Preparation of ds-DNA functionalized magnetic nanobaits for screening of bioactive compounds from medicinal plant

Li Yuan a,d, Pei-Li Xu a,c, Qiong Zeng a,d, Yi-Ming Liu a,b, Li-Sheng Ding a, Xun Liao a,*

a Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
b Department of Chemistry and Biochemistry, Jackson State University, 1400 Lynch St, Jackson, MS 39217, USA
c Shanghai Institute of Technology, Shanghai 201418, China
d University of Chinese Academy of Sciences, Beijing 100049, China

A R T I C L E   I N F O

Article history:
Received 9 March 2015
Received in revised form 23 May 2015
Accepted 25 June 2015
Available online 3 July 2015

Keywords:
ds-DNA
Immobilization
Magnetic nanoparticles
DNA binders

A B S T R A C T

A novel magnetic nanocomposite (MNPs@DNA) was synthesized by bonding double strand DNA (ds-DNA) onto Fe3O4 magnetic nanoparticles (MNPs) directly. MNPs@DNA was characterized by cyclic voltammetry (CV), differential pulse voltammetry (DPV), Fourier transform infrared spectroscopy (FT-IR) and thermo-gravimetric analysis (TGA), which indicated that ds-DNA was immobilized onto MNPs. Vibrating sample magnetometer (VSM) analysis indicated that the MNPs@DNA had a high saturation magnetization of 42.97 emu/g. A novel method for screening of active compounds from natural sources was developed by employing MNPs@DNA as a nanobait and high-performance liquid chromatography–mass spectrometry as detecting system. Columbamine, palmatine, jateorhizine, epiberberine and berberine were identified as DNA binders from the extract of Rhizoma coptidis. In addition, a comparison of the binding abilities among MNPs with different DNA strand lengths (25, 200 and 1200 bp) showed that the shortest one exhibited the highest binding ability. This is the first report on fast chemical characterization of active ingredients in medicinal plant using ds-DNA immobilized on magnetic nano-baits. This method can be used not only for screening of DNA binders from complex herbal matrices, but also for assessing the affinities between a specific ds-DNA and its potential binders.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

DNA is the carrier of genetic information which is responsible for gene expression, gene transcription and protein translation. The heteromorphosis and impairment of DNA can result in cancer and even death of the organisms, therefore, discovery of DNA binders and studies on interactions between them may be helpful to repair the impaired DNA. Small molecules interact with DNA in many ways, including intercalation [1,2], groove binding [3,4] and electrostatic binding [5,6]. A variety of techniques have been used to study the interactions between DNA and its binders, such as DNA-footprinting [7,8], nuclear magnetic resonance (NMR) [9,10], mass spectrometry (MS) [11,12], molecular modeling techniques [13], electrochemical measurement [14–17] and some spectroscopic methods [18–21]. It has been reported that the anti-cancer drug irinotecan can attach to DNA through electrostatic binding [22]; distamycin can form a monomer or dimer and bind in the minor groove of double helix DNA [23]; and berberine can bind to double helix DNA at AT-rich sequences preferentially [24].

Medicinal plant have been proved to be a rich source for discovering active compounds in treating and preventing various cancers and DNA binders were reported to be one group of such compounds responsible for the main therapeutic effects. Curcumin in the annual herb turmeric was found to be a minor groove binder which had therapeutic effects on cancer at low concentration [25]. Sixteen constituents in Trollius chinensis were proved to bind to DNA, which were proposed to be beneficial for the treatment of cancer, cardiovascular disease and neurodegenerative disorders [26]. As well known, due to the chemical complexity in the extract of medicinal plant, it was neither easy to identify the bioactive compounds nor to illuminate the pharmacological mechanism. Normally, screening bioactive compounds in medicinal plant is relied on bioactivity guided chromatographic separation. It is time-consuming, expensive and incomplete in many cases. Therefore, convenient and fast methods would be needed for screening effective components in medicinal plant.

Because of its characteristics such as superparamagnetism and suspension stability, easy surface modification, convenient solid–liquid separation, and excellent biocompatibility, magnetic nanoparticles (MNPs) had been recognized as an excellent support for biomolecules such as enzymes [27], DNA [28] and polypeptide [29], and have been widely used in targeted drug delivery [30], enzyme inhibitor screening [27,31] and catalysis [32,33]. We have developed some methods based on functional magnetic nanoparticles to study the bioactive components in medicinal plant. For example, polydopamine-coated magnetic nanoparticles had been used for selective extraction of berberine from Cortex Phellodendri [34]; human serum albumin functionalized magnetic nanoparticles had been used for ligand fishing of active steroid saponins.
from *Dioscorea nipponica* extract [35], and liver microsomes immobilized on MNPs for in vitro metabolic study of traditional Chinese medicine [36]. In those applications, bioactive compounds bound to the functionalized magnetic nanoparticles could be easily magnetically separated from the complex mixtures without the process of centrifugation or filtration, which made the screening fast and convenient. Therefore, DNA functionalized MNPs (MNPs@DNA) should possess promising effectiveness in screening DNA binders from medicinal plants.

