Assembly of citrate gold nanoparticles on hydrophilic monolayers

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ABSTRACT

Self-assembled monolayers (SAMs) as model surfaces were linked onto planar gold films thorough lipoic acid or disulfide groups. The molecules used were polyethylene glycol (EG-S-S), *N*-[tris-(hydroxymethyl)methyl]acrylamide polymers with and without lipoic acid (Lipa-pTHMMAA and pTHMMAA) and a lipoic acid triazine derivative (Lipa-MF). All the layers, but Lipa-MF with a primary amino group were hydroxyl terminated. The layers were characterized by contact angle measurements and atomic force microscopy, AFM. Citrate stabilized nanoparticles, AuNPs in water and phosphate buffer were allowed to assemble on the layers for 10 min and the binding was followed in real-time with surface plasmon resonance, SPR. The SPR resonance curves were observed to shift to higher angles and become increasingly damped, while also the peaks strongly broaden when large nanoparticles assembled on the surface. Both the angular shift and the damping of the curve was largest for nanoparticles assembling on the EG-S-S monolayer. High amounts of particles were also assembled on the pTHMMAA layer without the lipoic acid group, but the damping of the curve was considerably lower with a more even distribution of the particles. Topographical images confirmed that the highest number of particles were assembled on the polyethylene glycol monolayer. By increasing the interaction time more particles could be assembled on the surface.

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1. Introduction

Gold nanoparticles (AuNPs) exhibit interesting optical and electronic properties that have found promising applications in the fields of sensors, catalysis, electronics, photonics, solar cells, cancer diagnosis and therapy, drug delivery, biomedical imaging, implants, and recently as antioxidants, and anti-coagulants [1–10]. Two ways exist for optical excitation of electrons in noble metals: through surface plasmons in thin layers of noble metals or through bulk plasmons in nanoparticles [11]. Nanoparticles of noble metals exhibit strong resonant scattering of light due to plasmonic oscillation of free electrons in the bulk metal. On the other hand, Surface Plasmon Resonance (SPR) occurs when monochromatic light is directed onto a planar gold surface, which causes a collective oscillation of surface electrons at the interface between a dielectric material and the gold surface. Combinations of nanoparticle and surface excitation of plasmons are obtained through the use of well-defined nanostructured metal layers on dielectric substrates, generally referred to as Plasmonics [12].

The SPR phenomenon is based on free electrons resonating at the metal surface, the surface plasmons, which are excited with visible or near infrared light. There are basically four parameters of the layer that can change when the nanoparticles adsorb to the surface: the thickness (d), the refractive index (n), the absorption coefficient (k) and the degree of scattering (S). The SPR curve will shift upwards along the angle axis when d and n increase, while the SPR phenomenon will become damped by increase of k with an increase in reflection coefficient. The fitting of SPR curves according to Fresnel theory is only valid when using isotropic layers absent of scattering effects and only concerns d, n and k. Scattering will give further broadening of the SPR spectra.

In nanoparticles, a strong absorption can be observed in the UV–vis spectrum of a colloidal suspension. The absorbance spectra for Au nanoparticles have a maximum absorbance (λ_{max}) at around 520 nm. This plasmon mode absorption band gives the colloidal gold its characteristic deep red-purple color. The location of the absorbance peak is highly dependent upon the size and shape of the particles, but also their degree of aggregation. Controlled assemblies of AuNPs represent an area of growing interest. Nanoparticles can be assembled on the surface by physical and chemical assembly. Physical forces are involved in physical assemblies, which include spin coating, and various lithographic methods [13–16]. Often the physical methods combined with chemical ones utilizes coupling chemistries to direct and control the deposition of nanoparticles onto functionalized substrates [17–21]. This allows tailoring of the nanostructures and formation of more complex nanostructured assemblies [22]. Most of the supported nanoparticle assemblies are based on small-sized (2–8 nm) nanoparticles [23,24], whereas less studies are found on large particles. Although many methods have been described to fabricate AuNP assemblies, simple and effective routes is still very much pursued when targeting commercial applications.

In this study, bi-functional *N*-[tris-(hydroxymethyl) methyl]acrylamide polymers (Lipa-pTHMMAA and pTHMMAA), polyethylene glycol (EG-S-S) and Lipa-metformin (Lipa-MF) were used for linking gold nanoparticles to solid surfaces with a thin sputtered layer of gold (Scheme 1). The molecules were self-assembled on the gold surface by covalent linkage through lipoic acid or disulfide groups. The pTHMMAA polymer without the lipoic acid group was used as references. This polymer was also used to coat polystyrene. The coupling chemistries often use amino groups for nanoparticle immobilization. We chose Lipa-MF that possesses an amino group and the other molecules with hydroxyl groups for assembling nanoparticles on the surface. Atomic force microscopy (AFM) and contact angle was used for characterization of the layers and SPR for measuring the interaction of the AuNPs in real-time to the self-assembled layers.

Lipa-pTHMMAA is nonionic, very hydrophilic, biocompatible, and inert to biological fouling [25]. We have previously used Lipa-pTHMMAA conjugates to increase the stability of antibodies and avidin on gold surfaces and suppress non-specific binding in immunoassays [26–30]. Surface-bound poly(ethylene glycol) has been used for retarding the non-specific adsorption of proteins and other biological species [31,32]. During our immunoassay studies we had noted that uncoated AuNPs adhered to surfaces treated with Lipa-pTHMMAA. We wanted to investigate this further as we found no studies on the interaction of uncoated AuNPs with hydroxyl terminated layers.

