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Short communication

Synthesis of biofunctionalized silica nanospheres to separate GST-tagged proteins

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ABSTRACT

A Thiol-functionalized silica hollow nanospheres(denoted as SiO_2-SH NSs) were prepared through a hydrothermal route. The SiO_2-SH NSs were conjugated with glutathione group (denoted as—GSH) to afford SiO_2-GSH NSs. The as-prepared SiO_2-GSH sample has hollow structure and exhibits an average diameter of about 45 nm and a wall thickness of 10 nm. The SiO_2-GSH NSs were used to separate three kinds of GST-tagged proteins (GST-tagged GPX, GST-tagged LOV and GST-tagged 210-6P). These SiO_2-GSH NSs exhibit negligible non-specific adsorption, high binding capacity (89.9 μmol/g), the low detection limit (1.0×10^{-6} mol/L) and reuse property, showing great potentiality in purifying GST-tagged proteins.

1. Introduction

Glutathione S-transferase (denoted as GST) represents a major group of detoxification isoenzymes which participate in a wide range of processes including xenobiotic biotransformation, drug metabolism, degradation ofaromatic amino acids and so on [1]. In the meantime, GST can be used as a marker of early phase tumors such as brain tumor, ganglioglomas, protate cacer and so on [2–4]. This could partly account for the significance of the separation and purification of GST and GST-tagged proteins in biological medicine and disease detection. Recently many materials, such as microfiber, magnetic microparticles and gels, have been employed to separate and purify GST and GST-tagged fusion proteins [5,6]. These materials, although adaptable to many protein expression systems, have some limitations, such as the need for pretreatment to remove cell debris and colloid contaminants, a relatively long operation time, and poor protein solubility. Today, nanoparticles or nanorods as promising nano-devices for the separation of target proteins could be advantageous over conventional counterparts [7–10]; however, surface group density of solid nano-adsorbents was low due to the muti-step surface modification reaction. In the present research, we focus on the preparation of functionalized silica hollow NSs to enhance the surface group density. As-synthesized silica NSs can directly enrich and separate GST-tagged proteins from the *E. coil* cell lysate, showing great potential as novel adsorbents.

2. Experimental

2.1. Materials

3-Mercaptopropyltrimethoxysilane (MPS) was purchased from Alfa-Aesar, Amarica. Tetraethyl orthosilicate (TEOS) was from Tianjin Fuchen Chemicals; Hexadecyltrimethylammonium Bromide (CTAB) was purchased from Sinopharm Chemicals; Glutatione (GSH) was purchased from Amresco Company. Triethanolammine (TEA) was purchased from Tianjin Kermel Chemicals. Ethylene diamine tetraacetic acid (denoted as EDTA), absolute alcohol and aqueous ammonia (28 wt%) were purchased from Tianjin Kermel Chemical Reagent Company, China. Phosphate buffer salines (abridged as PBS; concentration 0.1 mol/L, pH 8.0; concentration 0.01 mol/L, pH 7.4) and 5,5′-dithiobis-(2-nitrobenzoic acid) (abridged as DTNB, purity ≥98%) were purchased from Sigma–Aldrich, Amarica. All the reagents are of analytical grade and used as-received.

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2.2. Preparation of SiO_2-GSH

Briefly, 0.03 g CTAB, 2.8 mL of ethanol and 1.1 mL of TEA were dissovled in 17 mL of H_2O to form a transparent solution. Then mixture of TEOS and MPS (different volume ratios) was slowly dropped into above solution at 60 °C. After the resultant solution heated at 60 °C for 3 h, the solution was transferred into a Teflon-lined stainless-steel autoclave, sealed and heated at 110 °C for 24 h. Upon completion of the reaction, the solution was cooled, centrifuged, washed and dried at 60 °C in an oven to afford SiO_2-SH product.

0.3 g of SiO_2-SH product was dispersed in 10 mL 80 mg/mL of GSH solution. The dispersion was oscillated at 37 °C for 24 h (60 rev/min), followed by centrifuging to afford desired SiO_2-GSH product. Subsequently, the obtained SiO_2-GSH product was fully washed with PBS solution to remove physically adsorbed GSH, followed by dispersion in ethanol (25%, v/v) and storing at 4 °C.

2.3. Separation and detection of GST-tagged proteins

The to-be-tested mixed proteins were collected from the cell lysate of *Escherichia coli*, which is by water lysis. (concentration 0.01 mol/L, pH 7.4). 1 mL 0.02 g/mL of SiO_2-GSH sample fully washed with PBS solution was directly introduced into 1000 μL of the cell lysate and shaken at a rotary speed of 90 rev/min at 4 °C for 1 h to capture GST-tagged proteins. Upon completion of capturing GST-tagged proteins, the SiO_2-GSH sample with captured GST-tagged proteins was isolated by centrifugation and fully washed with PBS solution to remove residual uncaptured proteins, followed by washing with 300 μL of 50 mmol/L GSH solution to disassociate the GST-tagged proteins from the surface. Then the separated GST-tagged proteins were detected with SDS-PAGE, with the preconcentration voltage of 70 V and the separation voltage of 120 V. The binding proteins concentration was analyzed at 280 nm and 400 nm by UV–vis spectrophotometer.