In this work, DNA was covalently immobilized on surface of magnetic nanoparticles by a new method and a screening method based on this nanocomposite was developed to selectively extract DNA binders from a traditional Chinese medicinal plant, *Rhizoma Coptidis*. The MNPs were synthesized by co-precipitation method, and DNA was then immobilized on MNPs by forming a phosphor amidate bond. Compared with previously reported [28,37] fabrication methods, in which a single strand DNA had to be end-modified firstly in order to be immobilized on the MNPs, and the target double or triple strand DNA was afterwards formed by base pairing, our method was much convenient and cheaper for the double strand DNA being firstly amplified by polymerase chain reaction (PCR) and then immobilized onto MNPs directly. The resultant MNPs@DNA was characterized by cyclic voltammetry, differential pulse voltammetry, Fourier transform infrared spectroscopy, vibrating sample magnetometer and thermo-gravimetric analysis. Furthermore, the DNA binders eluted from MNPs@DNA were detected by HPLC–MS. To our knowledge, this is the first report featuring ds-DNA directly immobilized on the surface of magnetic nanoparticles for screening ds-DNA binders from medicinal plant.

2. Materials and methods

2.1. Materials

N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC–HCl) were purchased from Sigma-Aldrich Chemistry Co., Ltd (United States). 3-Aminopropyltrimethoxysilane (APTMS) and tetraethyl orthosilicate (TEOS) were purchased from TCI (Tokyo, Japan). FeCl3·6H2O, FeCl2·4H2O, KH2PO4, K2HPO4, NaCl, acetone, ethidium bromide (EB) and ethylenediamine tetraacetic acid disodium salt (EDTA) were purchased from Chengdu Tianhua Chemical Technology Co., LTD (Chengdu, China). Primers and 25 bp ds-DNA were synthesized by Genewiz Biotechnology Co., Ltd (Suzhou, China). DNA of 200 bp and 1200 bp were synthesized by PCR and recycled with corresponding kit. 2× EasyTaq PCR SuperMix were purchased from TransGen Biotech Co., Ltd (Beijing, China). Tissue DNA kit and cycle pure kit were purchased from Tiangen Biotech Co., Ltd (Beijing, China). Fluorescamine was purchased from Alfa Aesar Co. (Tianjin, China). Purified water from a Milli-Q water system (Millipore Instruments Co., USA). Cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were carried out with an EC 550 electrochemical workstation (Wuhan Gaosun Instruments Co., China). HPLC analysis was carried out on a Shimadzu LC-20AD series HPLC system equipped with a Tianhe kromasil C18 column (250 × 4.6 mm, 5 μm particle) and operated with an ultraviolet detector at 280 nm wavelength. The DNA binders obtained by MNPs@DNA from ligand extraction library was eluted with solvent A (0.1% phosphoric acid in water, v/v) and B (acetonitrile) at a flow rate of 0.8 mL·min⁻¹. The solvent B was increased from 10% to 25% in 13 min and then increased to 70% in the next 15 min. For the extraction of R. Coptidis, the elution solvents and flow rate are same as DNA binders mentioned before, while the elution condition is different. The solvent B was kept 10% for 5 min and increased to 50% in 28 min, then increased from 50% to 80% in the last 3 min. Mass spectrometric detection was performed on a triple quadrupole mass spectrometry (Waters, USA) equipped with an ESI source. Nitrogen was supplied as the sheath gas and the capillary temperature was set at 400 °C for the experiments. All compounds were detected in the positive ion mode with a needle voltage 3.3 KV.

2.2. Apparatus

The detection of fluorescence was performed on a Varioskan Flash multifunctional microplate reader (Thermo, USA) at a wavelength of 450 nm. The concentration of DNA was measured by using a UV-1800 spectrophotometer (Shimadzu Instruments, Inc., Tokyo, Japan). The magnetic properties of the three types of nanoparticles were measured on a vibrating sample magnetometer (VSM, Quantum Design Company, USA). Fourier-transform infrared spectra (FTIR) were obtained using a Perkin-Elmer FT-IR Spectroscopy (KBr). Thermo-gravimetric analysis (TGA) was performed with a heating rate of 10 °C/min from room temperature to 700 °C under a high purity nitrogen atmosphere using a TGA Q500 V20.10 Build 36 thermo-analysis system (American TA Instruments Co., USA). Cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were carried out with an EC 550 electrochemical workstation (Wuhan Gaosun Instruments Co., China). HPLC analysis was carried out on a Shimadzu LC-20AD series HPLC system equipped with a Tianhe kromasil C18 column (250 × 4.6 mm, 5 μm particle) and operated with an ultraviolet detector at 280 nm wavelength. The DNA binders obtained by MNPs@DNA from ligand extraction library was eluted with solvent A (0.1% phosphoric acid in water, v/v) and B (acetonitrile) at a flow rate of 0.8 mL·min⁻¹. The solvent B was increased from 10% to 25% in 13 min and then increased to 70% in the next 15 min. For the extraction of R. Coptidis, the elution solvents and flow rate are same as DNA binders mentioned before, while the elution condition is different. The solvent B was kept 10% for 5 min and increased to 50% in 28 min, then increased from 50% to 80% in the last 3 min. Mass spectrometric detection was performed on a triple quadrupole mass spectrometry (Waters, USA) equipped with an ESI source. Nitrogen was supplied as the sheath gas and the capillary temperature was set at 400 °C for the experiments. All compounds were detected in the positive ion mode with a needle voltage 3.3 KV.

