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Iminodiacetic acid functionalized porous hydroxyapatite nanoparticles for capturing histidine-tagged proteins

Shasha Yao [a], Yanqin Huang [b], Yanbao Zhao [a,*], Yu Zhang [b], Xueyan Zou [a], Chunpeng Song [b]

[a] Key Laboratory for Special Functional Materials, Henan University, Kaifeng 475004, PR China
[b] Key Laboratory of Plant Stress Biology, Henan University, Kaifeng 475004, PR China

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ABSTRACT

A simple strategy has been developed to synthesize hydroxyapatite (HAP) nanoparticles (NPs) in a simulated body fluid (SBF). The HAP NPs have an average diameter of 50 nm and present porous structure. By taking advantage of surface hydroxyl groups, the HAP NPs are further modified with iminodiacetic acid (IDA), followed by chelating Ni^{2+} ions. The HAP/IDA-Ni^{2+} NPs as novel adsorbent can capture directly histidine-tagged (His-tagged) proteins from the mixture of lysed cells without sample pretreatment. Results indicated that the HAP/IDA-Ni^{2+} NPs present negligible nonspecific adsorption and high protein binding ability, and their specificity and affinity toward His-tagged proteins can remain after 5 times of recycling. The HAP/IDA-Ni^{2+} NPs are especially suitable for purification of His-tagged proteins with low molecule weight.

1. Introduction

Proteins play a variety of crucial roles in organisms, and the enrichment and purification of proteins, especially low-abundance proteins, are currently a hot topic [1–3]. The biotechnologies nowadays enable proteins to easily express with a tag, and many protein-purification methods are based on the specific interaction between immobilized ligands and affinity tags [4–6]. Among affinity tags, histidine tag is preferably considered in protein preparation, which can interact with the immobilized metal ions to create strong, yet reversible binding [7–9]. The purification of his-tagged proteins typically involves immobilizing metal ions, selectively binding proteins and effectively releasing recombinant proteins from the support surface. More recently, nanomaterials have emerged as a promising separation nano-tool in the proteome research [10–12]. Generally, iminodiacetic acid (IDA), *L*-cysteine, *L*-lysine and nitrolotriacetic acid (NTA) are used as metal chelators [13–16]. For example, the Ni-NTA complex-conjugated Fe_3O_4/Au and Fe_3O_4/SiO_2 NPs have been applied to separate His-tagged proteins [17,18]. However, a major limitation of current particle system is the low surface metal ion density, which restricts its practical application in protein separation, so it is necessary to develop novel particle adsorbent.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) is the main mineral constituent of teeth and bone, which consists of positively charged pairs of crystalline calcium ions and clusters of six negatively charged oxygen atoms associated with triplets of crystal phosphates. Due to its outstanding biocompatibility, it has been widely used as bone cavity filling, metallic implant coating and bone graft substitute [19–22]. In particular, HAP has rich surface active groups to conjugate ligands, which can increase effectively surface metal ion density. Compared with solid nano-adsorbents, porous HAP NPs can provide higher surface area, and would be practically used in the affinity purification of His-tagged proteins.

Herein, we report the synthesis of porous HAP NPs through a hydrothermal route. The obtained HAP NPs have plenty of hydroxyl groups and can be further modified with IDA molecules. After chelating Ni^{2+} ions, the resulting HAP/IDA-Ni^{2+} NPs as affinity adsorbent are used to directly separate His-tagged proteins from the mixture of *E. coil* cell lysate. Its protein binding ability was evaluated by three kinds of His-tagged proteins in detail.

2. Experimental section

2.1. Materials

Iminodiacetic acid (IDA) (≥98.0%) and 3-glycidyloxypropyltrimethoxysilane (GPTMS) were purchased from Aladdin Chemicals (Beijing, China). Phosphorus (V) oxide (P_2O_5), nickel chloride ($NiCl_2 \cdot 6H_2O$, ≥98.0%), calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$, ≥99.0%), sodium hydroxide (NaOH) and absolute alcohol were purchased from Tianjin Kermel Chemicals (Tianjin, China). Hydrochloric acid and ammonia solution ($NH_3 \cdot H_2O$) were from Luoyang Haohua Chemicals (Luoyang, 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

* Corresponding author. Tel./fax: +86 3783881358.
E-mail address: zhaoyb902@henu.edu.cn (Y. Zhao).

