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Design of green magneto-fluorescent γ-Fe_2O_3-methyldopa conjugate nanocrystal as a targeted probe for monitoring of esterase activity

Nahid Shahabadi [a,b,*], Maryam Maghsudi [a], Leila Nemati [a]

[a] Department of Inorganic Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran
[b] Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

One of the most important aspects of the biological systems is the retention of HSA activity. It is known that serum albumin, in addition to ligand binding capabilities, possesses some enzymatic properties such as esterase activity with p-nitrophenyl acetate substrate. The aim of this study was to synthesize and characterize the mono-dispersed magneto-fluorescent methyldopa coated (MNPs-MDP) which provides a unique opportunity to control and monitor the biological interactions by using magnetic force. An Organic fluorophore methyldopa (2-amino-3-(3,4-dihydroxyphenyl)-2-methyl acid, propanoic) (MDP) was introduced into γ-Fe_2O_3 particles and made the fluorescent and stable colloidal nanocrystals. As a biological host, human serum albumin (HSA) was chosen which is a major constituent of soluble human blood plasma proteins and is therefore considered as a suitable target for nanoparticle–protein interaction studies. MDP-γ-Fe_2O_3 nanocrystals showed inherent properties including excellent water solubility, and longtime stability against aggregation, biocompatibility and multifunctional surface rich in carboxyl groups. In addition, we tried to assess the influence of PMDP-γ-Fe_2O_3 binding on the activity of HSA. Such MDP-γ-Fe_2O_3 showed an increase in esterase activity in comparison with the free HSA. This method therefore provides a unique platform for preserving the protein structure and conformation.

1. Introduction

It is known that nanoparticles, when exposed to the biological fluid, coated with proteins and other biomolecules, but these nanoparticle–protein interactions have still poorly understood and the studies to characterize them are few in number. In biomedical applications, solubility and biocompatibility are among the crucial requirements. A significant challenge for the safety of nanoparticles in biomedical applications, either diagnostic or therapeutics, is that the human plasma proteins can be adsorbed onto the surface of nanoparticles which can cause the transmission of biological effects due to changes in protein conformation [1]. The conformational changes of the proteins can alter their functions and molecular mechanisms, and could contribute to disease pathogenesis [2].

Any practical applications of nanoparticles in biological fluids, such as blood plasma, should ensure the mono-dispersity of the material under such harsh conditions, as particle agglomeration and sedimentation [3] may affect the bio-distribution of the material and lead to harmful side effects [4]. Bare magnetic nanoparticles often tend to aggregate in tissue fluids. Therefore, improvement of the water dispersity and biocompatibility of magnetic nanoparticles is necessary. In this content, water-soluble luminescent nanoparticles may be a promising candidate for bio-labeling applications.

Issues of biological targeting, bio-distribution and in vivo toxicity of nanomaterials in general, highly depend on the stability of such nanocrystals in complex biological media. Therefore, additional processing using of ligand exchange is required to transfer these materials to buffer media and to impart biocompatibility. Dopamine (DA) is an important neurotransmitter in the brain containing both of catechol and amine functional groups. It is also the main adhesion composition of mussel foot proteins which can self-polymerize under alkaline conditions and generate polydopamine (PDA) organic nanoparticles [5]. The possible mechanism for self-polymerization of DA is considered to be the oxidation of catechol in an alkaline environment [6] due to the unique physicochemical properties and excellent biocompatibility of PDA [7]. Methyldopa (2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid) (MDP) is a catechol derivative (catecholamine) which is widely used as an antihypertensive agent. It

* Corresponding author at: Department of Inorganic Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran, Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail address: nahidshahabadi@yahoo.com (N. Shahabadi).

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has recently been demonstrated that catechol derivatives such as the neurotransmitter dopamine and L-3,4-dihydroxyphenylalanine (L-Dopa), a precursor to dopamine that is also used as a component of adhesives generated by marine mussels, exhibit strong affinity to metal oxide nanocrystals [5]. MDP was chosen because the chemical difference between dopamine and other investigated catechol derivatives is only in one carboxyl group, encouraging us to review the role of carboxylates and methyl groups in iron oxide binding of this anchor (Scheme 1). Magnetic nanocrystal was prepared by a simple two step reaction: first, hydrophobic oleic acid (OA)–Fe_3O_4 was synthesized via the thermal decomposition of $Fe(OA)_3$ in tetradecene solvent in the presence of oleic acid ligand and second water-soluble MDP-γ-Fe_2O_3 was obtained by an one-step ligand exchange process. The strategic approach to modify the surface composition is ligand exchange.

The objective of this study was to investigate the interaction of MDP-γ-Fe_2O_3 with biological host, namely human serum albumin (HSA), to show the result of conformational or structural changes and its influence on the activity of. To date, no research has been focused on investigation of the green fluorescence coated with the amphiphilicanti-hypertensive polymer of drug shell for ultra-sensing of the esterase activity in the biological host. Due to high affinity of protein to bind to various surfaces, it is often used as a blocking agent. According to the complexity of this protein, its interaction with nanoparticles is by no means trivial. It is difficult to deliver a generalized statement for protein nanostructure interactions. It is probably due to an ensemble of various parameters having significant impacts on protein–nanoparticle interactions. Essentially, for biomedical applications, nanoparticles in general should not have any adverse effect on the structure and activity of the biological milieu. Any conformational changes in protein would lead to loss of biological activity or altered immune response and our results would have implications for bio applications involving nanoparticles. To design more efficient detection assays based on HSA–MDP-γ-Fe_2O_3 interactions, HSA binds to the negatively charged nanoparticles with nanomolar affinity.

