



Spectrofluorometric and thermal gravimetric study on binding interaction of thiabendazole with hemoglobin on epoxy-functionalized magnetic nanoparticles

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ARTICLE INFO

Article history:

Received 22 November 2014
Received in revised form 26 March 2015
Accepted 2 May 2015
Available online 5 May 2015

Keywords:

Magnetic nanoparticles
Hemoglobin
Thiabendazole
Stern–Volmer
Horowitz–Metzger

ABSTRACT

The interaction of thiabendazole (Tbz) with hemoglobin (Hb) on epoxy-functionalized iron oxide nanoparticles was presented in this study. The binding capacity of Tbz was determined by measuring at an excitation wavelength of 299 nm using fluorescence spectroscopy. The thermodynamic parameters of the Hb–Tbz interaction were calculated from Stern–Volmer and van't Hoff equations. The values of enthalpy change, ΔH , and entropy change, ΔS , were found to be 0.20 kJ mol^{-1} and $0.70 \text{ J mol}^{-1} \text{ K}^{-1}$, respectively, which indicates that the hydrophilic interaction plays a main role in the binding process. The interaction ability was confirmed by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). Also, the thermal behavior of the Hb–Tbz interaction on functionalized iron oxide nanoparticles was studied by using the thermogravimetric analysis (TGA) technique in the temperature range of 25–950 °C, and then the kinetic parameters for the thermal decomposition were determined using the Horowitz–Metzger method.

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1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) have wide applications in many fields due to their magnetism and low toxicity. Magnetic nanoparticles also offer many attractive possibilities for biomedical applications in medical diagnosis and therapy, such as magnetic resonance imaging, hyperthermia, magnetic separation, and transportation of drugs to the places of diseases, due to their biocompatibility [1]. Non-covalent binding of magnetic nanoparticles to biological macromolecules like proteins, enzymes and drugs which has been recently described, provides several biological aspects such as binding constant and site stoichiometry [2,3]. Magnetic separation is a recently developing technology and mostly applied in the field of bioseparation. The principle of this method is to utilize magnetic particles to bind the target molecules via a ligand to form a complex that can be separated from the bulk solution by magnetic field gradient which is a variation in the magnetic field with respect to position. Compared to conventional separation, the advantages of magnetic separation are attributed to its speed, accuracy, and simplicity [3]. The surface of Fe_3O_4 nanoparticles can be modified with functional molecules such as silane compounds and crosslinkers such as thioglycolic acid, oleic acids and N,N' -methylenebisacrylamide for enhancing their stability, dispersity and adjusting surface properties like hydrophilicity [4–6].

Hemoglobin (Hb) has been one of the most extensively studied proteins. It is well known for being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [7]. Hb is the major hemoprotein of the red blood cell, which can reversibly bind with many kinds of endogenous and exogenous agents [8]. The emission of tryptophan is highly sensitive to its local environment, and is thus often used as a reporter group for protein conformational changes [9]. Fluorescence quenching is an important method to study the interaction of substances with protein because it is sensitive and relatively easy to use [10–12]. In literature, most studied proteins for drug binding are bovine serum albumin and human serum albumin. However, there are several studies on interactions between hemoglobin and drugs that have been reported by fluorescence and thermogravimetry techniques [12,13]. Identification of interactions between drugs and hemoglobin is very crucial since these interactions may have undesirable effects on their main function. Studies on interactions between albumin and drugs have been widely reported. But, the reports on hemoglobin are very limited. Identification of the interactions may help us understand and prevent the side effect of the drugs in human metabolism. This study reveals that the material has still a good potential for the planning of new applications. Hemoglobin which is an abundant protein in serum plasma was used for the preparation of a protein–iron oxide nanoparticle system as a drug carrier. Superparamagnetic iron oxide nanoparticle–protein biocompatible aggregates served as a nanocarrier for the hydrophobic anticancer drug thiabendazole. In considering the role of this protein in drug metabolism, the determination of the amount of drug that interacted

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with this serum protein can provide information about the structural characteristic and the therapeutic effectiveness of the drug. The analysis of thermal decomposition is an important tool for estimating thermal stability and calculating the kinetic parameters of nanoparticle–biomolecule systems. In this study, the interaction of Tbz with Hb was evaluated by using these techniques. The characterization of the protein–nanoparticle system was also examined by an FT-IR spectrometer and a scanning electron microscope (SEM).