2.3. Synthesis of amino group functionalized magnetic nanoparticles

Firstly, FeCl3·6H2O (2.0271 g) and FeCl2·4H2O (0.7455 g) were dissolved in 250 mL of deionized water, and ammonia water was added dropwise under vigorous stirring till the pH value reached to 10, and kept stirring for 30 min. The black nanoparticles were collected with an external magnet and washed with water and ethanol alternately for three times. Secondly, the MNPs were re-dispersed in 150 mL of ethanol, to which 400 μL of TEOS was added and the pH value was adjusted to about 10 by ammonia water dropwise, and then vigorously stirred at 35 °C for 5 h to produce the core-shell structured MNPs@SiO2. The MNPs@SiO2 nanoparticles were collected with an external magnet and washed with ethanol three times. Thirdly, the MNPs@SiO2 nanoparticles were re-dispersed into 165 mL ethanol, to which 2 mL of APTMS was added, and after vigorous stirring at 35 °C for 24 h, amino groups were introduced on the surface of MNPs@SiO2 to produce MNPs@NH2. Finally, the MNPs@NH2 nanoparticles were collected with an external magnet, washed three times with water and dried in a lyophilizer.

2.4. Quantification of amino groups on MNPs@NH2

To determine the amount of DNA fit for the immobilization reaction (amino groups: DNA = 1:1 in concentration), the amino groups on MNPs@NH2 were firstly quantified based on the fluorescamine quantitative detection method [38]. In brief, 10 mg of MNPs@NH2 were suspended in 5 mL NaOH (0.02 M) and sonicated for 30 min. After vortexing oscillation 60 h, the nanoparticles were separated from the solution with an external magnet, and the supernatant was kept before use. The APTMS was diluted with NaOH (0.02 M) to obtain different concentrations of APTMS standard solution: 0.4, 0.3, 0.2, 0.1, and 0.05 mM. Four microliters of each APTMS standard solution were pipetted into 116 μL of PBS (0.2 M, pH 8.0) respectively, followed by adding 20 μL of fluorescamine which was dissolved in acetone (0.3 g/L) to react for 20 min in darkness. Finally, the fluorescence was measured by a multifunctional microplate reader at a wavelength of 450 nm. According to the standard curve of APTMS, the concentration of the amino groups on the MNPs@NH2 can be calculated.

2.5. Immobilization of ds-DNA onto MNPs@NH2

Three different lengths of ds-DNAs (25, 200 and 1200 bp) were immobilized on MNPs@NH2. The immobilization procedure is illustrated in Fig. 1. Firstly, three kinds of ds-DNAs were diluted with PBS to the same concentration with that of amino groups of MNPs@NH2. Secondly, 20 mg of MNPs@NH2 was firstly suspended in 200 μL of PBS buffer (10 mM sodium phosphate, 0.15 M NaCl, 10 mM EDTA, pH 7.2) to which 25 μL
of imidazole (0.1 M, pH 6) was added. Then, 1.25 mg of EDC was dissolved into 20 μL DNA solution and the mixture was added into the above MNPs@NH2 suspension. Finally, the whole reaction mixture was vortexed at room temperature overnight to obtain the MNPs@DNA. The MNPs@DNA was magnetically separated and washed several times with PBS until no free DNA was detected in the washing solution. The final MNPs@DNA was re-dispersed in 500 μL PBS and stored at 4 °C before use.

2.6. Extraction of ds-DNA binders with MNPs@DNA (200 bp)

A testing ligand library containing EB, columbamine, magnoflorine and berberine (all are 80 μg/mL) in PBS was prepared for the validation of the ligand extraction. One hundred microliters of the testing ligand library was added to 500 μL MNPs@DNA (200 bp) suspensions to vortex for 10 min at room temperature, and then the MNPs@DNA was separated from the solution with an external magnet. The MNPs@DNA was washed three times with PBS to remove the unspecific bound compounds. The MNPs@DNA was suspended into 200 μL deionized water and vortexed for 30 s, followed by heating the suspension to 80 °C for 5 min to release any compound bound to the MNPs@DNA. Finally, the MNPs@DNA was magnetically separated and the supernatant was collected and analyzed by HPLC-MS. The protocol for extracting ds-DNA binders is shown in Fig. 2.

One gram dried powder of *R. Coptidis* root was extracted with 100 mL 20% methanol, the extraction was concentrated to dryness and finally diluted to 0.1 mg/mL with PBS. The ligand extraction procedure was carried out using MNPs@DNA (200 bp) with the same way as described for the testing ligand library.

