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Facile fabrication and selective detection for cysteine of xylan/Au nanoparticles composite

Yuqiong Luo[a,1], Zuguang Shen[a,1], Pai Liu[a,b], Lihong Zhao[a], Xiaoying Wang[a,*]

[a] State Key Laboratory of Pulp & Paper Engineering, South China University of Technology, 381 Wushan Road, Tianhe District, Guangzhou 510640, People's Republic of China

[b] School of Life Science, Beijing Institute of Technology, No. 5 Yard, Zhong Guan Cun South Street, Haidian District, Beijing 100083, People's Republic of China

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ABSTRACT

This work reported a facile and green method to prepare highly stable and uniformly distributed Au nanoparticles (AuNPs), using biopolymer xylan as stabilizing and reducing agent. Full characterizations were performed and the results revealed that AuNPs were well dispersed with the diameters of 10–30 nm. The optimal condition was as follows: the ratio of xylan to $HAuCl_4$ was 150 mg:15 mg, reaction temperature was 80 °C and reaction time was 40 min. The xylan/AuNPs composite exhibited highly selective and sensitive sensing of cysteine in aqueous solution, it could distinguish cysteine among dozens kinds of amino acids, and the limit of detection (LOD) for cysteine was calculated as 0.57 μM. Besides, the xylan/AuNPs composite was applied for Cys detection in human serum. This study provides a new way for high-value utilization of the rich biomass resource and a cheap, rapid and simple method for Cys detection in real biological samples.

1. Introduction

As a sulfur-containing amino acid, cysteine (Cys) plays a crucial biological role in the human body, including protein synthesis, detoxification and metabolism (Gazit, Ben-Abraham, Pick, Ben-Shlomo, & Katz, 2003). However, abnormal levels of Cys have been implicated in various diseases such as slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness (Shahrokhian, 2001). Various methods based on analytical techniques have been proposed for the detection of Cys, including HPLC, electrochemical method, fluorescence spectroscopy, FT-IR spectroscopy and so on (Tseng, Chen, & Ho, 2006). Most of them, however, require complicated instrument and sample preparation in certain cases, which limits the scope of their practical applications (Hou, Chen, Tang, & Long, 2014). Thus, there is an intense demand for a simple and rapid sensor for the detection of Cys with high sensitivity and specificity.

Recently, colorimetry using gold nanoparticles (AuNPs) emerges as a promising method for the Cys sensing based on their high extinction coefficient and the distance-dependent optical property (Zhang, Xu, Yuan, Yang, & Yang, 2011). The well-dispersed AuNPs solution is red color, but the aggregation of AuNPs results in a color change of AuNPs solution from red to blue/purple and a red shift in surface plasmon resonance (SPR) (Li, Hu, Liu, & Liu, 2011; Liu et al., 2010). Due to the low cost and simple experimental process, several studies based on designing colorimetric gold nanosensors have been reported. For example, Vinod Kumar and Philip Anthony (2014) have reported that AuNPs with different surfactants were synthesized and explored for cysteine colorimetric sensing, the modified AuNPs solution showed a color change after the addition of cysteine (10^{-7} M). Fu, Liu, Wu, and Zhang (2014) have proposed a sensitive and selective method based on the rhodamine B-covered gold nanoparticle with dual-readout (colorimetric and fluorometric) detection for L-cysteine, the detection limit was as low as 10 nM. Xiao et al. (2011) have reported the specific detection of cysteine and homocysteine (Hcy) in biological fluids by the fluorosurfactant-stabilized gold colloidal solution, the selective detection of Cys was as low as 1.0 μM in the presence of Hcy. However, in order to achieve satisfactory cysteine detection effect, the above AuNPs were synthesized using chemical reducing agents and modified with various chemical stabilizers. Therefore, it is promising to use the green synthesized AuNPs to detect cysteine.

Compared with physical methods and conventional chemical methods adapting sodium borohydrate or hydrazine as reducing agents, the approaches for biomass-assisted synthesis and stabilization of gold nanoparticles (AuNPs) have attracted much

* Corresponding author.
E-mail address: xyw@scut.edu.cn (X. Wang).
[1] The authors contributed to the work equally and should be regarded as co-first authors.

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Table 1
Xylan/AuNPs composite obtained under different reaction conditions of time, temperatures and ratios.

Samples	t (min)	T (°C)	Xylan:Au^{3+} (g:mg)	Samples	t (min)	T (°C)	Xylan:Au^{3+} (g:mg)
XylAu1	30	70	0.15:5	XylAu10	30	100	0.15:15
XylAu2	30	70	0.15:10	XylAu11	10	80	0.15:15
XylAu3	30	70	0.15:15	XylAu12	20	80	0.15:15
XylAu4	30	70	0.15:20	XylAu13	30	80	0.15:15
XylAu5	30	70	0.15:25	XylAu14	40	80	0.15:15
XylAu6	30	60	0.15:15	XylAu15	50	80	0.15:15
XylAu7	30	70	0.15:15	XylAu16	60	80	0.15:15
XylAu8	30	80	0.15:15	XylAu17	70	80	0.15:15
XylAu9	30	90	0.15:15	XylAu18	80	80	0.15:15

attention, since they are non-toxic, non-hazardous and environmental friendly approaches (Bhattacharya & Gupta, 2005; Gurunathan, Han, Park, & Kim, 2014; Ling, Luo, Luo, Wang, & Sun, 2013). Ganeshkumar, Ponrasu, Raja, Subamekala, and Suguna (2014) have synthesized pullulan stabilized gold nanoparticles for cancer targeted drug delivery. Lahr and Vikesland (2014) reported the intracellularly biosynthesised gold nanoparticles used in surface-enhanced Raman spectroscopy (SERS) cellular images. Engelbrekt et al. (2009) synthesized AuNPs using glucose and starch as reducing and protecting agents, respectively. However, the green synthesized AuNPs used for cysteine detection has not been reported up to now.