2. Experimental

2.1. Materials

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, >99%), ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, >99%), [3-(2,3-epoxypropoxy)-propyl]-trimethoxy silane, sodium hydroxide ($\geq 97\%$), hydrochloric acid (37%), tris (hydroxymethyl)-aminomethane (99.8–100.1%), ammonium hydroxide (25% w/w) and ethanol (>99.2%) were obtained from Merck (Germany). Thiabendazole and hemoglobin were obtained from Sigma (USA). All aqueous solutions were prepared with deionized water via a Millipore Milli-Q Plus water purification system. All chemicals were of analytical and molecular grade.

2.2. Instrumentation

All fluorescence measurements were carried out on a Perkin-Elmer LS 55 Luminescence Spectrometer (Waltham, USA). The emission spectra were scanned from 200 to 800 nm. The excitation and emission slit width were both set to 5 nm. Thermogravimetric analysis was performed with a Setaram SETSYS thermal analyzer at a temperature range of 25–950 °C at a heating rate of 10 °C min^{-1} under an argon atmosphere with a gas flow rate of 20 mL min^{-1} . FT-IR spectra were recorded on a Perkin-Elmer spectrum 100 FT-IR spectrometer (ATR) (Perkin-Elmer Inc., USA). The magnetization curves of samples were measured with a vibrating sample magnetometer (VSM, LakeShore-7407, USA) at room temperature. SEM images were obtained using a Zeiss LS-10 field emission SEM instrument equipped with an Inca Energy 350 X-Max (Oxford Instruments) spectrometer. Samples were sputter-coated with a Au (60%) and Pd (40%) alloy using a Q150R (Quorum Technologies) instrument. Images were obtained at 3×10^{-4} Pa working pressure and 15 keV accelerating voltage using InLens detection mode (2 mm working distance). The size and shape of the SPIONs was determined by transmission electron microscopy (TEM, FEI Company-Tecnai™ G2 Spirit/Biotwin).

2.3. Synthesis and modification of SPIONs

SPIONs were prepared as reported elsewhere [14], with some alterations: 2.0 mL of 1 M FeCl_3 and 0.5 mL of 2 M FeCl_2 aqueous solutions were mixed and stirred vigorously, and then 25 mL of 0.5 M aqueous NaOH was added dropwise while the mixture was stirred, resulting in the formation of an iron oxide precipitate. The precipitate was then separated using a magnet and washed extensively with water until the supernatant reached pH 7. The obtained nanoparticles were dried at 70 °C.

SPIONs were sonicated in 150 mL of dry toluene for 30 min to achieve a uniform dispersion. Then, an adequate amount of [3-(2,3-epoxypropoxy)-propyl]-triethoxysilane (GPTS) was added to the solution under N_2 atmosphere at 80 °C and stirred for 6 h. The optimal surface modification molar ratio of silane to SPIONs was found to be 4:1 [15]. After that, the solution was cooled to room temperature. The prepared GPTS-SPIONs were collected with a magnet, and then washed with toluene and ethanol three times. Finally, the silane modified SPIONs were dried under vacuum at 70 °C.

