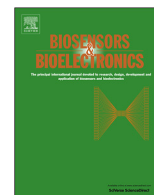
Biosensors and Bioelectronics 50 (2013) 441–446

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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



Molecular sensing with magnetic nanoparticles using magnetic spectroscopy of nanoparticle Brownian motion

Xiaojuan Zhang [a], Daniel B. Reeves [b], Irina M. Perreard [a], Warren C. Kett [c], Karl E. Griswold [c], Barjor Gimi [a], John B. Weaver [a,b,c,*]

[a] Department of Radiology, Geisel School of Medicine, Dartmouth College, 1 Rope Ferry Road. Hanover, NH 03755-1404, United States
[b] Department of Physics, Dartmouth College, 6127 Wilder Laboratory, Hanover, NH 03755-3528, United States
[c] Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755, United States

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form
20 June 2013
Accepted 24 June 2013
Available online 4 July 2013

Keywords:
Magnetic nanoparticles
Brownian motion
Relaxation time
Biosensing

ABSTRACT

Functionalized magnetic nanoparticles (mNPs) have shown promise in biosensing and other biomedical applications. Here we use functionalized mNPs to develop a highly sensitive, versatile sensing strategy required in practical biological assays and potentially *in vivo* analysis. We demonstrate a new sensing scheme based on magnetic spectroscopy of nanoparticle Brownian motion (MSB) to quantitatively detect molecular targets. MSB uses the harmonics of oscillating mNPs as a metric for the freedom of rotational motion, thus reflecting the bound state of the mNP. The harmonics can be detected *in vivo* from nanogram quantities of iron within 5 s. Using a streptavidin–biotin binding system, we show that the detection limit of the current MSB technique is lower than 150 pM (0.075 pmole), which is much more sensitive than previously reported techniques based on mNP detection. Using mNPs conjugated with two anti-thrombin DNA aptamers, we show that thrombin can be detected with high sensitivity (4 nM or 2 pmole). A DNA–DNA interaction was also investigated. The results demonstrated that sequence selective DNA detection can be achieved with 100 pM (0.05 pmole) sensitivity. The results of using MSB to sense these interactions, show that the MSB based sensing technique can achieve rapid measurement (within 10 s), and is suitable for detecting and quantifying a wide range of biomarkers or analytes. It has the potential to be applied in variety of biomedical applications or diagnostic analyses.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

One of the major challenges in biosensing, diagnostics and pharmaceutical drug development is the rapid, accurate and sensitive monitoring of the concentration of biomarkers, drugs or pathogens in biological samples. Driven by this challenge, a number of new biosensing platforms have been reported using a variety of detecting techniques. Some of these techniques provide high sensitivity. For example, an optical biosensor based on wavelength shifts using porous silicon has demonstrated sensitivity for streptavidin and DNA at pico- and femto-molar concentrations (Lin et al. 1997). An electrochemical biosensor based on silicon nanowire field-effect transistors has achieved sensitivity for streptavidin down to hundreds of femtomolar concentrations (Duan et al. 2012). An aptamer sensor based on silicon microring resonators showed the detection limit of 1.4 nM for thrombin(Park et al. 2013). An electrochemical aptamer biosensor using chitosan–Au nanocomposites has demonstrated the capability to detect thrombin with a detection limit of 5.5 fM(Zhao et al. 2012). However, the disadvantages of these methods limit their biomedical application. For example, the traditional techniques are usually time-consuming, requiring large sample volumes and multiple washing steps (Adler et al., 2008; Homola, 2003). In addition, optical sensors based on fluorescence detection usually suffer from high background signals (Owicki, 2000).

