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Short communication

Cholesterol aided etching of tomatine gold nanoparticles: A non-enzymatic blood cholesterol monitor

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ABSTRACT

Colloidal gold is extensively used for molecular sensing because of the wide flexibilities it offers in terms of modifications of the gold nanoparticles (GNPs) surface with a variety of functional groups. We describe a simple, enzyme free assay for the detection of cholesterol, and demonstrate its applicability by estimating cholesterol in human serum samples. To enable cholesterol detection, we functionalized GNPs with tomatine, a glycoalkaloid found in the leaves and stem of tomato plants. The binding of cholesterol onto tomatine functionalized gold nanoparticles (TGNPs) was characterized by a blue shift in the plasmon absorption spectra (SPR) followed by reduction in the particle size. The TGNPs have been core etched with increasing concentration of cholesterol and with 800 ng/mL of cholesterol particles in the size range of 10–12 nm have been obtained. This behavior was attributed to the enhanced hydrophobicity of the surface acquired by cholesterol binding resulting in the folding or shrinkage of molecule in turn leading to core etching. The method was successfully applied for the detection of cholesterol in real samples and agrees well with values obtained from the conventional method. Because of its significant plasmonic shift and simplicity, this biosensor could be used for cholesterol detection as it does not demand either any hazardous and costly chemicals or any complex synthetic routes.

1. Introduction

Cholesterol is an essential lipid for human body (Chart 1, Supplementary information) and an important component of intracellular membranes of mammalian cells. The estimation of blood cholesterol is one of the most widely performed assays in biochemistry since cholesterol plays a vital role in the initiation and progression of cardiovascular diseases. The introduction of enzymes as reagents into the methodologies of cholesterol determination (Stadtman et al., 1954; Turfitt, 1984; Richmond, 1973) has drastically altered the chemical methods using acid approaches taken by most analysts of the past 90 years (Abell et al., 1952; Bloor, 1916; Pearson et al., 1952; Tschugaev, 1900). Although enzyme based sensors show good selectivity and high sensitivity, the activity of the enzyme decreases with use, and the enzyme is easily denatured during its immobilization procedure due to its intrinsic instability (Park et al., 2006). These drawbacks prevent the application of enzymatic cholesterol biosensors. It still remains a challenge to achieve non-enzymatic, sensitive, simple, rapid and inexpensive detection of blood cholesterol.

In order to overcome the disadvantage of enzymatic cholesterol sensors, non-enzymatic cholesterol sensor has been designed and fabricated, which has several attractive advantages, such as stability, simple fabrication, reproducibility, low cost, etc. Nanostructured noble metal constitutes an ideal candidate as a platform for the preparation of non-enzymatic cholesterol sensor and has been reported by some groups (Li et al., 2010; Lee and Park, 2010; Lee et al., 2009). Recently our group has reported digitonin gold nanoparticle based cholesterol sensor (Raj et al., 2011). Apart from this, detection of cholesterol based on synthetic receptors are rather scanty.

Colloidal gold is extensively used for molecular sensing due to the wide opportunities it offers in the design of easy to perform methods (Aslan and Prez-Luna, 2002). One of the approaches for the design of functional nanomaterials is based on the assembly of NPs with specific ligands or biomolecules such as proteins, lipids and nucleic acid (Pan et al., 2003; Choi et al., 2007; Rosi and Mirkin, 2005; Raj et al., 2014). The modification process or alignment of NPs leads to tunable optoelectronic properties which in fact is used in sensing applications. In our strategy for developing a cholesterol sensor we functionalized GNPs with tomatine (Chart 2, Supplementary Information), a glycoalkaloid found in the stems and leaves of tomato plants (Seipke and Loria, 2008). Tomatine is a virtually nonabsorbable saponin which has been used as an antifungal agent and analytically as cholesterol precipitant (Edwards et al., 1964). Herein, we report a

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non-enzymatic detection of blood cholesterol based on the etching of TGNPs. Alternatively this report seems to be a different approach for the detection of cholesterol based on the etching of GNPs. The size of the GNPs reduces after addition of cholesterol such that a blue shift on the SPR peak can be observed. The sensitivity of the developed cholesterol-TGNPs core etching technique for cholesterol detection is experimentally investigated using UV–visible spectrometry. Human serum samples were analyzed to demonstrate the practical application of this plasmonic blood cholesterol sensor. The results emerged demonstrate that this method could hopefully be integrated into clinical labs, as this does not demand either any hazardous and costly chemicals or any complex synthetic routes.

2. Methods

The list of used reagents can be found in Supplementary information (S11).

2.1. Apparatus and equipments

The list of used apparatus and equipments can be found in Supplementary information (S12).

2.2. Synthesis and functionalization of GNPs

The detailed method for the synthesis of GNPs, GNP2, TGNP, interaction of cholesterol with TGNP, interference studies and analysis of cholesterol in real blood samples are available in Supplementary information (S13–S18).

