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Journal of Colloid and Interface Science 321 (2008) 484–492
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Synthesis of cobalt ferrite core/metallic shell nanoparticles for the development of a specific PNA/DNA biosensor

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Received 25 January 2008; accepted 6 February 2008

Available online 13 February 2008

Abstract

Controlled synthesis of cobalt ferrite superparamagnetic nanoparticles covered with a gold shell has been achieved by an affinity and trap strategy. Magnetic nanoparticles are functionalized with a mixture of amino and thiol groups that facilitate the electrostatic attraction and further chemisorption of gold nanoparticles, respectively. Using these nanoparticles as seeds, a complete coating shell is achieved by gold salt-iterative reduction leading to monodisperse water-soluble gold-covered magnetic nanoparticles, with an average diameter ranging from 21 to 29 nm. These constitute a versatile platform for immobilization of biomolecules *via* thiol chemistry, which is exemplified by the immobilization of peptide nucleic acid (PNA) oligomers that specifically hybridize with complementary DNA molecules in solution. Hybridation with DNA probes has been measured using Rhodamine 6G fluorescence marker and the detection of a single nucleotide mutation has been achieved. These results suggest the PNA-nanoparticles application as a biosensor for DNA genotyping avoiding commonly time-consuming procedures employed.

Keywords: Magnetic nanoparticle; Gold shell; Peptide nucleic acid; DNA–PNA hybridization; Mutation detection; Rhodamine 6G

1. Introduction

In recent years, the synthesis of composite magnetic nanomaterials has received increasing attention due to their electronic, magnetic, catalytic and chemical or biological sensing properties [1]. Currently, magnetic nanoparticles offer widespread applications in biotechnology, such as deoxyribonucleic (DNA) and ribonucleic (RNA) acid purification [2], cell separation, drug delivery, magnetic resonance imaging [3] and hyperthermia cancer treatments [4,5]. In this sense, magnetic nanoparticles show high magnetic susceptibility and good dispersibility, however resistance to oxidation and stability in physiological environments are also required [5]. Covering magnetic nanoparticles with an external inert shell such as gold has been attempted in order to add new properties (inertness, protection of the magnetic core against oxidation) to those of the particles, without modifying their superparamagnetic behavior [6–8]. Moreover, gold allows a number of possibilities for detection and provides an optimized platform for chemical functionalization through the attachment of thiolated molecules, which form self-assembled monolayers (SAMs). Therefore, surface-modified nanoparticles constitute a fully functional material that can be used either as an *in vivo* probe or as a nanostructured biosensor [9]. Although magnetic and metallic nanoparticles have been widely investigated [10,11], few works have been published on the development of a combined approach to achieve this composite material [12–15]. Some methods commonly used for obtaining metal oxide core/Au shell magnetic nanoparticles are based on a reversed micelle system. These systems are less suitable for biological applications due to the use of harmful surfactants or organic solvents [16–18]. Recently, a water-based synthesis method involving ultravio-

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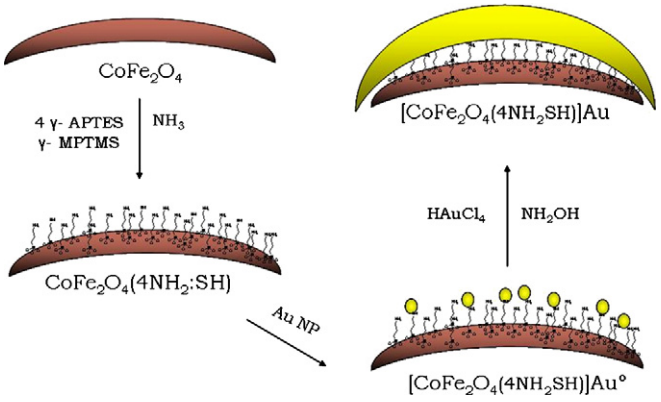
0021-9797/$ – see front matter
doi:10.1016/j.jcis.2008.02.010



Scheme 1. Reaction strategy showing the successive steps for gold covering process onto 4:1 amino:mercapto functionalized cobalt ferrite nanoparticles.

let (UV) radiation was used to cover iron oxide/titanium oxide nanoparticles with gold [11], however it yielded incomplete gold coverage and required several complicated steps.

