Biosensors and Bioelectronics 49 (2013) 249–255

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Facile one-pot synthesis of L-proline-stabilized fluorescent gold nanoclusters and its application as sensing probes for serum iron

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ARTICLE INFO

Article history:
Received 3 December 2012
Received in revised form
7 May 2013
Accepted 19 May 2013
Available online 25 May 2013

Keywords:
L-proline
Gold nanocluster
Fluorescence
Ferric ion
Serum iron

ABSTRACT

Gold nanoclusters (Au NCs) possess outstanding physical and chemical attributes that make them excellent scaffolds for the construction of novel chemical and biological sensors. In this study, a simple one-pot synthesis method, employing L-proline as the stabilizer, was presented for preparation of fluorescent Au NCs. This strategy allowed the generation of water-soluble Au NCs within a short time of 15 min. The as-prepared Au NCs exhibited a bluish fluorescence emission at 440 nm and a quantum yield of 2.94%. Based on the aggregation-induced fluorescence quenching mechanism, the Au NCs provided favorable biocompatibility, high sensitivity and good selectivity for the measurement of ferric ion (Fe^{3+}). Furthermore, serum samples were analyzed for the serum iron contents by using this proposed biocompatible fluorescent sensor, indicating the potential value of this Au NCs-based fluorescent sensor for application in biological and clinical analysis.

1. Introduction

Noble metal nanoclusters (NCs) typically possess sizes less than 2 nm and have attracted substantial research interest in the fields of chemistry, materials, biology, and medicine (Han and Wang, 2011; Wen et al., 2011; Wu and Jin, 2010; Negishi et al., 2004; Lin and Tseng, 2010). Their fundamental physicochemical properties are much dependent on the sub-nanometer structure (Xie et al., 2009; Zhu et al., 2008). When the size is comparable to the Fermi wavelength of an electron, the continuous density of states breaks into discrete energy levels, and these metal NCs exhibit molecule-like properties in the absorption and fluorescence features (Zheng et al., 2003; Bao et al., 2007). Compared with the quantum dots, which have larger size (about 3 to 100 nm) and commonly consist of some toxic metal species, noble metal NCs show great superiority for fabrication of sensitive and efficient biological sensors due to their ultra-small size, favourable biocompatibility, high fluorescence and non-toxicity (Lin et al., 2009a, 2009b; Derfus et al., 2004; Guo et al., 2009). Therefore, it is of great significance to facilely functionalize the metal NCs and further broaden their application as promising biosensors.

Up to now, there has been a great deal of research work focused on metal NCs, especially Au NCs (Shang and Nienhaus, 2012; Shang et al., 2011). Numerous preparative protocols for Au NCs have been developed over the past few decades, including both "top–down" and "bottom-up" methods (Ott and Finke, 2007). For "top–down" manufacturing approach, Au NCs in smaller sizes are introduced by etching the crystals in larger sizes during various scaling-down processes (Saheb et al., 2008). And for "bottom-up" fabrication approach, the nano-structures are formed by assembling individual atoms one-by-one, into a multi-atom unit, by a more or less controlled growth process (Seeman and Belcher, 2002). However, most of these methods involved multiple reaction steps and needed some non-biocompatible precursors or etching reagents, resulting in limited application (Yang et al., 2011).

Nowadays, considerable efforts have been devoted to exploration of facile one-pot synthesis strategies mostly focused on control over the size, shape, stability, functionality and solubility of Au NCs (Liu et al., 2008; Chen et al., 2009). For example, monodisperse Au NCs with high quantum yield (QY) have been successfully synthesized by using a poly(amidoamine) dendrimer as the template (Zheng et al., 2003). Thiol-protected Au NCs with size less than 1.2 nm have been reported and showed favourable potential for further application (Wu et al., 2009). In addition to these as-described chemical species, biological macromolecules with naturally defined structures such as DNA, peptide and protein have been continuously used as the ligands to induce the nucleation and growth of nano-crystals based on biomineralization, or as the templates to construct nano-structures due to their inherent reducing properties (Petty et al., 2004; Mao et al., 2004; Liu et al.,

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http://dx.doi.org/10.1016/j.bios.2013.05.019

2012; Baron et al., 2005; Xie et al., 2009; Jin et al., 2011). Recently, some biological micromolecules were testified to be good reducing reagents exhibiting both stabilizing and protecting behavior for fabricating Au NCs in a facile process (Ho et al., 2012; Yu et al., 2012). Considering the advantages of easy synthesis, low cost, good biocompatibility and chiral structure, amino acids are expected to be the favourable and powerful reagents for functionalization of Au NCs. Especially considering the inherent reducing ability of some amino acids, it is highly promising for one-pot synthesis of amino acids-stabilized Au NCs without additional reducing reagents (such as borohydride or hydroxylamine hydrochloride) (Ho et al., 2012).