2. Experimental

2.1. Chemicals and synthesis

Chloroauric acid (HAuCl$_4$ aq) 99.999% grade was purchased from Aldrich. Biograde sodium citrate was from Sigma-Aldrich. 10 mM phosphate buffer (PB) pH 7.4 was used. Poly(ethylene glycol) with a disulphide linkage (P8735-EG-disulfide, Mw 6500 g/mol), hereafter named EG-S-S, was from Polymer Source Inc. Quebec, Canada (Scheme 1c). The polymers pTHMMAA and Lipa-pTHMMAA were prepared according to the method by Albers et al. (Mw in the range of 4500 and 6000 g/mol) [25,28]. For the preparation of the pTHMMAA polymer, 4,4′-azobis(cyanovaleric acid) from Sigma-Aldrich was used as the azo-initiator. α-Lipoic acid *N*-succinimidyl ester was prepared as described by Tappura et al. [33]. Metformin (1,1dimethylbiguanide) was prepared from the hydrochloride salt by the method of Huttunen et al. [34]. The compound Lipa-MF was synthesized according to the following procedure: To a mixture of 1,1-dimethylbiguanide (1.15 g, 8.87 mmol) in acetonitrile (50 mL) was added a mixture of lipoic acid NHS ester (1.0 g, 3.3 mmol) in acetonitrile (50 mL). A white precipitate was formed and the mixture was stirred for 1 h. The reaction was judged completed by TLC analysis (eluent: ethyl acetate). The mixture was filtered and the clear yellow filtrate was concentrated by rotary evaporation to about 15 mL. The mixture was purified by chromatography (silica gel, eluent dichloromethane/methanol, 20/1, v/v). The fractions containing the pure product were combined and the solvent removed to give a yellow solid, 6-[4-(1,2-dithiolan-3-yl)butyl]-*N*,*N*-dimethyl-1,3,5-triazine-2,4-diamine, **Lipa-MF** (0.74 g, 75%). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 5.06 (2H, br, s, NH_2), 3.57 (1H, m, H-3), 3.23-3.01 (8H, m, H-1, H-11, H-12), 2.49 (2H, t, J = 7.4 Hz, H-7), 2.45 (1H, m, H-2′), 1.90 (1H, m, H-2″), 1.73 (4H, m, H-4, H-5), 1.51 (2H, m, H-6). ESI-MS: accurate mass, MH$^+$ 300.1324, calcd. 300.1317; MNa$^+$ 322.1122, calcd. 322.1136.

2.2. Preparation of gold nanoparticles

Gold nanoparticles were prepared according to the method by Frens [35]. Briefly, a 50 mL aqueous solution of HAuCl$_4$ (0.25 mM) was heated to boiling and trisodium citrate (138 mM) was added. The solution was allowed to boil for another 20 min, and cooled down during continuous stirring. The resultant gold colloids were stored in 4 °C. The pH of the AuNP solution was adjusted to a pH of 6.4. These particles are termed "AuNPs citrate". A set of AuNPs was centrifuged at 4 °C at 4000 rpm for 30 min. The supernatant was discarded, phosphate buffer, PB (10 mM sodium phosphate at pH 7.4) was added and the tube was centrifuged once more. This procedure was repeated twice to change to PB buffer. These nanoparticles are termed "AuNPs PB".

2.3. Instrumentation

The ^{1}H NMR spectra were recorded with a 300 MHz Varian Mercury NMR spectrometer. High-resolution MS spectra were recorded with a Waters LCT premier XE ESI-TOF mass spectrometer in the positive ion mode. UV–vis absorption spectra were recorded using a Perkin-Elmer UV–vis Envision Spectrophotometer at room temperature. A Zetasizer Nano ZS (Malvern Instruments) was used for dynamic light scattering and zeta potential measurements.

Butwar-coated copper grids were made hydrophilic by glow discharging with an EMS/SC7620 Mini sputter coater according to the instructions by the manufacturer. Gold nanoparticle samples (5 μL) were added on the grids and incubated for about 1 min after which the excess sample was blotted with Whatman 3 mm paper. The samples were dried overnight and imaged with a JEM-1400 (JEOL) transmission electron microscope.

SPR measurements were performed with SPR Navi^{TM} 210A-L instrument (BioNavis, Tampere, Finland), using two different wavelengths. A flow-rate of 10 μL/min was used throughout the experiments. SPR curves were recorded every 11th second, in total about 60 curves were recorded for the interaction with AuNPs. The slides were rinsed with water and stored in room temperature until further measurements were performed when removed from the SPR instrument.

An NTEGRA Prima (NT-MDT, Russia) atomic force microscope (AFM) was used to analyze the topography of the samples in intermittent-contact mode. The images (1024 × 1024 pixels) were recorded in ambient conditions in air (RH = 35 ± 3%, T = 25 ± 1 ∘C) using rectangular cantilevers (Model: NSG01, NT-MDT, Russia) with a nominal tip radius of 10 nm. A scan speed was set to 0.39 Hz. The obtained images were processed and analyzed with commercial image analysis software (SPIP 5.1.3, Image Metrology, Denmark).