2.4. Characterization

The morphology and composition were characterized by transmission electron microscopy (TEM, JEM-2010, Japan), Fourier transform infrared (FT-IR, AVATAR360, America) and thermogravimetric analysis (TG, EXSTAR 6000, Japan), respectively. The surface area was measured by the Brunauer-Emmett-Teller (BET) method (QUADRASORB, American). The separated GST-tagged proteins were detected with SDS-PAGE (Power PAC 300, China). The content of–SH group of the prepared nanospheres and the binding proteins concentration was analyzed by UV–vis spectrophotometer (nanodrop 2000c, America).

3. Results and discussion

3.1. TEM images of SiO_2-SH NSs<

A sereies of SiO_2-SH samples were prepared under different TEOS/MPS volume ratios so as to investigate the effects of reaction condition on the morphology of the SiO_2-SH products. As shown in Table 1 and Fig. 1, SiO_2-SH sample obtained at a TEOS/MPS volume ratio of 10:1 (TEOS 1.4 mL, MPS 0.14 mL) exhibits hollow spherical shape as well as an average diameter of about 45 nm and a wall

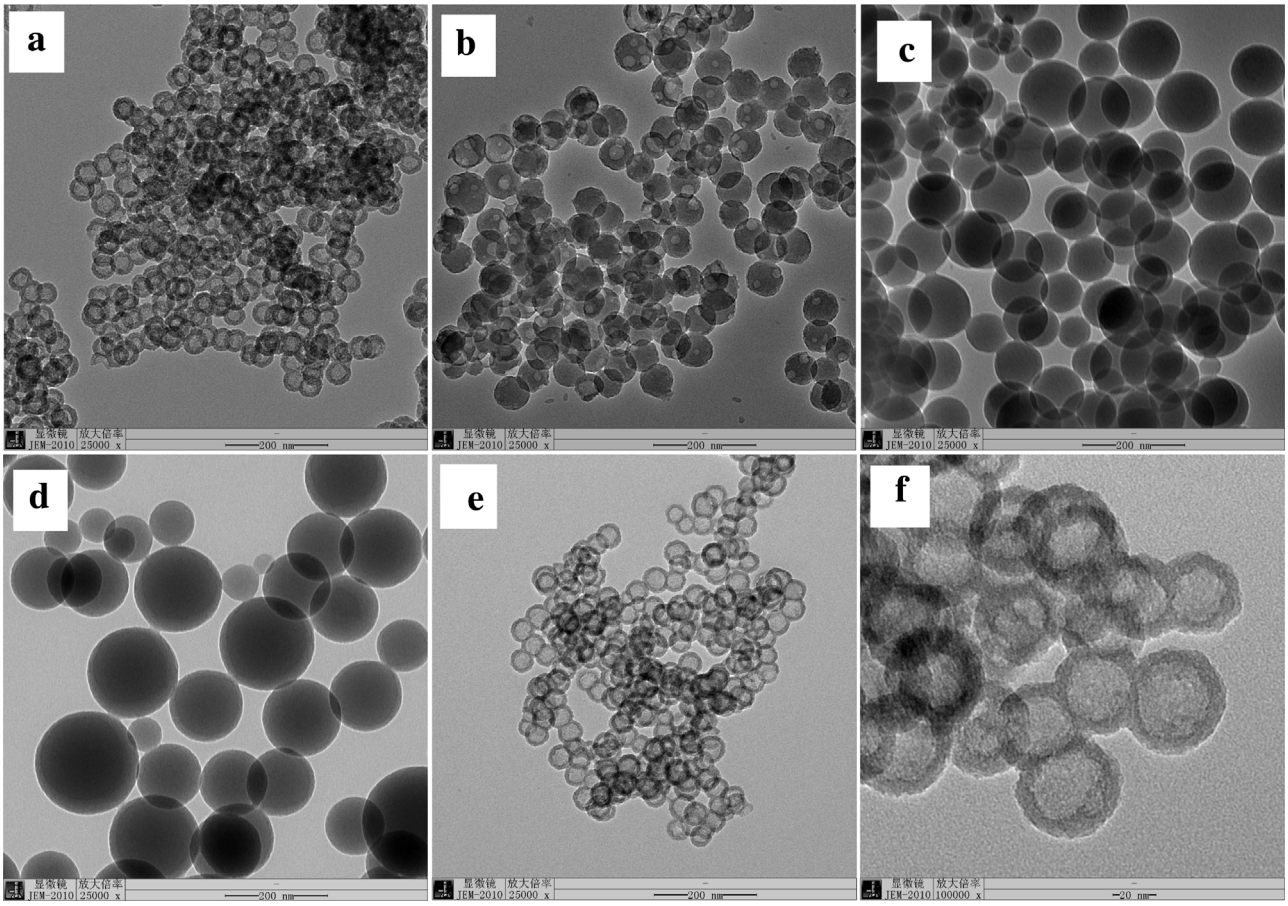

Fig. 1. TEM images of SiO_2-SH and SiO_2-GSH product obtained under different reaction conditions: a- SiO_2-SH at TEOS/MPS volume ratio 10:1, b- SiO_2-SH at TEOS/MPS volume ratio 1:1, c- SiO_2-SH at TEOS/MPS volume ratio 1:10, d- SiO_2-SH with 1.4 mL of MPS alone, e,f-the SiO_2-GSH NSs prepared from the SiO_2-SH obtained at a TEOS/MPS volume ratio of 10:1.