Furthermore, as an essential component of iron–sulfur protein and heme groups, iron is the most affluent transition metal in cellular systems and is of outstanding significance owing to its essential roles in biological systems, including oxygen transportation, oxygen metabolism, transcriptional regulation and electron transfer (Aisen et al., 1999; Lohani and Lee, 2010). Both the iron deficiency and overload can lead to various diseases, such as anemia, Parkinson's syndrome, Alzheimer's disease and cancer (Zecca et al., 2004; Altamura and Muckenthaler, 2009; Wang et al., 2012). Serum iron content is an objective assessment of human nutritional status and for diagnosis of some relevant diseases, such as anemia and liver diseases, thus much efforts have been focused on development of powerful protocols for routine determination of serum iron (Klein et al., 1970; O'Malley et al., 1970). So far, several methods, such as UV spectrometry and atomic absorption spectrophotometry, have been developed for the detection of serum iron (Mikac-Dević, 1969; Kingsley and Getchell, 1956; Rodgerson and Helfer, 1966; Olson and Hamlin, 1969). However, these techniques often require a long analysis time, significant special skills and complicated procedures (Valcour et al., 1990; Fujita et al., 1994). Fluorescent spectrometry, in contrast, has proved to be a more powerful optical method for trace analysis of important biological samples due to its low cost, high sensitivity and rapid implementation (Wang et al., 2012; Xiang and Tong, 2006; Lee et al., 2011).

In this work, we demonstrate a facile one-pot strategy to successfully synthesize the water-soluble, stable and light blue-emitting Au NCs stabilized by L-proline (Scheme 1). Compared to the previously reported protocols, the prepared Au NCs showed obvious superiority. First, the Au NCs could be obtained by both "heating method" and "ambient temperature method". It is noteworthy that the heating synthesis approach was really convenient and rapid, exempted from pressuring, special treatment and media. Second, these reactions are green and atom-economic processes, only involving two reactants of $HAuCl_4$ and L-proline without extra catalysts or templates. To the best of our knowledge, this is the first report for synthesis of L-proline-stabilized Au NCs through a simple reaction. Further, the resultant Au NCs were applied as the selective sensor for serum iron. Moreover, the synthesis of biomolecule-protected Au NCs could promote future research and practical application of this novel type of NCs materials in real sample analysis.

2. Experimental

2.1. Chemicals and materials

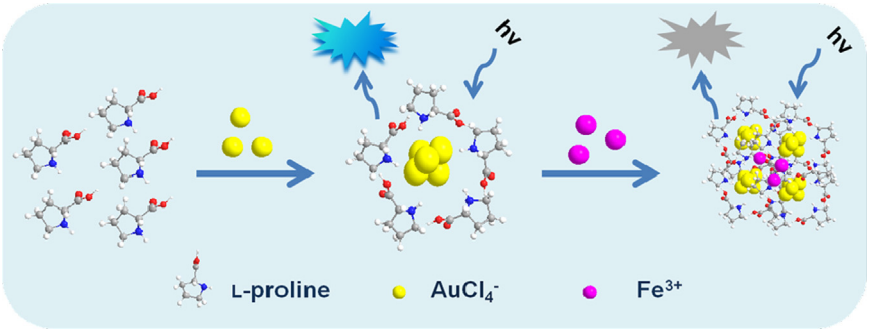
L-proline, trichloroacetic acid, 4-aminoantipyrine, ammonium iron(III) sulfate dodecahydrate, manganese(II) chloride tetrahydrate, copper(I) chloride, zinc chloride, cadmium chloride hemi (pentahydrate), chromium(III) chloride, silver nitrate, lead(II) nitrate, cobalt(II) nitrate hexahydrate, nickel(II) nitrate hexahydrate and iron(II) chloride tetrahydrate were all purchased from Sigma-Aldrich (St. Louis, MO). Quinine sulfate dihydrate, sodium chloride, potassium chloride, aluminum chloride, copper(II) nitrate trihydrate, magnesium chloride hexahydrate, calcium chloride and ethylenediaminetetraacetic acid disodium salt dihydrate were from Aladdin Chemistry Company (Shanghai, China). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), mercuric chloride, sodium acetate, hydrogen peroxide solution (30%), sodium hydroxide, hydrochloric acid, acetic acid and nitric acid were bought from Beijing Chemical Factory (Beijing, China). All chemicals used in this work were of analytical grade and used as received. Water was purified using a Milli-Q-system (Millipore, Bedford, MA, USA). The normal human serum samples were kindly donated by the volunteers.

2.2. Instrumentation

Absorption spectra were recorded using a TU-1900 UV–vis double-beam spectrometer (Purkinje General, China). All fluorescence measurements were performed using an F-4500 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence lifetimes were measured with a compact fluorescence lifetime spectrometer C11367 (Hamamatsu, Japan) at room temperature. Electrospray ionization (ESI) mass spectra of Au NCs solutions were conducted on a Micromass quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, USA). The spectrum was collected in the positive mode. Transmission electron microscopy (TEM) images were taken on a JEM-2010 (Jeol Ltd, Japan) at an accelerating voltage of 100 kV. The TEM specimens were prepared by dropping the sample solutions onto carbon coated copper grids.

2.3. Synthesis of Au NCs

All glassware used in this work was thoroughly cleaned with freshly prepared aqua regia (3 parts HCl and 1 part HNO_3) and rinsed with water prior to use. The fluorescent Au NCs were prepared by both "heated method" and "ambient temperature method". For the former method, briefly, the L-proline solution



Scheme 1. Schematic of the formation and the Fe^{3+}-mediated fluorescence quenching of Au NCs.