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Surface interactions of gold nanorods and polysaccharides: From clusters to individual nanoparticles

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ABSTRACT

Gold nanorods (AuNRs) are suitable for constructing self-assembled structures for the development of biosensing devices and are usually obtained in the presence of cetyltrimethylammonium bromide (CTAB). Here, a sulfated chitosan (ChiS) and gum arabic (GA) were employed to encapsulate CTAB/AuNRs with the purpose of studying the interactions of the polysaccharides with CTAB, which is cytotoxic and is responsible for the instability of nanoparticles in buffer solutions. The presence of a variety of functional groups such as the sulfate groups in ChiS and the carboxylic groups in GA, led to efficient interactions with CTAB/AuNRs as evidenced through UV–vis and FTIR spectroscopies. Electron microscopies (HR-SEM and TEM) revealed that nanoparticle clusters were formed in the GA-AuNRs sample, whereas individual AuNRs, surrounded by a dense layer of polysaccharides, were observed in the ChiS-AuNRs sample. Therefore, the presented work contributes to the understanding of the driving forces that control the surface interactions of the studied materials, providing useful information in the building-up of gold self-assembled nanostructures.

1. Introduction

Gold nanoparticles (AuNPs) have increasingly been given extensive attention due to their unique properties making those materials useful in catalysis, nanoelectronics and, more interestingly, in optical sensing and diagnostics in the biomedical field (Garabagiu & Bratu, 2013; Kopwitthaya et al., 2010; Mitamura, Imae, Saito, & Takai, 2007; Pierrat, Zins, Breivogel, & Sonnichsen, 2007).

Among the AuNPs, considerable attention has been dedicated to gold nanorods (AuNRs). The coherent oscillation of the electrons along the short axis (transversal SPR) and the long axis (longitudinal SPR) of the nanorods causes two surface plasmon resonance (SPR) bands. At least one of these bands can be found in the visible spectra. The transversal SPR band has a maximum absorption around 520 nm (Rayavarapu et al., 2010), whereas the longitudinal SPR band is observed in the range from 650 nm (shorter rods) to 950 nm (longer rods) (Eutis & El-Sayed, 2006; Rayavarapu et al., 2010). As transversal and longitudinal SPR are shape and size dependent (Murphy & Jana, 2002; Xie et al., 2011), the AuNRs are particularly suitable for building up self-assembled structures for the development of biosensors (Yu et al., 2014), nanodevices (Xie et al., 2011), and non-invasive probes (Charan et al., 2012).

Regarding the applications of AuNPs in biological environments, some important issues arise concerning the maintenance of their morphological stability, cytotoxicity, and interactions with different organisms or their components. Therefore, the choice for appropriate stabilizing agents is of the utmost importance in obtaining AuNRs that are stable in different environmental conditions (pH and ionic strength) and that exhibit low toxicity.

The seed mediated method in the presence of the surfactant cetyltrimethylammonium bromide (CTAB) is the most commonly employed procedure to obtain AuNRs, although some other methods have been recently proposed (da Silva, Nunes, Meneghetti,

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& Meneghetti, 2013; Pérez-Juste, Pastoriza-Santos, Liz-Marzán, & Mulvaney, 2005). In general, the method initially proposed by Murphy (Gole, Orendorff & Murphy, 2004; Jana, Gearheart & Murphy, 2001a; Jana, Gearheart, & Murphy, 2001b; Jana, Gearheart, & Murphy, 2001c; Johnson, Dujardin, Davis, Murphy & Mann, 2002; Murphy & Jana, 2002) and El-Sayed groups (Nikoobakht & El-Sayed, 2001) consists of the formation of the AuNRs from small sized spherical AuNPs (seed solution), which then acts as nucleation centers in the AuNRs synthesis. In the presence of CTAB, the AuNRs growth is mainly unidirectional since the interactions between the polar head groups of the surfactant, i.e. the quaternary ammonium bromide moiety, and the crystallographic {110} facet of the growing particle is preferential, causing the growth in the longitudinal direction, parallel to the {001} planes (da Silva, Meneghetti, Denicourt-Nowicki, & Roucoux, 2014; Meena & Sulpizi, 2013; Nikoobakht & El-Sayed, 2003). Therefore, the different growth rates of the facets are the factors that determine the final shape of the nanoparticle (short *versus* long NRs). In addition, CTAB is responsible for maintaining the colloidal stability since the bilayer structure, which is formed by the self-interaction of the alkyl groups of CTAB, promotes the suitable protection against particle agglomeration in aqueous media through electrostatic and steric interactions (Boca & Astilean, 2010). However, CTAB is cytotoxic and causes AuNRs instability in buffer solutions, which then restricts their use in biological applications (Boca & Astilean, 2010; Hamon, Bizien, Artzner, Even-Hernandez, & Marchi, 2014; Rayavarapu et al., 2010).

Therefore, when focusing on the obtention of AuNRs that safely can be used in the biomedical field, it is fundamental to replace part of CTAB or encapsulate the CTAB/AuNRs to obtain particles with reduced toxicity that are also stable under different environments. Kopwitthaya et al. (2010) and Rayavarapu et al. (2010) synthetized CTAB/AuNRs and showed that the replacement of CTAB by thiolated poly(ethylene glycol) (PEG-SH) produced particles with lower cytotoxicity compared to the as-prepared AuNRs (Kopwitthaya et al., 2010; Rayavarapu et al., 2010). In addition, other molecules have been used to replace CTAB intending to reduce the cytotoxicity, such as polystyrene sulfonate, polyethylene glycol (Rayavarapu et al., 2010), 1-Mercaptoundec-11-yl)hexa(ethylene glycol) (EG_6OH) (Xie et al., 2011), thio-polyethylene glycols (Bogliotti et al., 2011), polyacrylic acid, poly(allylamine) hydrochloride (PAH) (Huang, Jackson & Murphy, 2012), and 3-mercaptopropionic acid (MPA) (Garabagiu & Bratu, 2013).

