Journal of Immunological Methods 349 (2009) 38–44

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Research paper

A washing-free and amplification-free one-step homogeneous assay for protein detection using gold nanoparticle probes and dynamic light scattering

Xiong Liu [a,b], Qun Huo [a,b,c,*]

[a] NanoScience Technology Center, University of Central Florida, 12424 Research Parkway Suite 400, Orlando, FL 32826, United States
[b] Department of Chemistry, University of Central Florida, 12424 Research Parkway Suite 400, Orlando, FL 32826, United States
[c] Department of Mechanical, Materials and Aerospace Engineering, University of Central Florida, 12424 Research Parkway Suite 400, Orlando, FL 32826, United States

ARTICLE INFO

Article history:
Received 17 June 2009
Received in revised form 23 July 2009
Accepted 28 July 2009
Available online 6 August 2009

Keywords:
Gold nanoparticles
In vitro diagnostic
Dynamic light scattering
Heidelberger–Kendall
Hook effect
Competitive assay

ABSTRACT

In this study, we developed a one-step, washing-free and amplification-free assay for protein analysis using gold nanoparticle probes (GNPs) and dynamic light scattering (DLS) technique. The target protein concentration was determined by analyzing the level of GNP aggregation caused by antibody–antigen interactions using DLS. Two formats of assays were designed for mouse IgG detection. In the first format of assay, mouse IgG was directly mixed with GNPs conjugated to goat anti-mouse IgG. Due to the multiple binding sites of primary mouse IgG by the secondary antibody, mouse IgG caused nanoparticle aggregation. Mouse IgG can be detected at a concentration as low as 0.5 ng/mL and the dynamic range of this assay is between 0.5 and 50 ng/mL. A second format of assay developed in this study is a competitive assay conducted by using both mouse IgG and goat anti-mouse IgG conjugated GNPs. In this assay format, mouse IgG was detected within a dynamic range of 100 ng/mL to 10 μg/mL. The CV% of these assays is generally well within 10%. In conclusion, we demonstrated here that by using GNPs as a light scattering enhancer and selecting the proper assay formats, low cost, easy-to-conduct, and highly sensitive bioassays can be developed for protein detection and analysis.

1. Introduction

Protein detection and analysis play an extremely important role in *in vitro* diagnostics. With the tremendous progresses made in proteomics in the last few decades, there has been a growing demand for highly sensitive, fast and simple techniques for protein analysis using minimum volume of samples and cost effective instrumentations. Many protein biomarkers associated with cancer and other diseases exist in biological fluids and systems with low abundance, typically in the range of fg/mL to ng/mL (Sahab et al., 2007; Wu et al., 2007; Paliouras et al., 2007). How to detect these low abundance biomarker proteins has raised tremendous challenges for bioanalytical chemists. Traditional methods address this difficulty by using amplification labels such as enzymes, radioactive isotopes or fluorescent probes to achieve the required high sensitivity (Alivisatos, 2004; Daniel and Astruc, 2004; Rosi and Mirkin, 2005; Wilson, 2008; Diamandis and Christopoulos, 1996; Elshal and McCoy, 2006). These methods, however, often involve many steps in the assay, including labeling, multiple washing and separation cycles, signal amplification and measurement. These complicated procedures not only make it difficult to automate the assay, but also increase the cost of protein analysis.

Since the middle 1990s, gold nanoparticles (GNP) have been investigated extensively for biomolecular imaging and detection (Tsai et al., 2005; Bonham, et al., 2006; Mirkin et al., 1996; Nam et al., 2003; Pingarron et al., 2008; Das et al., 2006; Wu et al., 2008; You et al., 2007; Bonham et al., 2007). The unique optical properties of GNPs, including their strong absorption and intense light scattering at the surface plasmon resonance wavelength region, aggregation-induced color

* Corresponding author. Tel.: +1 407 882 2845; fax: +1 407 882 2819.
E-mail address: qhuo@mail.ucf.edu (Q. Huo).

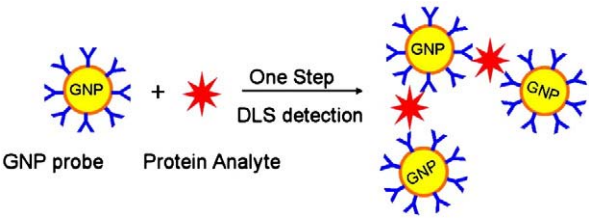
0022-1759/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2009.07.015