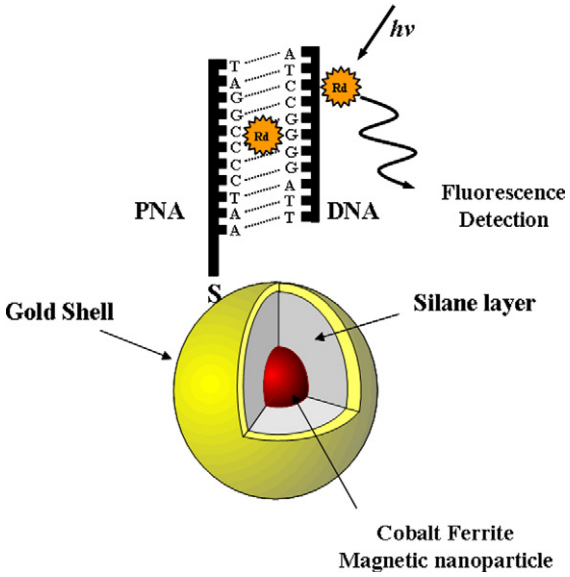
Here we describe the synthesis of water-soluble gold-covered magnetic nanoparticles as well as their use for the development of specific DNA biosensors. One of the present work aims has been the optimization of the metallic covering stage which was achieved by bonding gold seeds through an affinity and trap strategy (Scheme 1). Magnetic nanoparticles are functionalized with a mixture of amino and thiol groups that facilitate the electrostatic attraction and further chemisorption of the gold nanoparticles, respectively. Using these gold nanoparticles as seeds, a complete coating shell is achieved by gold salt-iterative reduction [19].

Thiol-modified single stranded peptide nucleic acid (ssPNA) oligomers can be directly immobilized onto Au-magnetic nanoparticles following a procedure previously optimized for the formation of PNA SAMs on gold surfaces [20]. These PNA-modified, gold-covered magnetic nanoparticles can interact specifically with a DNA target molecule complementary to the attached PNA probe (Scheme 2). The specific PNA–DNA hybridization is monitored by the detection of an external fluorescent molecule, Rhodamine 6G, which intercalates in the PNA–DNA double helix and also associates electrostatically to the phosphate groups present in the DNA backbone [21].

2. Experimental

2.1. Materials

All chemicals were used as purchased. $CoCl_2{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, γ-aminopropyltriethoxysilane (APTES), γ-mercaptopropyltrimethoxysilane (MPTMS) and $HAuCl_4$ were acquired from Sigma with ACS Reagent purity grade. Tetrakis (hydroxymethyl)phosphonium chloride (THPC) and hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) were purchased to Fluka. The ssPNA molecule used was supplied by Isogen Life Science. It contained a sequence of 11 nucleotides (nt) complementary to a characteristic region of one of the proteins that constitute the capsid of the foot-and-mouth disease virus (FMDV). The PNA sequence is: Cys-O-O-AATCCCCGGAT where each of the two "O" spacer units is a 1.5 nm long molecule of 8-amino-3,6-dioxaoctanoic acid, and the amino acid cysteine (Cys) that provides the terminal thiol group to the PNA molecule that allows further chemisorption on a gold surface. ssDNA oligomers containing the fully complementary sequence (5′-ATCCGGGGATT-3′) (HIBR), or with an oligomer identical in all positions but the central one, with a single nucleotide mutation (5′-ATCCGAGGATT-3′) (SMM) were purchased to Isogen Life Science.



Scheme 2. Functionalized gold shell–cobalt ferrite nanoparticles with thiolated PNA for hybridation with complementary DNA target molecules and further fluorescence detection by intercalated or electrostatically associated Rhodamine 6G.

2.2. Synthesis of cobalt ferrite magnetic nanoparticles

The cobalt ferrite synthesis was carried out according to previous work [22–24]. 5 mL of 2 M $CoCl_2{\cdot}6H_2O$ (Sigma, ACS reagent) in HCl 7.4% solution and 40 mL of 0.5 M $FeCl_3{\cdot}6H_2O$ in Milli Q water were pre-heated to 50 °C, mixed and poured into a boiling solution of 200 mL 1 M NaOH under vigorous stirring. The boiling was maintained for 30 min and the solution was cooled down to room temperature without stirring. After five water-cleaning stages by magnetic sedimenting and decanting of supernatant, the ferrofluid was treated with an oxidative reaction to passivate the surface by redispersion in 30 mL of 2 M HNO_3 solution containing 0.35 M $Fe(NO_3)_3{\cdot}9H_2O$ and heating to 100 °C for 45 min under continuous stirring. The resulting product was magnetically sedimented overnight. Finally, the supernatant was decanted and substituted by 100 mL of Milli Q water.

2.3. Functionalization of magnetic nanoparticles with amine and thiol groups

The reaction was carried out at room temperature by mixing in a round-bottomed flask 288 mL of absolute ethanol, 2 mL of the cobalt-ferrite ferrofluid ($\approx$16 mg mL^{-1}) and 12 mL of 30%