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was from Beijing CoWin Biotech (Beijing, China). Protein molecular weight marker (Low) was from TakaRa Biotech (Dalian, China).

2.2. Preparation of HAP/IDA NPs and adsorption of Ni^{2+} ions

In a typical synthesis, first, 0.07 g P_2O_5 was dissolved in 25 mL of alcohol and 0.4 g $Ca(NO_3)_2 \cdot 4H_2O$ was dissolved in 25 mL of SBF solution at 60 °C, respectively. Second, the P_2O_5 solution was added slowly into the $Ca(NO_3)_2$ solution and the pH value of the solution was tuned to 10. After reaction for 0.5 h, the solution was transferred into a Teflon-lined stainless-steel autoclave, sealed and heated at 165 °C for 12 h. Finally, the solution was cooled, centrifuged and washed to get the HAP product.

For the preparation of the GPTMS–IDA silane [23], 1.7 g IDA was dissolved in 20 mL of deionized water in a flask, and the pH value of this solution was tuned to 11. The flask containing the IDA solution was placed in ice-bath at 0 °C, and subsequently 2 mL of GPTMS was added dropwise into the IDA solution under stirring. The mixed solution was heated to 65 °C and kept at this temperature for 12 h while stirring continuously.

For surface modification, the pH value of the GPTMS–IDA solution (3 mL) was adjusted to 2 and 0.1 g of HAP NPs was added. After reaction at 90 °C for 3 h, the suspension was centrifuged and washed to get the HAP/IDA product. Subsequently, 5 mg of HAP/IDA was dispersed in 50 mL of the 2 M $NiCl_2$ solution. After shaking for 2 h, the solution was centrifuged and washed to get HAP/IDA-Ni^{2+} NPs.

2.3. Preparation and separation of His-tagged proteins

In this study, three kinds of His-tagged proteins were prepared: His-tagged ASCORBATE PEROXIDASE3 (His-tagged APX3) [24], His-tagged calcium protein kinases (His-tagged CPK4) [25] and His-tagged thioredoxin 9 (His-tagged TRX9) [26]. We cloned APX3 and CPK4 from *Arabidopsis thaliana* and constructed them into the pET-28a plasmid. His-tagged TRX9 was from PET-32a plasmid [27]. His-tagged recombinant plasmids were transformed into *E. coli* strain Rosetta (DE3) (Novagen) for protein expression using standard protocols [28]. The *E. coli* cells are lysed by 20 mM Tris (including 150 mM NaCl, pH 8).

After being washed three times with binding buffer (20 mM Tris–HCl, 0.2 mol/L NaCl), HAP/IDA-Ni^{2+} NPs were added directly into 1.0 mL mixture of cell lysate and shaken for 2 h at a rotation speed of 90 rpm at room temperature. Then, the HAP/IDA-Ni^{2+} NPs having captured His-tagged proteins were isolated from the solution by centrifugation and washed three times to remove uncaptured proteins. Finally, the targeting NPs were washed with 300 μL imidazole solution (1.0 mol/L) to disassociate His-tagged proteins from their surface.

2.4. Characterization

The morphology and composition were characterized by transmission electron microscopy (TEM, JEM-2010, Japan), Fourier transform infrared (FTIR, AVATAR360, America), X-ray diffraction (XRD, X-Pertpro, Holland) and thermogravimetric analysis (TG, EXSTAR 6000), respectively. The surface area was measured by the Brunauer–Emmett–Teller (BET) method using nitrogen adsorption and desorption isotherms on automated surface area and pore size analyzer (QUADRASORB, SI, American). X-ray photoelectron spectroscopy (XPS) spectra were collected on an axis ultra X-ray photoelectron spectrometer, and XPS analyses were corrected with reference to C 1s (284.6 eV). The absorbed Ni^{2+} density was carried out using inductively coupled plasma atomic emission spectrometer (ICP-AES, Optima 2100 DV). The separated His-tagged proteins were detected with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE, Power PAC 300, China), with a preconcentration voltage of 70 V and a separation voltage of 120 V. The binding proteins concentration was analyzed by UV–vis spectrophotometer (Nanodrop 2000c, America).