change (Tsai et al., 2005), and the surface-enhanced Raman scattering (Bonham, et al., 2006), have been studied in much detail in both direct and amplified biomolecular assays (Mirkin et al., 1996; Nam et al., 2003). GNPs, when conjugated with a detector antibody, may be used in a traditional heterogeneous sandwich-type immunoassay. The GNP probes immobilized on a surface due to biomolecular binding may be detected optically or electrochemically (Mirkin et al., 1996; Nam et al., 2003; Pingarron et al., 2008; Das et al., 2006; Wu et al., 2008). GNPs have also been used to develop homogeneous biomolecular assays (Tsai et al., 2005; Lee et al., 2008). Such assays are based on the detection of nanoparticle aggregation caused by protein–protein interactions or DNA hybridization. GNP aggregation or de-aggregation often leads to optical property changes such as wavelength shifts of the surface plasmon resonance (SPR) bands (or color change) (Tsai et al., 2005), melting temperature increases of the hybridized DNA (Mirkin et al., 1996; Nam et al., 2003), fluorescence quenching or enhancement (You et al., 2007; Bonham et al., 2007), and surface-enhanced Raman scattering (Bonham, et al., 2006). Homogeneous assays offer some unique advantages compared to heterogeneous assays, especially in the simplicity of the assay. However, the sensitivity of homogeneous assays, including the assays involving GNPs as mentioned above, is often not as high as heterogeneous amplification assays. Mirkin et al. developed a bio-bar-code amplification method using DNA bar-code modified GNPs for both protein and DNA detection. Although this method has extremely high sensitivity, the assay involves a complicated multiple-step process that is very difficult to conduct and automate (Mirkin et al., 1996; Nam et al., 2003).

Laser light scattering immunoassays (LIA) have been known for more than 3–4 decades (Bangs, 1996; Berne and Pecora, 1976; Cohen and Benedek, 1975; Antony et al., 1998). Most of these assays are based on immunoaffinity interaction-induced particle aggregation. The level of particle aggregation may be measured using turbidity, nephelometry, angle-dependent scattering intensity, or dynamic light scattering technique. Turbidity and nephelometry are still used substantially in medical diagnostic and environmental testing labs for biomolecule and microorganism detection. In these two assays, light transmitted through or scattered from a turbid assay solution due to particle aggregation is measured and correlated to analyte concentration. However, these two assays typically have very low sensitivity, and are mostly used for high abundance protein analysis. Dynamic light scattering (DLS), a technique used extensively for characterizing particle size and size distribution, was also applied for particle agglutination-based immunoassays in the late 1970s (Berne and Pecora, 1976; Cohen and Benedek, 1975; Antony et al., 1998). However, this technique never took off as a main stream immunoassay method for protein analysis due to multiple limitations. First and most important above all, these LIAs including DLS, turbidity, and nephelometry assays, use micron or submicron polymer beads such as latex particles as light scattering enhancers. The light scattering intensity of polymer latex particles, although substantially stronger than biomacromolecules, is still not strong enough to overcome the background scattering from sample matrices. Biological fluids such as blood serum or plasma contain large amounts of nanoparticles and micron scale particles. The intense light scattering from these matrix particles can cause significant interference to the assay. Second, when early attempts were made on using DLS for latex particle-based light scattering immunoassays, DLS was still a relatively high cost instrument, difficult to operate, with limited sensitivity and data analysis capability. Due to these multiple reasons, DLS was not considered as a promising tool for immunoassay applications.

Recently, the extensive study and further understanding on the optical properties of gold nanoparticles (GNPs) have opened many new doors in biomolecular imaging and detection. Gold nanoparticles, including spherical particles, nanorods and nanoshells with a size ranging from tens to hundreds of nanometers, are known to have a large light absorption and scattering cross section in the surface plasmon resonance (SPR) wavelength region (Jain et al., 2006; Link and El-Sayed, 1999; Pecora, 2000; Xie et al., 2007). In normalized terms, the scattering cross section of a 30 nm gold particle at its SPR region is about 250 times larger than a 30 nm polystyrene particle. As compared to fluorophores, the scattering light intensity from a 60 nm gold nanoparticle is four to five orders of magnitude higher than a strongly fluorescent fluorescein molecule (Jain et al., 2006; Link and El-Sayed, 1999). Furthermore, the scattering light from nanoparticles does not suffer from the many problems of fluorophores, such as photobleaching, extreme sensitivity to environmental changes. The strong light scattering of gold nanoparticles has enabled many important and promising applications in biomedical field, such as molecular and cell imaging (Wang et al., 2007; Aslan et al., 2005; Huang et al., 2006), biosensing, and bioassays (Yguerabide and Yguerabide, 1998; Schultz, 2003; Huo, 2007; Du et al., 2006; Russier-Antoine et al., 2008; Ray, 2006; Hirsch et al., 2003; Xu et al., 2007).

With its strong light scattering, we hypothesized that GNPs could be an excellent light scattering enhancer to replace polymer latex particles for immunoassay development using light scattering techniques. We recently conducted some exploratory studies to demonstrate the proof-of-concept of using GNPs for light scattering assay of both proteins and DNA targets as illustrated in Scheme 1 (Liu et al., 2008; Dai et al., 2008). Using a non-competitive assay format, a sandwich-type antibody–antigen binding or DNA hybridization was transduced into GNP aggregation formation, which was detected by DLS and subsequently correlated to the target analyte concentration. Due to the extremely strong light scattering intensity of GNPs around their SPR band region, this assay can potentially become highly sensitive for biomolecular detection and analysis. It is a single-step, washing-free and amplification-free process, therefore, it is very easy-to-



Scheme 1. Illustration of a one-step homogeneous biomolecular assay using gold nanoparticle probes as light scattering enhancers coupled with dynamic light scattering detection.