Polysaccharides are part of a very promising family of naturally occurring molecules that have also been described to interact with gold nanoparticles. The presence of a variety of functional groups in their structure assists the favorable interactions between the AuNRs and the surrounding media, which is responsible for the AuNRs stabilization and also provides sites for further chemical modifications (Erathodiyil & Ying, 2011; Liu et al., 2013). In addition to being natural products, polysaccharides have inherent properties such as biodegradability, biocompatibility, and low toxicity (Liu et al., 2013). Surprisingly, there is a relatively low number of scientific works reporting the use of polysaccharides as stabilizing agents of AuNRs (Wang, Chang & Peng, 2011; Yu et al., 2014), although many works have reported the efficient stabilization of spherical gold nanoparticles by polysaccharides.

Chitosan (Chi) is a linear polysaccharide extracted from the exoskeleton of crustaceans, obtained by deacetylation of chitin. Medium and high molar mass chitosan is only soluble in water at pH lower than 6.0 (Williams & Phillips, 2000, Chp. 21). Chitosan (Boca et al., 2011) and its derivatives have been successfully employed to cap AuNPs for photothermal therapy (Wang, Chang & Peng, 2011; Yang et al., 2015), for optoacoustic tomography (Wang et al., 2015), among other applications. Gum arabic (GA) is a highly branched natural polysaccharide exuded from the trunks and barks of acacia trees. This polysaccharide has been extensively used for the stabilization of spherical AuNPs (Chanda et al., 2010; Kattumuri et al., 2007; Wu & Chen, 2010), displaying optimal performance in a wide pH range (Barros et al., 2016).

In order to reduce the cytotoxicity inherent to CTAB stabilized AuNRs and simultaneously improve the AuNRs stability in physiological media, we used a sulfated chitosan (ChiS) or GA to encapsulate CTAB/AuNRs. The non-toxicity and biocompatibility of Chi and GA were evaluated in previous works (Bicho, Roque, Cardoso, Domingos, & Batalha, 2009; Boca et al., 2011). The chemical modification of Chi to obtain ChiS is of interest because it does not only keep the Chi main chain backbone intact, but it also improves its solubility in aqueous media (Jayakumar, Nwe, Tokura, & Tamura, 2007). Moreover, it can potentially infer new functionalities to the modified Chi since sulfated polysaccharides, like heparin for example, are known to present important biological functions such as anticoagulant and/or antithrombotic actions (Asif et al., 2016; Jayakumar et al., 2007; Maas et al., 2012).

Herein we demonstrate by UV–vis (Ultraviolet–visible) and FTIR (Fourier Transform Infrared) spectroscopies, and also by transmission and scanning electron microscopies that ChiS and GA interact differently with the AuNRs. The differences are discussed in terms of the different functional groups present in each polysaccharide that leads to distinct polysaccharide/AuNRs structures. Therefore, this study contributes to understanding and controlling the self-assembling behavior of AuNRs, mediated by the capping agent.

2. Materials and methods

2.1. Materials

Tetrachloroauric acid ($HAuCl_4{\cdot}3H_20$, 30% in dilute HCl, 99,9%), CTAB (≥98%), GA ($M_w = 9.3 \times 10^5\ g\ mol^{-1}$; uronic acid content of 17%) (Grein et al., 2013), chitosan (≥75% deacetylated), and silver nitrate ($AgNO_3$, > 99%) were purchased from Sigma-Aldrich. Sodium borohydride ($NaBH_4$, ≥ 98%) was purchased from Nuclear (São Paulo, Brasil) and ascorbic acid (AA, >99%) was purchased from Dinâmica (São Paulo, Brasil). Milli-Q grade water (18.2 MΩ cm, Millipore, USA) was used in the preparation of all solutions. Prior to use, GA powder was solubilized in water, left overnight at 4 °C and subsequently dialyzed for 48 h against distilled water through a dialysis membrane (12–14 kDa cut-off) and freeze-dried.

2.2. Sulfation of a commercial chitosan

Commercial chitosan (Chi) underwent the sulfation reaction according to Terbojevich, Carraro, and Cosani (1989)'s sulfuric acid:chlorosulfonic acid method. Briefly, 1.00 g of commercial chitosan was added to the pre-cooled (4 °C) reaction mixture (40 mL of sulfuric acid (H_2SO_4 95%) and 20 mL of chlorosulfonic acid ($HClSO_3$ 98%)). Then, the reaction was carried out at room temperature under stirring for 1 h. The sulfation was stopped by pouring 250 mL of cold diethyl ether (Et_2O) into the reaction mixture. The precipitate formed was washed with cold Et_2O, then suspended in distilled water, neutralized with saturated $NaHCO_3$, dialyzed against tap water through a 3500 kDa cut-off membrane, and freeze-dried. The final product was fully characterized (ChiS, $M_w = 1.4 \times 10^4\ g\ mol^{-1}$; $SO_4 = 48\%$; SO_3 *O*–3 = 6.8% and SO_3 *O*–6 = 41.2%) (Supplementary material—Fig. S1 and Table S1) and resulted in a pale yellow powder that was stored in a moisture free environment.

The surface charge was obtained using a Zetasizer Nano ZS instrument by solubilizing the polysaccharides (ChiS and GA) using Milli-Q water.