### 3. Results and discussion

#### 3.1. Characterization of MNPs@SiO2, MNPs@NH2 and MNPs@DNA (200 bp)

The ds-DNA (200 bp) was covalently immobilized onto the surface of amino modified MNP, and it was characterized in comparison with MNPs@SiO2 and MNPs@NH2 with the following techniques. The FT-IR spectra of amino groups functionalized MNPs (a), MNPs with ds-DNA (200 bp) immobilized on (b) and pure ds-DNA (200 bp) (c) were shown in Fig. 3. In Fig. 3(a), a sharp and strong absorption peak at 584.58 cm⁻¹ was attributed to Fe–O bond vibration, and the peak at 998.04 cm⁻¹ could be attributed to the Si-O-Si asymmetric vibration absorption. The peak at 1622.48 cm⁻¹ was attributed to the characteristic N–H bending vibration of amino groups, and the peak at 1358.26 cm⁻¹ was attributed to the characteristic C–N stretching vibration [39]. In Fig. 3(c), the absorption peak at 3348.84 cm⁻¹ was attributed to the characteristic N–H bending vibration of amino groups, and the peak at 1358.26 cm⁻¹ was attributed to the characteristic C–N stretching vibration [40]. In Fig. 3(b), the absorption peak at 3448.84 cm⁻¹ was attributed to the characteristic C–N stretching vibration of the nucleic acid base [40]. The absorption peak at 1118.48 cm⁻¹ was attributed to the characteristic C–H vibration band in phenyl ring. Those at 1661.68 and 1590.81 cm⁻¹ were C=O characteristic stretching vibrations which were split because of the antiparallel orientation of the polynucleotide double helix [40]. The moderate absorption peak at 1056.10 cm⁻¹ was the typical P–O–C bonds stretching vibration. After immobilization of ds-DNA on the surface of MNPs@NH2, the FT-IR spectrum (Fig. 3(b)) showed the bands for both MNPs@NH2 and ds-DNA, indicating that the ds-DNA was successfully immobilized on the surface of MNPs@NH2.
deployed to investigate the three types of MNPs synthesized. The cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were measured by electrochemical workstation in 10 mM PBS (pH 7.2) containing 10 mM EDTA and 5 mM [Fe(CN)₆]³⁻/⁴⁻. Each of the three types of MNPs was suspended in PBS at a concentration of 10 μg/mL, and then 100 μL of the suspension was added into the reaction pool of screen-printed carbon electrode. An external magnet was placed under the electrode so that the MNPs@DNA can be adsorbed tightly onto the upper surface of the electrode. The potential range was set from −0.1 to 0.6 V at a scan rate of 50 mV/s. The PBS buffer with 10 mM EDTA and 5 mM [Fe(CN)₆]³⁻/⁴⁻ was used for the control for this experiment. Fig. 5(a) shows that the oxidation–reduction peaks of MNPs@SiO₂, MNPs@NH₂ and MNPs@DNA (200 bp) were in correspondence with the oxidation–reduction peak of [Fe(CN)₆]³⁻/⁴⁻, i.e. 325 and 160 mV, indicating that all of them had no electrical activity. With the subsequent introductions of amino groups and ds-DNA onto the surface of MNPs@SiO₂, the peak currents were significantly increased accordingly. The increase of the peak currents for the MNPs@NH₂ was caused by the amino groups which can attract more [Fe(CN)₆]³⁻/⁴⁻. The peak currents for the MNPs@DNA were even higher than that of MNPs@NH₂ due to the conductivity of DNA. Although the possible electrostatic repulsion between ds-DNA and [Fe(CN)₆]³⁻/⁴⁻ on the electrode surface can lead to the decrease of current response, it might be overcome by the significant conductivity of DNA which raised especially from the fact that their double helices can lead to the flow of positively charged counter ions along the negatively charged phosphate backbone [45–47]. In addition, DPV was employed to confirm the surface modification of the MNPs. In Fig. 5(b), the peak currents were increased with the subsequent modifications of amino groups and ds-DNA on the surface of MNPs@SiO₂. The peak current of MNPs@SiO₂ was found to be lower than that of [Fe(CN)₆]³⁻/⁴⁻. It could be attributed to the MNPs@SiO₂ absorbed on the surface of electrode, which had certain resistance than bare electrode. From the changes of current responses, it was obvious that DNA were immobilized onto the surface of MNPs@NH₂.

3.2. Effectiveness of the MNPs@DNA nanobaits

The feasibility and specificity of the MNPs@DNA were evaluated by using the ligand extraction library mentioned before. Columbamine is present in many species of plants of the Berberidaceae family, and has been reported to be able to interfere with DNA [48]. EB could insert into the base pairs of double strands DNA and has been used as nucleic acid dye for a long time. Berberine was reported to be a minor groove binder for DNA, and it specifically bind to AT-rich sequences with high

---

**Fig. 3.** FT-IR spectra of (a) MNPs@NH₂, (b) MNPs@DNA (200 bp), and (c) pure ds-DNA (200 bp).

The concentration of amino groups attached on the MNPs was quantified according to the standard calibration curve of APTMS, \( F = 31.304C + 2.7751 (R^2 = 0.9952) \). Here, \( F \) was the fluorescence value of amino which reacted with fluorescamine, and \( C \) was the concentration of APTMS. The concentration of amino was calculated to be 0.0034 mmol per mg of magnetic nanoparticles.