2.4. Immobilization of Hb

Functionalized SPIONs at the particle concentration range of 2.5–25 mg mL^{-1} were mixed with 1 mg mL^{-1} of hemoglobin (Hb) in 2 mL of 20 mM $\times$ Tris-HCl (pH 7.4, in 150 mM NaCl) for 2 h at 4 °C [13]. Hb immobilized GPTS-SPIONs were separated magnetically from the supernatant. Then, Hb immobilized particles were washed with 20 mM $\times$ Tris-HCl (pH 7.4) twice. The supernatant was kept at 4 °C for the analysis of protein concentration. The amount of immobilized Hb was measured by using fluorescence spectroscopy. For this aim, the intrinsic fluorescence of the protein was recorded at 280 and 342 nm of excitation and emission wavelengths [16]. Protein concentration after immobilization was determined from the regression equation of the calibration curve.

2.5. Binding of Tbz

1 mM of standard solution of thiabendazole was prepared by dissolving in a solvent mixture of 20 mM $\times$ Tris-HCl (pH 7.4)-DMF (90%–10%, v/v). Excitation and emission wavelengths of the drug were scanned by fluorescence spectroscopy. Drug solution was added to the appropriate amount of Hb immobilized GPTS-SPIONs in 20 mM $\times$ Tris-HCl (pH 7.4). Each mixture was mixed for 30 min at a particle concentration range of 2.5–25 mg mL^{-1} [13]. The nanoparticles were separated using a 0.5 T magnet. Emission spectra of the remaining solution were recorded at 299 nm and 358 nm of excitation and emission wavelengths. The binding amount of the drug was determined from the regression equation of the calibration curve.

3. Results and discussion

3.1. Characterization

The immobilization of hemoglobin (Hb) on GPTS-SPIONs was confirmed by FT-IR spectroscopy comparing with the spectra of structures before and after the immobilization process (Fig. 1). The FT-IR bands at low wave numbers ($\leq 700 \text{ cm}^{-1}$) come from vibrations of Fe–O bonds of iron oxide. The presence of magnetite NPs can be seen by two strong absorption bands at around 632 and 585 cm^{-1} [15]. The peaks at 3373 cm^{-1} and 1615 cm^{-1} are assigned to the O–H bonds on the structure of SPIONs (Fig. 1a). When compared with the spectrum of SPIONs, several new bands appear at 2913 cm^{-1} , 2854 cm^{-1} , 1190 cm^{-1} and 1080 cm^{-1} in the spectrum of GPTS-SPIONs (Fig. 1b). The peaks at 2913 cm^{-1} and 2854 cm^{-1} come from C–H stretching vibration [17,18]. The presence of the anchored epoxy silane group is confirmed by the stretching vibration of Si–O that appears at 1080 cm^{-1} [19]. The peak at 1190 cm^{-1} is attributed to the vibrational band of the C–O bond for the epoxy group. Fig. 1c shows the FT-IR spectrum of hemoglobin immobilized GPTS-SPIONs. The peak at 1151 cm^{-1} comes from the stretching vibration of C–O in the structure of hemoglobin [20]. The peak at 1205 cm^{-1} is assigned to the amide bond. The broad band at 3302 cm^{-1} is due to the stretching vibrations of N–H in $-\text{NH}_2$ and O–H in $-\text{COOH}$, which indicates that hemoglobin was immobilized successfully on GPTS-SPIONs [19].

In order to study their magnetic behavior, the magnetic properties of the magnetic nanoparticles were measured at room temperature with VSM. Fig. 2 shows the hysteresis loops of the samples. The saturation magnetization was found to be 42.3 emu/g for Tbz-Hb-GPTS-SPIONs, less than the pure SPIONs (54.4 emu/g) and GPTS-SPIONs (51.3 emu/g). This difference suggests that a large amount of drug and hemoglobin was coated on the surface of SPIONs [15]. As shown in the figure, no reduced remanence and coercivity being zero were detected, indicating that all unmodified, GPTS-modified and Tbz-Hb modified SPIONs are superparamagnetic. When the external magnetic field was removed, the magnetic nanoparticles could be well dispersed by gentle shaking.

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