Furthermore, it is noted that among various biopolymers, hemicelluloses are gaining great attention as one of the most abundant forestry and agricultural residues (Xu & Huang, 2014). The hemicelluloses possess many hydroxyl groups and reducing aldehyde groups and are in the form of helical chains or random-coil chains in aqueous solution (Mazeau & Charlier, 2012). This provides a possibility for hemicelluloses to act as reducing agent and stabilizing agent by capping the generated nanoparticles in the special structure. But there is still no report about synthesizing AuNPs by adopting hemicelluloses as reducing and stabilizing agent.

In this paper, we investigated xylan—the model compound of hemicelluloses, acted as reducing and stabilizing agents for the preparation of AuNPs, and optimized the synthesis condition of AuNPs. Moreover, we discussed the L-cysteine detection effect of xylan/AuNPs composite in order to explore the practical value of the AuNPs in medical field.

2. Experiment

2.1. Materials and apparatus

Xylan isolated from bagasse was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Its *Mw* was 4.9×10^4 g/mol. The sugar composition (relative weight percent) was determined by the sugar analysis: 87.35% xylose, 9.28% arabinose, 0.81% glucose, 0.50% galactose and 2.06% glucuronic acid. Chloroauric acid hydrate ($HAuCl_4{\cdot}4H_2O$) was purchased from ShangHai Hushi Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade.

2.2. Preparation and characterization of xylan/AuNP composites

2.2.1. Preparation of xylan/AuNP composites

First, xylan (0.15 g) was dissolved in the 2% NaOH aqueous (3 mL) to obtain 5% xylan solution. Different amounts of $HAuCl_4{\cdot}4H_2O$ (5, 10, 15, 20, 25 mg) were dissolved in 12 mL of distilled water and heated to the reacted temperature. Then the xylan solution was added into the above $HAuCl_4$ solution, the mixture was reacted under 60–100 °C for 30–80 min. When color shifted from yellow to red wine, the xylan/AuNPs composites were obtained. The effects of different mass ratios of xylan to $HAuCl_4{\cdot}4H_2O$ (XylAu1–XylAu5), different reaction temperatures (XylAu6–XylAu10), and different reaction time (XylAu11–XylAu18) on the formation of AuNPs were investigated. And the samples are listed in Table 1.

2.2.2. Characterization of xylan/AuNP composites

UV–vis spectra were obtained by TU-1810 (Beijing, China) with scan range of 800–300 nm. Size distribution was carried out on a Zetasizer 3000HSA apparatus (Malvern, England). The concentration of colloidal xylan/AuNPs was fixed to be 0.1% (w/v). JEM-2010HR transmission electron microscopy (TEM) (JEOL, Japan) was used to investigate the microstructure of the AuNPs at an accelerating voltage of 200 kV. TEM samples were prepared by diluting colloidal xylan/AuNPs composite with water after sonication. Then every sample was dropped on a copper grid coated a layer of ultrathin carbon film.

The crystal structure of AuNPs was investigated by a D8 advance X-ray diffractometer (Bruker, Germany), in which the nickel-filtered Cu Kα radiation ($\lambda = 0.15406$ nm) was generated at 40 kV and 40 mA. Samples were scanned at a step size of 0.0195°/min in the range 5–90° (2θ).

X-ray photoelectron spectroscopy (XPS) analysis was recorded by Axis Ultra DLD (Kratos, UK) surface analysis equipment with Mg Kα ($h\gamma = 1253.6$ eV) in steps of 0.1 eV. The core-level data were analyzed with nonlinear fitting software (XPSPEAK 4.1).

2.3. Detection of L-Cys using xylan/AuNPs composites

2.3.1. Detection of L-Cys in distilled water

First, the obtained xylan/AuNPs composites solution was diluted 10 times with distilled water. Then, 0.5 mL of Cys solution with various concentrations (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μM) was added to the xylan/AuNPs composite solution (2.5 mL) respectively, and the corresponding UV–vis spectra were recorded.

The limit of detection (LOD) for Cys were calculated by $3S_0/S$, where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the blank measurements ($n = 12$), and S is the slope of the calibration curve (Zhang et al., 2011).

The selectivity for Cys was confirmed by adding other amino acid solutions (Val, His, Asn, Gly, Tyr, Met, Phe, Glu, Trp, Leu, Ile, Thr, Ala, Lys and Pro, 0.5 mL, 500 μM) to the xylan/AuNPs composite solution (2.5 mL) instead of Cys.

2.3.2. Detection of L-Cys in deproteinized plasma samples

The standard addition technique was used for the determination of Cys in human serum samples according to described procedures with slight modification (Wu et al., 2012). Human blood, obtained from healthy volunteers, was centrifuged at 4000 rpm for 30 min at 4 °C. The supernatant, containing proteins and amino acids amongst other components, was used as the source of the plasma. Then 1.2 mL acetonitrile was added to a 2 mL serum sample, followed