MNPs@SiO₂, MNPs@NH₂ and MNPs@DNA (200 bp) were analyzed by VSM at room temperature. The magnetization (emu/g) versus magnetic field (oe) for these three kinds of magnetic nanoparticles is presented in Fig. 4(a). Both coercivity and remanence for them were zero, indicating that the magnetic nanoparticles had no hysteresis and thus the superparamagnetism of them. The saturation magnetization of MNPs@SiO₂ was 51.02 emu/g, and those for MNPs@NH₂ and MNPs@DNA were decreased to 47.65 emu/g and 42.97 emu/g, respectively. The results could be explained by the diamagnetic of the NH₂ and DNA surrounding the MNPs@SiO₂.

The TGA analysis of MNPs@SiO₂, MNPs@NH₂ and MNPs@DNA (200 bp) was carried out from room temperature to 700 °C (Fig. 4(b)). The weight loss of the three types of MNPs accounted for the organic functional groups and DNA on the MNPs. Compared to MNPs@NH₂ (red curve), the weight loss of MNPs@NH₂ (red curve) was 6.96 wt.%, which could be ascribed to the loss of the ethyldiamine cations of amino groups and ds-DNA on the surface of MNPs@SiO₂, while the weight loss of MNPs@DNA (blue curve) was ascribed to the loss of DNA (3.68 wt.%) compared to MNPs@NH₂ (red curve).

Electrochemical methods have been applied in DNA analysis in which immobilization of DNA on electrode usually results in a change of current response [41–43]. In this work, a similar electrochemical method was employed to investigate the three types of MNPs synthesized. The cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were measured by electrochemical workstation in 10 mM PBS (pH 7.2) containing 10 mM EDTA and 5 mM [Fe(CN)₆]³⁻/⁴⁻. Each of the three types of MNPs was suspended in PBS at a concentration of 10 μg/mL, and then 100 μL of the suspension was added into the reaction pool of screen-printed carbon electrode. An external magnet was placed under the electrode so that the MNPs@DNA can be adsorbed tightly onto the upper surface of the electrode. The potential range was set from −0.1 to 0.6 V at a scan rate of 50 mV/s. The PBS buffer with 10 mM EDTA and 5 mM [Fe(CN)₆]³⁻/⁴⁻ was used for the control for this experiment. Fig. 5(a) shows that the oxidation–reduction peaks of MNPs@SiO₂, MNPs@NH₂ and MNPs@DNA (200 bp) were in correspondence with the oxidation–reduction peak of [Fe(CN)₆]³⁻/⁴⁻, i.e. 325 and 160 mV, indicating that all of them had no electrical activity. With the subsequent introductions of amino groups and ds-DNA onto the surface of MNPs@SiO₂, the peak currents were significantly increased accordingly. The increase of the peak currents for the MNPs@NH₂ was caused by the amino groups which can attract more [Fe(CN)₆]³⁻/⁴⁻. The peak currents for the MNPs@DNA were even higher than that of MNPs@NH₂ due to the conductivity of DNA. Although the possible electrostatic repulsion between ds-DNA and [Fe(CN)₆]³⁻/⁴⁻ on the electrode surface can lead to the decrease of current response, it might be overcome by the significant conductivity of DNA which raised especially from the fact that their double helices can lead to the flow of positively charged counter ions along the negatively charged phosphate backbone [45–47]. In addition, DPV was employed to confirm the surface modification of the MNPs. In Fig. 5(b), the peak currents were increased with the subsequent modifications of amino groups and ds-DNA on the surface of MNPs@SiO₂. The peak current of MNPs@SiO₂ was found to be lower than that of [Fe(CN)₆]³⁻/⁴⁻. It could be attributed to the MNPs@SiO₂ absorbed on the surface of electrode, which had certain resistance than bare electrode. From the changes of current responses, it was obvious that DNA were immobilized onto the surface of MNPs@NH₂.

3.2. Effectiveness of the MNPs@DNA nanobaits

The feasibility and specificity of the MNPs@DNA were evaluated by using the ligand extraction library mentioned before. Columbamine is present in many species of plants of the Berberidaceae family, and has been reported to be able to interfere with DNA [48]. EB could insert into the base pairs of double strands DNA and has been used as nucleic acid dye for a long time. Berberine was reported to be a minor groove binder for DNA, and it specifically bind to AT-rich sequences with high
binding degree [24]. All the three compounds could bind to DNA with high affinity, while magnoflorine had little affinity to ds-DNA. A solution containing the four compounds was incubated with MNPs–DNA and the binders extracted were analyzed with HPLC as shown in Fig. 6(a).

