (HAP) is a material very similar to the mineral component of bone, and more than 60% of the currently available bone graft substitutes involve calcium phosphate-based materials [24–26]. HAP exists as a crystalline structure of associated calcium, phosphate and hydroxyl groups, and contains multiple adsorption sites: the so-called P-sites that are negatively charged and the so-called C-sites that are positively charged. In general, positively charged amino groups on proteins interact with negatively charged P-sites and protein carboxyl groups interact by coordination complexation to C-sites. Thus, HAP has attracted much attention in the chromatographic adsorbent [27–31]. Compared to polymer, magnetic and silica based nanoparticle-adsorbent, few effort was focused on HAP based nanoparticle-adsorbent.

Herein, we report the synthesis of novel affinity adsorbent, $Ni(OH)_2$/HAP nanorods through a hydrothermal route. To increase the density of Ni^{2+} ions, the HAP nanorods were one-step formed by reaction of $(NH_4)_2HPO_4$ and $Ca(NO_3)_2$ in the presence of

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Ni^{2+} ions, and Ni^{2+} ions were in situ immobilized on the surface of HAP nanorods. The HAP nanorods with exposed Ni^{2+} sites can be used to selectively separate His-tagged proteins from the mixture of cell lysate. The schematic presentation of the synthesis of Ni $(OH)_2$/HAP nanorods and their purification of His-tagged proteins are shown in Scheme 1. Their protein binding ability and purifying efficiency were evaluated through separating three kinds of His-tagged proteins. The present method is facile and its enrichment efficiency is much higher than that of commercial microbeads, which may provide many potential applications for enrichment and separation of different types of proteins.

2. Experimental

2.1. Materials

Ammonium hydrogen phosphate ($(NH_4)_2HPO_4$), nickelous chloride ($NiCl_2{\cdot}6H_2O$, ≥98.0%), calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$, ≥99.0%), sodium hydroxide (NaOH, ≥96.0%), ammonia solution ($NH_3{\cdot}H_2O$) and absolute alcohol were purchased from Tianjin Kermel Chemicals (Tianjin, China). All chemical agents used in these experiments were of analytical grade and used directly without further purification. Ni-NTA agarose was from QIAGEN (Beijing, China); BCA protein Assay Kit was from Beijing CoWin Biotech (Beijing, China); Protein molecular weight marker (Low) was purchased from TakaRa Biotech (Dalian, China).

2.2. Preparation of $Ni(OH)_2$/HAP nanorods

In a typical synthesis, 50 mL calcium–nickel mixed solution was added dropwise into 50 mL $(NH_4)_2HPO_4$ solution (0.6 mol/L) under magnetically stirring condition, then the pH value of this solution was tuned to 10 with ammonia solution and keep the reaction for 0.5 h at the room temperature. Subsequently, the solution was transferred into a Teflon-lined stainless-steel autoclave, sealed and heated at 180 °C for 15 h. Finally the solution was cooled down to room temperature, centrifuged and washed to get HAP product. The molar ratio of Ca/P was set to 10/6 (the ratio is same as that of apatite) and the molar ratio of Ni/Ca was 0/1, 1/4, 1/2, 1/1, 2/1 and 1/0, respectively.

2.3. Preparation and separation of his-tagged proteins

In this study, three different 6 × His-tagged proteins were prepared: His-tagged CPK4 [32], His-tagged APX3 [33] and His-tagged Thioredoxin 9 (His-tagged TRX9) [34]. We cloned APX3 and CPK4 from *Arabidopsis thaliana* and constructed them into the pET-28a plasmid. His-TRX is from PET-32a plasmid [35]. His-tagged recombinant plasmids were transformed into *E. coli* strain Rosetta (DE3) (Novagen) for protein expression using standard protocols [36].

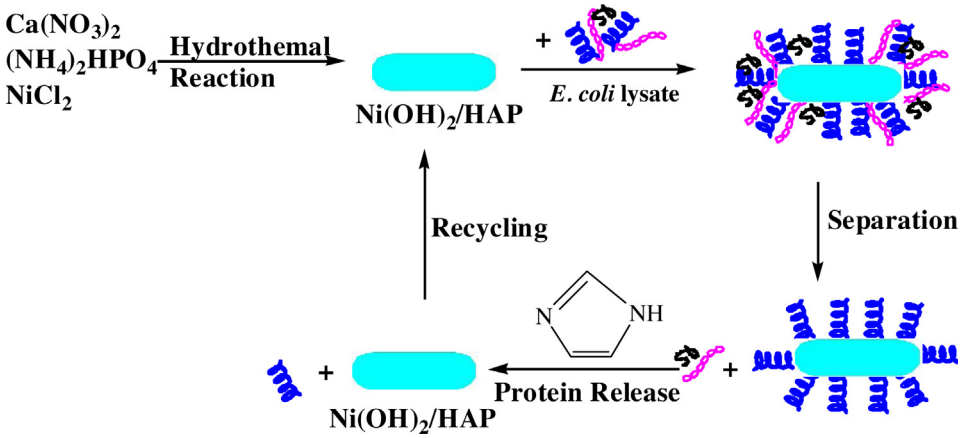
Theoretically, all the proteins with His-tagged would be captured by metal-chelate affinity separation method. After being washed three times with tris buffer (20 mM). First, the HAP nanorods were added directly into 1.5 mL mixture of cell lysate and shaken for 2 h at a rotation speed of 90 rpm at room temperature. Second, the $Ni(OH)_2$/HAP nanorods having captured His-tagged proteins were isolated from the solution by centrifugation and washed three times with tris buffer in order to remove any uncaptured proteins. Then, the targeting nanorods were washed with 300 μL imidazole solution (1.0 M) to disassociate His-tagged proteins from their surface. Final, separately collected protein solutions were detected by SDS-PAGE.

2.4. Characterization

The morphology and composition were characterized by Transmission electron microscopy (TEM, JEM-2010, Japan), field emission scanning electron microscopy (FESEM, JSM-7001F, Japan), Fourier transform infrared (FT-IR, AVATAR360, America), X-ray diffraction (XRD, X-Pertpro, Holland) and thermogravimetric analysis (TG&DTA, EXSTAR 6000), respectively. The separated His-tagged proteins were detected with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE, Power PAC 300, China), with the preconcentration voltage of 70 V and the separation voltage of 120 V. The protein concentrations were measured by a UV–vis spectrophotometer (Lambda Bio40, PerkinElmer, USA) at 280 nm.

3. Results and discussion

Fig. 1 shows the XRD pattern of the six samples prepared at different mole ratio of Ni/Ca. In the absence of Ni^{2+}, the as-obtained sample displays multiple broad peaks and all the diffraction peaks can be well attributed to pure HAP (JCPDS 09-432). Introduction of Ni^{2+} ions, the collected samples show broad HAP peaks and other strong peaks, and the strong peaks belong to hexagonal nickel hydroxide (JCPDS 14-0117), indicating the formation of nickel hydroxide and HAP. With the increase of Ni/Ca ratio, the intensity of $Ni(OH)_2$ diffraction peaks increases while that of HAP peaks decreases, implying that a part of Ca^{2+} ions in the HAP framework might be substituted by Ni^{2+} ions and most Ni^{2+} ions exists in the



Scheme 1. Schematic representation of the preparation procedure of $Ni(OH)_2$/HAP nanorods and their affinity separation of His-tagged proteins.