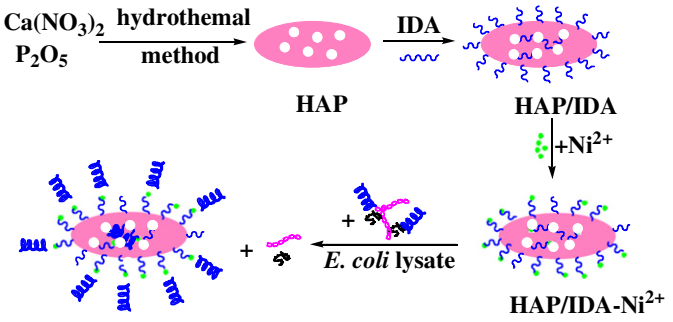
3. Results and discussion

The preparation of HAP/IDA-Ni^{2+} NPs and the purification of His-tagged proteins involve the following four steps (shown in Scheme 1): preparation of HAP NPs by hydrothermal route, surface modification with IDA, adsorption of Ni^{2+} ions and purification of His-tagged proteins from the mixture of cell lysate.

Fig. 1 gives the TEM images of the obtained HAP (a) and HAP/IDA (b) NPs. It can be seen that the obtained HAP sample displays wide size distribution, with an average diameter of 40 nm. The HAP sample is nearly spherical and presents porous or hollow structure. After modification with IDA, the HAP/IDA sample is also spherical and its size increases slightly, which indicates that IDA is conjugated on the HAP surface. In addition, the HRTEM image (insert) reveals that the sample has a well crystalline nature. The N_2 adsorption/desorption isotherms of the prepared HAP/IDA sample (Fig. 1c) exhibited a type IV isotherm demonstrating the porous characteristics of the HAP/IDA NPs. The BET surface area and pore volume of the sample are 42.9 m^2/g and 0.32 cm^3/g, respectively. It is clear that the HAP/IDA sample presented wide pore diameter distribution, and the pore diameter was mainly focused on 2.9 nm and 30 nm.

Fig. 2a shows the XRD patterns of the prepared HAP/IDA NPs. It is clear that the HAP/IDA NPs display multiple intense peaks and all the peaks can be perfectly indexed to crystalline HAP (JCPDS 09-0432), not only in peak position but also in their relative intensity, indicating that the obtained sample is crystalline HAP. Fig. 2b gives the FTIR spectra of HAP and HAP/IDA NPs. In HAP FTIR spectrum, the characteristic bands at 3431 and 1633 cm^{-1} are attributed to the adsorbed water. The intense bands located at 561, 601 and 1039 cm^{-1} correspond to PO_4^{3-} ions in apatitic structure [29]. The band at 1456 cm^{-1} belongs to CO_3^{2-} absorption bands, which might be caused by carbon dioxide from the air [30]. Compared to HAP, the absorption peaks of water and carbonate in HAP/IDA sample are decreased due to IDA modification. There appears a sharp peak at 3571 cm^{-1}, which is assigned to the stretching vibration of the OH^- group present in HAP. In addition, the weak $-CH_2-$ absorption peak at 2930 cm^{-1} indicates that the IDA content is not too much.

Fig. 3a gives TG analyses of HAP and HAP/IDA samples. For HAP sample, there appears a successive mass loss process from room temperature to 800 °C. The initial mass loss below 200 °C can be attributed to the desorption of adsorbed water from the surface of the sample powder and the following mass loss is related to the further release of the inner adsorbed or crystal water (total mass loss 17%). For HAP/IDA sample, the sample also presents successively mass loss and the total mass loss is about 7%. The initial mass loss below 250 °C is due to releasing adsorbed water from the sample powder (3%) and the mass loss above 300 °C is mainly attributed to the decomposition of organic precursors. The decrease in mass loss indicates that IDA is coating on the surface of HAP NPs and the IDA coating layer can effectively reduce the adsorption of HAP NPs on the water. XPS is a powerful technique for the study of transition metal compounds having localized valence d



Scheme 1. Preparation of HAP/IDA-Ni^{2+} NPs and their separation of His-tagged proteins.