Columbine, EB and berberine were detected in the elution solution because of the high affinity with DNA, but magnoflorine was not detected. The affinities of ds-DNA binders were characterized by relative binding degree which was calculated according to the following equation [37],

\[
\text{Relative binding degree} = \left( \frac{C_i - C_c}{C_0} \right) \times 100\%
\]

where \(C_i\) and \(C_c\) were the concentrations of a compound in the experiment and control group, respectively; \(C_0\) was the total concentration of a compound.

![Diagram](image-url)

**Fig. 5.** (a) Cyclic voltammograms (CV) and (b) differential pulse voltammograms (DPV) of MNPs@SiO₂, MNPs@NH₂ and MNPs@DNA (200 bp).

![Diagram](image-url)

**Fig. 6.** (a) The HPLC-UV chromatograms of the testing ligand library (red line) and the binders extracted with MNPs@DNA (200 bp) (black line). (b) Relative binding degree of the compounds in ligand fishing library using MNPs@DNA (200 bp).
in the extract. The concentration of each compound was calculated according to the standard calibration curve. The standard calibration curves for the four compounds were as follows: \( A = 374804C-41662 \) \((R^2 = 0.9999)\) for magnoflorine, \( A = 104471C-121007 \) \((R^2 = 0.9991)\) for columbamine, \( A = 726365C-466517 \) \((R^2 = 0.9995)\) for EB, and \( A = 53593C-51432 \) \((R^2 = 0.9991)\) for berberine. Here \( C \) denotes the concentration of ligands and \( A \) is the corresponding peak areas. The results of relative binding degree are shown in Fig. 6(b).

3.3. Screening for DNA binders from R. Coptidis extract with MNPs@DNA

*R. Coptidis* extract is effective in treating tumor and cancer [49,50]. Its main bioactive constituents are berberine, palmatine and jatrorrhizine, and they have been reported to possess high affinity with duplex, triplex and G-quartet DNA [37,51]. To compare the binding ability of different lengths of ds-DNAs toward the DNA binders, ds-DNA including 25 bp, 200 bp and 1200 bp were immobilized onto MNPs@NH\(_2\) respectively, and then used for extraction of DNA binders in *R. Coptidis* extract.

Fig. 7 shows the chromatograms of the DNA binders extracted from *R. Coptidis* extract with MNPs@DNA in different length (25 bp, 200 bp and 1200 bp), and MNPs@NH\(_2\) was used as a control. Five compounds had been extracted and their retention time, molecular ion peaks and MS fragmentation data are listed in Table 1.

The standard calibration curves for the five compounds were as follows: \( A = 104471C-121007 \) \((R^2 = 0.9991)\) for columbamine, \( A = 56569C-53373 \) \((R^2 = 0.9998)\) for epiberberine, \( A = 74871C-102780 \) \((R^2 = 0.9993)\) for jateorhizine, \( A = 651586C-462185 \) \((R^2 = 0.999)\) for palmatine, and \( A = 53593C-51432 \) \((R^2 = 0.9991)\) for berberine. The results of relative binding degree were listed in Table 2. Columbamine exhibited the strongest affinity toward MNPs@DNA, followed by palmatine, jateorhizine, epiberberine and berberine. In the mean time, different types of MNPs@DNA exhibited different binding abilities to DNA binders. The order of the binding ability of MNPs@DNA is: MNPs@DNA (25 bp) > MNPs@DNA (200 bp) > MNPs@DNA (1200 bp). This difference might be caused by the steric hindrance between ds-DNA and nanoparticles which allow less amount of ds-DNA be immobilized on MNPs. The shorter the DNA length is, the more amount of DNA can be immobilized on the MNPs. On the other hand, as in the case of the longest DNA (1200 bp), the binding ability did

---

**Table 1**

| Peak | Compounds  | Rt (min) | [M + H]+ (m/z) | MS/MS (m/z) |
|------|------------|----------|----------------|-------------|
| 1    | Columbamine| 23.6     | 338            | 322, 294, 280|
| 2    | Epiberberine| 23.8     | 336            | 320, 292    |
| 3    | Jateorhizine| 24.3     | 338            | 322, 294, 280|
| 4    | Palmatine  | 26.3     | 352            | 336, 308    |
| 5    | Berberine  | 26.8     | 336            | 320, 292    |

Fig. 7. HPLC chromatograms for the DNA binders in *R. Coptidis* extracted by MNPs@DNA in different length (above) and the binders’ chemical structure. Upper: (a) *R. Coptidis* extract; (b) binders extracted by 25 bp DNA; (c) binders extracted by 200 bp DNA; (d) binders extracted by 1200 bp DNA; and (e) binders extracted by MNPs@NH\(_2\) (e). Below: 1 columbamine; 2 epiberberine; 3 jateorhizine; 4 palmatine; 5 berberine.
not decrease too much, it could be attributed to the double helix structure of DNA — longer DNA had more binding sites.

This is the first report on fast chemical characterization of active ingredients in medicinal plant using ds-DNA immobilized on magnetic nano-baits. Compared to other similar works [28,37], the immobilization procedure was more convenient through phosphor amidate bond, the screen method was quicker due to the magnetic separation and the cost was cheaper because of amplifying ds-DNA without any end-modification.