In comparison, sensing methods based on magnetic nanoparticles (mNPs) have advantages including biocompatibility, environmental safety, and low cost to synthesize; moreover, mNP-based sensing methods provide less background noise, because there is little or no magnetic signal from biological samples (Haun et al., 2010; Shao et al., 2012). Hence, they have received considerable attention for developing biosensing and diagnostic tools. To date, various mNPs based detection methods have been reported, including AC susceptometry (Park et al., 2011), Hall effect measurements (Mihajlovic et al., 2005), magnetoresistance measurements (Baselt et al., 1998), superconducting quantum interference devices (SQUIDs; Kotitz et al., 1999), and MRI spin–spin relaxation time (T_2) assay (Perez et al., 2002). However, many of these

* Corresponding author at: Geisel School of Medicine, Department of Radiololgy, One Medical Center Drive, Lebanon, NH 03756, United States.
Tel.: +1 603 650 8270; fax: +1 603 650 5455.
E-mail addresses: John.B.Weaver@Dartmouth.edu, John.B.Weaver@hitchcock.org (J.B. Weaver).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.06.049

established techniques suffer from extreme conditions requiring low temperatures or long measurement times while admitting low sensitivity and limited types of target molecules. The most important limitation is that all are incapable of *in vivo* use with high sensitivity (Haun et al., 2010; Shao et al., 2012).

A relatively new mNP sensing method, magnetic spectroscopy of nanoparticle Brownian motion (MSB), has shown to be capable of sensing the change in any property influencing the rotational Brownian motion of the mNPs within seconds (Rauwerdink et al., 2010; Rauwerdink and Weaver, 2010b; Weaver and Kuehlert, 2012; Weaver et al., 2009). Particularly of interest to biosensing applications, MSB enables quantitative measurement of the bound fraction (Rauwerdink and Weaver, 2011) and relaxation times (Weaver and Kuehlert, 2012). The measurements rely on the fact that mNPs tend to align with the applied magnetic field, but that tendency is countered by Brownian motion that randomizes the mNPs' alignment. The extent of disorder caused by Brownian motion is linked directly to environmental conditions, including temperature (Weaver et al., 2009), bound state (Rauwerdink and Weaver, 2010a, 2011), and viscosity (Rauwerdink and Weaver, 2010b). Higher harmonics were used instead of the fundamental frequency to avoid picking up the signal of the applied field. The odd harmonics are of interest in describing the shape of magnetization and its saturation. The detection using harmonics can be achieved with high sensitivity and it has been shown that in imaging applications (Gleich and Weizenecker, 2005), the harmonics can be measured *in vivo* with ng of NPs (Weizenecker et al., 2007; Weizenecker et al., 2009). The harmonics increase linearly with the number of mNPs so the ratio of the harmonics was used as a concentration-independent metric to characterize the relaxation time (Weaver and Kuehlert, 2012). The ratio of 5th to the 3rd harmonics is an appropriate parameter to reflect the bound state of the mNPs.

In this study, we first verified the feasibility of MSB sensing the mNPs' restricted rotation secondary to the analyte binding multiple mNPs together. Linking mNPs together produces larger changes in the MSB signal than simply binding the analyte to the mNP (Rauwerdink and Weaver, 2010a). The well characterized biotin and streptavidin system with high affinity was chosen as the first model system, demonstrating the validity of the technique and allowing us to explore some of the parameters affecting the measurement. We then tested DNA aptamer–thrombin system, and a DNA–DNA interaction system. The results show that MSB can achieve specific detection with high sensitivity, and is capable of detecting analyte directly in biological samples. Thus there is potential for MSB biosensing to be utilized for a wide range of biomarkers in a variety of biomedical applications or diagnostic analyses, both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Material