Zeta potential and ultraviolet–visible spectrophotometry were employed to monitor the colloidal stability of MDP-γ-Fe_2O_3 nanoparticles at different pH, different buffers and different concentrations of the NaCl. The ligand exchange on the nanoparticle surface was characterized by using Transmission Electron Microscope (TEM) images, Dynamic Light Scattering (DLS), FT-IR Fourier Transform Infrared, UV–Vis spectroscopy, and zeta potential measurements. For quantitative assessment of HSA adsorption onto the fluorescent nanoparticles, further studies are needed and fluorescence and circular dichroism (CD) techniques are very important in this field. The esterase activity of HSA in the presence of MDP-γ-Fe_2O_3 under semi-physiological conditions (pH 7.4, 37 °C) was highlighted.

2. Experimental

2.1. Materials and methods

Iron(III) chloride, $Fe(Cl)_3$, oleic acid (OA), NaOH, tetradecene and dimethyl sulfoxide (DMSO) were purchased from Merck. MDP was purchased from Aldrich Chemical Co. HEPES, PBS, Tris–HCl and p-nitrophenyl acetate (PNPA) was purchased from Sigma–Aldrich.

The particle size and morphology was performed using Transmission Electronic Microscopy (TEM). Dynamic Light Scattering and ξ-potential measurements were carried out with a Nano ZS (red badge) ZEN 3600 (Malvern) with a laser wavelength of 633 nm and a Zeta sizer Malvern Nano ZS instrument. DLS was performed to evaluate the hydrodynamic diameter of suspended particles in a solution. Zeta potential was recorded for the solution of MNPs in water with pH values ranging from 1.5 to 12. Stability of the MDP-γ-Fe_2O_3 in various buffers (HEPES, Tris–HCl and PBS) was studied by UV–vis absorption spectra. FT-IR spectra of the MDP-γ-Fe_2O_3, OA–Fe_3O_4 and MDP were carried on a Bruker ALPHA, and prior to KBr-pellet fabrication; the samples were dried in a vacuum.

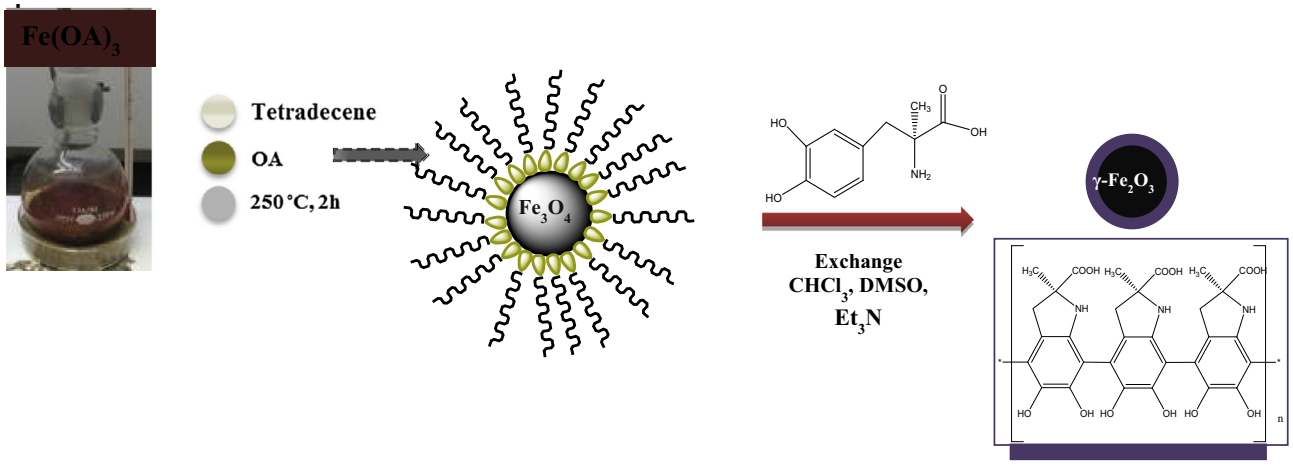
Cyclic voltammetry (CV) measurements were performed using a μ-Autolab TYPE III (Eco Chemie B.V., Ultrecht, The Netherlands) and driven by the NOVA software (Version 1.8) in conjunction with a conventional three-electrode system and a personal computer for data storage and processing. UV–Vis spectroscopy was conducted on UV Win T80 spectrophotometer.

The effect of MDP-γ-Fe_2O_3 on the esterase activity of HSA was measured with the synthetic substrate p-nitrophenyl acetate (PNPA). The formation of p-nitrophenol was monitored at λ_{abs} = 400 nm (molar absorption coefficient, ε = 17700 M^{-1} cm^{-1}) on UV Win T80 spectrophotometer.

CD measurements were performed by a Jasco J-810 spectropolarimeter. The concentration of HSA was fixed (3 μM) and the MDP-γ-Fe_2O_3 concentration varied from 0 to 100 μg/mL. The secondary structural data of the CD spectra were analyzed using CDNN deconvolution program.

Fluorescence measurements were recorded on a JASCO spectrofluorimeter (FP 6200).

For statistical analyses, replicated data from at least two or three experiments were taken into account.



Scheme 1. The synthetic route for making the OA–Fe_3O_4 and one-step exchange of making the MDP-γ-Fe_2O_3 nano crystals.