4. Conclusions

The ds-DNA functionalized magnetic nanoparticles were evaluated for the first time as magnetic nano-baits of ligand fishing in this work. The ds-DNA binders from medicinal plant were easily isolated and identified by means of the proposed ds-DNA based magnetic nano-baits in combination with HPLC–MS. The synthesized magnetic nanoparticles were characterized by CV, DPF, FT-IR, VSM, and TGA, which have shown high saturation magnetization. A ligand extraction library which was composed of palmitic acid, EB, magnofolrine and berberine was developed to investigate the feasibility and specificity of the MNPs@DNA. This method was applied to screening active compounds in R. Codiptis extract. Five compounds were successfully extracted from R. Codiptis and identified by HPLC–MS. The MNPs@DNA exhibited high extracting capability with ds-DNA binders, and it could be applied in preliminary process for screening and identifying ds-DNA binders from complicated extraction of traditional Chinese medicine. This work provided a convenient, rapid, and cost effective procedure for screening DNA binders from medicinal plant.

Acknowledgments

Financial supports from National Natural Science Foundation of China [21072184 and 81173536] and West Light Foundation of The Chinese Academy of Sciences are gratefully acknowledged.

References

[1] M. Trieb, C. Rauch, F.R. Wilkowo, B. Wellenzohn, K.R. Liedel, Cooperative effects on the formation of intercalation sites, Nucleic Acids Res. 32 (2004) 4696–4703.
[2] L.S. Wu, A. Reymer, C. Persson, K. Kazimierzczuk, T. Brown, P. Lincoln, B. Norden, M. Billerle, Initial DNA interactions of the binuclear threading intercalator \(\mu\)-bidppz(bipy)\(\mu\)-Ru2\(\text{II}\) – \(\text{II}\)Ru2\(\text{II}\) and its host DNA, J. Am. Chem. Soc. 116 (1994) 4154–4165.
[3] A. Agostini, E. Marco, V. Garca-A-Herna\'endez, A. Domingo, Antimutagen activity, X-ray crystal structure, and DNA binding properties of thioicaroline A, a natural bisintercalating thiophedipsideptide, J. Med. Chem. 50 (2007) 3322–3333.
[4] L. Li, Y. Ma, C. Yang, Y. X. Yang, Interaction of anticancer drug mitoxantrone with DNA analyzed by electrochemical and spectroscopic methods, Biophys. Chem. 116 (2005) 199–205.
[5] G. Zhang, X. Hu, P. Fu, Spectroscopic studies on the interaction between carbyl and calf thymus DNA with the use of ethidium bromide as a fluorescence probe, J. Photochem. Photobiol. B 108 (2012) 53–61.
[6] H.J. Reza, H.T. Guan, Electrochemical study on the interaction of irinotecan with calf thymus double stranded DNA, Chin. J. Chem. 30 (2012) 738–742.
[7] M.A. Churchill, J. Haagensen, Detection of ds-DNA formation by hydroxyl radical footprinting, relationship of distamycin binding sites to DNA structure and positioned nucleosomnes on 5 s RNA genes of Xenopus, Biochemistry 29 (1990) 6043–6050.
[8] S. Mazzin, M.C. Bellucci, R. Mondelli, Mode of binding of the cytokidal anticancer berberine with the double helix oligonucleotide D(AAAGGTCCT)\(\text{II}\), Bioorg. Med. Chem. 11 (2003) 505–514.
[9] A. Kumar, U. Bora, Interactions of curcumin and its derivatives with nucleic acids and their implications, Mini-Rev. Med. Chem. 13 (2013) 256–264.
[10] Z.L. Song, H. Wang, B.B. Ren, B.B. Zhang, Y.K. Hashi, S.Z. Chen, On-line study of flavonoids of Troilus chinensis Bunge binding to DNA with ethidium bromide using a novel combination of chromatographic, mass spectrometric and fluorescence techniques, J. Chromatogr. A 1282 (2013) 102–112.
[11] Y.T. Zhu, X.Y. Ren, Y.M. Liu, Y. Wei, L.S. Qiong, X. Liao, Covalent immobilization of porcine pancreatic lipase on carboxyl-activated magnetic nanoparticles: characterization and their implications, Mini-Rev. Med. Chem. 13 (2013) 256–264.
[12] L.J. Cai, F. Bu, X. Fu, Y. Yang, Analysis of tyrosinase binders from Glycyrrhiza uralensis root: evaluation and comparison of tyrosinase immobilized magnetic fishing–HPLC and reverse ultrafiltration–HPLC, J. Chromatogr. B 932 (2013) 19–25.
[13] J. Govan, V.K. Guruk\'o, Recent advances in the application of magnetic nanoparticles as assay support for homogeneous enzyme-catalyzed assays, Nanomaterials 4 (2014) 222–241.
[14] S. He, W. Shi, X. Zhang, J.Y. Liu, Huang, Beta-cyclodextrin-based inclusion complexes of CoFe\(\text{II}\)\(\text{II}\) oxide magnetic nanoparticles as catalyst for the luminal cholinumuliciumnescence system and their applications in hydrogen peroxide detection, Talanta 82 (2010) 186–192.
[15] H.L. Shi, S.L. Peng, J. Sun, Y.M. Liu, Y.T. Zhu, L.S. Qiong, X. Liao, Selective extraction of berberine from Cortex Phellodendri using polydopamine-coated magnetic nanoparticles, J. Sep. Sci. 37 (2014) 704–710.
[16] L.X. Liu, S. Shi, X. Chen, M. Peng, Analysis of tyrosinase binders from Glycyrrhiza uralensis root: evaluation and comparison of tyrosinase immobilized magnetic fishing–HPLC and reverse ultrafiltration–HPLC, J. Chromatogr. B 932 (2013) 19–25.
[17] J. Govan, V.K. Guruk\'o, Recent advances in the application of magnetic nanoparticles as assay support for homogeneous enzyme-catalyzed assays, Nanomaterials 4 (2014) 222–241.
[18] S. He, W. Shi, X. Zhang, J.Y. Liu, Huang, Beta-cyclodextrin-based inclusion complexes of CoFe\(\text{II}\)\(\text{II}\) oxide magnetic nanoparticles as catalyst for the luminal cholinumuliciumnescence system and their applications in hydrogen peroxide detection, Talanta 82 (2010) 186–192.
[38] M. Weigele, S. Debernardo, W. Leimgruber, Fluorometric assay of secondary amino acids, Biochem. Biophys. Res. Commun. 50 (1973) 352–356.