Iron oxide nanoparticles functionalized with amine groups or streptavidin were obtained from MicroMod (Micromod Partikeltechnologie GmbH, Germany). EZ-link Sulfo-NHS-LC-Biotin was obtained from Thermo. Streptavidin protein and human thrombin protein were purchased from Sigma-Aldrich (St. Louis, MO) and Abcam (Cambridge, MA), respectively. All the chemicals used in this research were analytical grade reagents. Previously reported 15 mer (Bock et al., 1992) and 29-mer (Tasset et al., 1997) anti-thrombin aptamer sequences with biotin modification at 5′ were used in the thrombin targeted system. For the DNA–DNA binding system, two 12 base single-strand DNAs (ssDNA), S1 and S2, with biotin modification at 5′ and 3′ respectively were designed. Complementary strands were synthesized so half of each was complementary to S1 and the other half to S2. All the aptamer and DNA sequences in this study were custom synthesized by Integrated DNA technologies, Inc. (Coralville, IA) Spacers of 10 Ts were added between the biotin and the aptamer sequence to enhance the accessibility of the aptamer towards its target protein by reducing steric effects. The DNA sequences are listed in the supplementary material.

2.2. Preparation of functional mNPs

Before conjugation, mNPs were washed using a magnetic separator, and reconstituted in PBS buffer with 0.005% tween 20 (pH 7.4). To prepare biotin modified mNP, EZ-link Sulfo-NHS-LC-Biotin was added to the amine labeled mNP solution and incubated at room temperature for 2 h. Aptamer and ssDNA modified mNPs were achieved by conjugating the terminal biotin moieties on the aptamer/ssDNA to the streptavidin labeled mNPs. The molar ratio of the DNA aptamer to the streptavidin groups was 50:1. This reaction mixture was incubated at room temperature in PBS at pH 7.4 for 2 h. After conjugation, the modified mNPs were washed 3 times to remove all the free biotin, aptamers or ssDNA, and resuspended in PBS or Tris–HCl, correspondingly. The mNPs were stable in solution without precipitation for months.

Each sample contained approximately 150 μg mNP diluted in 500 μl buffer. PBS (pH 7.4) buffer was used for streptavidin detection; buffer containing 50 mM Tris–HCl, 140 mM NaCl, and 1 mM $MgCl_2$ (pH 7.4) was used for thrombin detection; 20 mM Tris–HCl was used for DNA detection. All the samples were prepared in triplicate. MSB measurements were taken before and 10 min after the addition of the analyte to allow binding to occur.

2.3. Characterization of amine surface density on mNPs

The number of amine groups on each mNP was evaluated using the biotin quantitation kit purchased from Thermo Pierce (Rockford, IL). Briefly, the biotinylated NP was added to a mixture of 4′-hydroxyazobenzene-2-carboxylic acid (HABA) and avidin. Because of its higher affinity for avidin, biotin displaces the HABA and results in a decrease of absorbance at 500 nm, which is proportional to the concentration of biotin in the sample.

2.4. Detection of biomolecule interactions via MSB

mNPs suspended in solution have a dynamic response to a magnetic field that depends on the balance between magnetic forces tending to align the mNPs and thermal effects that hinder the alignment of the mNPs. Relaxation times are used to characterize these mechanisms. We employed large mNPs where Neel relaxation is minimized and Brownian relaxation is dominant (Connolly and St Pierre, 2001) to maximize the sensitivity to binding because binding affects only the Brownian relaxation. In this study, mNPs with 50 nm iron oxide composite cores and 113 nm hydrodynamic radii were used. MSB is most sensitive to a given biomarker when the biomarker links multiple mNPs together, restricting rotation motion and increasing the relaxation time. When the biomarker has multiple binding sites, ligands from 2 different mNPs can bind the same biomarker molecule, assembling the mNPs into clusters or aggregates. Such an interaction leads to an increasing effective size of the mNP cluster, resulting in increased Brownian relaxation time, which could be sensed through the MSB measurement (Scheme 1). The harmonics were immediately recorded at 290, 510, 737, 1050, 1270, 1740 and 2110 Hz and 10 mT, and data acquired at 1270 Hz was used to analyze the detection sensitivity. The ratio of the 5th to the 3rd harmonic ($R53$) at each frequency was used as a concentration-independent metric(Weaver and Kuehlert, 2012).

Download English Version:

https://daneshyari.com/en/article/7234055

Download Persian Version:

https://daneshyari.com/article/7234055

Daneshyari.com