3. Results and discussion

3.1. Synthesis and functionalization of GNPs

GNPs synthesized (Turkevich et al., 1981) yielded spherical particles with an average diameter of 22 ± 2 nm (Fig. S1a) and SPR maxima at 519 nm (Fig. S2). After adding Tween 20, the SPR maxima of GNPs was shifted to 522 ± 1 nm due to the physical adsorption of surfactant and was consistent with the reported shifts of the band upon formation of dielectric layers around colloidal metals. The SPR maxima further shifted to 524 ± 2 nm upon chemisorptions of 16-MHDA, indicating the formation of a thicker monolayer around the GNPs (Fig. S3). The particle size also increased to 24 ± 2 nm further confirming the modification process (Table 1). On further conjugation with tomatine, the SPR further red shifted to 533 ± 1 nm (Fig. S3), with an increase in the particle size to 28 ± 2 nm (Fig. S1a). The spectral shift is not accompanied by any broadening, confirming non-aggregation of particles at this stage of modification. The modification steps were further confirmed by Fourier transform infrared spectroscopy (Fig. S4), particle size by dynamic light scattering (DLS) (Fig. S1b) and zeta potential measurements (Table 1). DLS shows an increase in the particle size during different modification process. The negative charge on the gold nanoparticles (-57 ± 2 mV) was found to be reduced on conjugation of 16-MHDA (-42 ± 1 mV) which was further reduced to (-30 ± 3 mV) when tomatine was assembled on the surface.

Table 1
Particle size and zeta potential as a function of surface modification.

System	Size (nm)	Zeta potential (mV)
GNP	22 ± 2	-57 ± 2
GNP2	24 ± 2	-42 ± 1
TGNP	28 ± 2	-30 ± 3
TGNP+cholesterol	12 ± 2	-9 ± 2

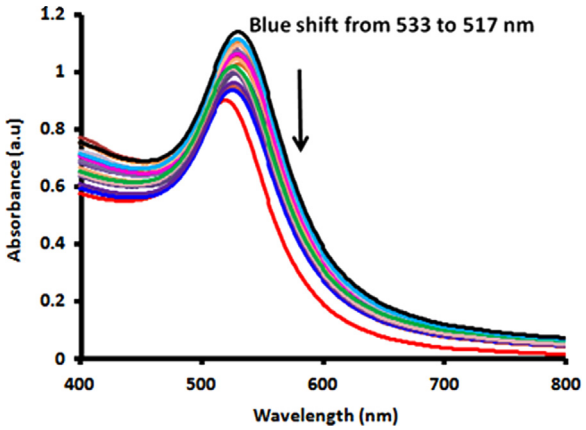

Fig. 1. Plasmon absorption peak of TGNPs in the presence of different cholesterol concentrations (concentration from 100 to 850 ng/mL of cholesterol).

3.2. Interaction of cholesterol with TGNPs

At first, we investigated the effect of cholesterol on TGNP and the results are shown in Fig. 1. The core of most of the studies on the use of GNPs as colorimetric sensor is the red shift in the SPR absorption peak on the event of NPs-biomolecule interaction leading ultimately to a color change. Contrary to these reports, we observed a blue shift in the SPR peak of TGNPs on interacting with cholesterol. To study the effect in more detail, different concentrations of cholesterol ranging from 100 to 800 ng/mL were added and the SPR shifted proportionally from 533 ± 1 nm to 517 ± 2 nm. The shift towards the blue region is a manifestation of the decrease in the particle size upon cholesterol binding. Further investigation showed that upon addition of cholesterol, TGNPs also tend to split into smaller ones.

Several researchers have reported that alkane thiol molecules in solution can etch GNPs due to the formation of self-assembled monolayers (SAMs) and surface tension repulsion of the attached molecules (Pettibone and Hudgens, 2011). GNPs are then separated into smaller gold clusters from the outermost surface layers. The mechanism of formation of GNPs of smaller size is not well understood. We present below our tentative suggestions for the etching of TGNPs in the presence of cholesterol. There are two possible routes for etching. In the presence of cholesterol, etching takes place resulting in the formation of smaller GNPs. This behavior is attributed due to the enhanced hydrophobicity of the surface resulting in the shrinkage or folding of the molecule. As a result of folding the innumerous hydroxyl groups on the surface of tomatine comes in direct contact with the core of the GNP resulting in the etching of the gold core. The proposed mechanism is depicted in Scheme 1.

Cholesterol binding forces the diffused out tomatine molecules to assume a condensed shape resulting in an overall reduction in size. In the second possible route, upon addition of cholesterol to TGNPs due to the strong affinity of cholesterol towards TGNPs they may come closer to one another. Under such a situation the hydroxyl groups on the surface of one TGNP may interact with the core of the neighboring TGNPs causing the etching of the gold core. Similarly a number of TGNPs interact with one another resulting in etching and the reduction in the particle size. However as we proposed in our first route, enhanced hydrophobicity of the surface ultimately leading to shrinkage of the molecule may be the prime cause for the size reduction. Our findings agree with that of Lee et al. (2012) where 4-dimethyl amino pyridine (DMAP) capped