[39] J.L. Zhang, R.S. Srivastava, D.K. Misra, Core–shell magetite nanoparticles surface encapsulated with smart stimuli-responsive polymer: synthesis, characterization, and LCST of viable drug-targeting delivery system, Langmuir 23 (2007) 6342–6351.

[40] K. Arora, A. Chauhey, R. Singhul, R.P. Singh, M.K. Pandey, S.B. Samanta, B.D. Malhotra, S. Chand, Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films to DNA biosensor, Biosens. Bioelectron. 21 (2006) 1777–1783.

[41] O.A. Arotiba, P.G. Baker, B.B. Mamba, E.I. Iwuoha, The application of electrodeposited poly(propylene imine) dendrimer as an immobilization layer in a simple electrochemical DNA biosensor, Int. J. Electrochem. Sci. 6 (2011) 673–683.

[42] V. Ferreira, A. Tenreiro, L.M. Abrantes, Electrochemical, microgravimetric and AFM studies of polythionine films — application as new support for the immobilisation of nucleotides, Sens. Actuators, B 119 (2006) 632–641.

[43] G.A. Nascimento, E.V.M. Souza, D.S. Campos-Ferreira, M.S. Arruda, Electrochemical DNA biosensor for bovine papillomavirus detection using polymeric film on screen-printed electrode, Biosens. Bioelectron. 38 (2012) 61–66.

[44] N.N. Zhu, H. Gao, Q. Xu, Y.Q. Lin, L. Su, L.Q. Mao, Sensitive impedimetric DNA biosensor with poly (amidoamine) dendrimer covalently attached onto carbon nanotube electronic transducers as the tether for surface confinement of probe DNA, Biosens. Bioelectron. 25 (2010) 1498–1503.

[45] O. Arotiba, J. Owino, E. Songa, N. Hendricks, T. Waryo, N. Jahed, P. Baker, E. Iwuoha, An electrochemical DNA biosensor developed on a nanocomposite platform of gold and poly (propyleneimine) dendrimer, Sensors 8 (2008) 5791–5809.

[46] H.W. Fink, C. Schönberger, Electrical conduction through DNA molecules, Nature 398 (1999) 407–410.

[47] B. Giese, J. Amaudrut, A.K. Köhler, M. Spormann, S. Wessely, Direct observation of hole transfer through DNA by hopping between adenine bases and by tunnelling, Nature 412 (2001) 318–320.

[48] X.Y. Su, L. Kong, X. Li, X.G. Chen, M. Guo, H.F. Zou, Screening and analysis of bioactive compounds with biofingerprinting chromatogram analysis of traditional Chinese medicines targeting DNA by microdialysis/HPLC, J. Chromatogr. A 1076 (2005) 118–126.

[49] T. Schnell, B. Latz-Bruing, M. Wink, Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores, Phytochemistry 44 (1997) 257–266.

[50] M.M. Bao, Z.F. Cao, D. Yu, S.L. Fu, G.H. Zhang, P. Yang, Columbamine suppresses the proliferation and neovascularization of metastatic osteosarcoma U2OS cells with low cytotoxicity, Toxicol. Lett. 215 (2012) 174–180.

[51] M. Ebrahimi, T. Khayamian, Interactions of G-QUADRUPLEX DNA binding site with berberine derivatives and construct a structure-based QSAR using docking descriptors, Med. Chem. Res. 23 (2014) 1327–1339.