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Direct interaction of hydrophilic gold nanoparticles with dexamethasone drug: Loading and release study

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ABSTRACT

Water-soluble gold nanoparticles functionalized by sodium 3-mercapto-1-propansulfonate (Au-3MPS) were synthesized with different Au/thiol molar ratios for their ability to interact with biomolecules. In particular, a synthetic glucocorticoid steroid, *i.e.* dexamethasone (DXM) was selected. Herein, the formation of the Au-3MPS/DXM bioconjugate is reported. Au-3MPS nanoparticles show a plasmon resonance at 520 nm, have a spherical morphology and average size of 7–10 nm. The total number of gold atoms was estimated to be about 10600, with a surface component of 8800 atoms and a number of thiol ligands of about 720, roughly one anchored thiol every 10 surface gold atoms. The drug-nanoparticle interaction occurs through the fluorine atom of DXM and Au(I) atoms on the gold nanoparticle surface. The 3MPS ligands closely pack apart each other to leave room for the DXM, that lies at the gold surface in an unusual, almost parallel feature. The loading efficiency of DXM on Au-3MPS was assessed in the range 70–80%, depending on the thiol content. Moreover, our studies confirmed the drug release of about 70% in 5 days. Thanks to their unique properties, *i.e.* high water solubility, small size and almost monodispersity, Au-3MPS display high potential in biotechnological and biomedical applications, mainly for the loading and release of water insoluble drugs.

1. Introduction

Nanostructured materials play a key role in the scientific community for their fascinating properties that allow their use in advanced applications [1–3]. The transport and release of therapeutic materials to specific target, represents one of the most interesting challenges to which the biomedicine is called; an example is represented by gold nanoparticles (AuNPs) that can be used in the hyperthermia treatment [4,5]. For this purpose several delivery systems have been investigated, including polymers, liposomes, polymeric micelles and vesicles, dendrimers and metallic nanoparticles [6–9]. Gold nanoparticles have proved to be a promising carrier towards different cell types, thanks to a variety of physical–chemical properties, that make these particles a privileged candidate in drug transport and release applications.

More specifically, the gold core is substantially inert, non-toxic and biocompatible, and represents an ideal starting point for the building up of a composite carrier. Gold nanoparticles can be produced with a good control of shape or size dispersity and functionality [10]. Several techniques for AuNPs characterization allow a good estimation of the grafting density, in particular for covalent functionalization [11,12]. Moreover, it is possible to address the synthesis towards a specific target, with size comparable with biomolecules, such as proteins or DNA, facilitating particle integration into biological systems. Furthermore, the high surface/volume ratio allows a tunable degree of functionalization, both with molecules able to bind the target and with therapeutic and drug compounds [13].

Dexamethasone (DXM), a synthetic glucocorticoid steroid, is one of the most widely used drugs for the treatment of myeloma [14]. This drug has been used as a therapeutic agent for several inflammatory diseases, because of the suppression of the expression of inflammatory genes [15,16]. DXM is also used in the treatment of inflammatory diseases such as asthma [17], meningitis [18], and rheumatoid arthritis [19]. Finally, DXM is an hydrophobic bioactive compound largely used in tissue engineering applications [20]. Because of the above stated characteristics of this bioactive molecule, DXM-loaded AuNPs may represent a promising candidate to be an effective carrier in drug delivery. However, the drug

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loading efficiency and, especially, the release kinetic in an appropriate aqueous medium, must be accurately investigated.

In this study, we extend our previous investigation on the characterization of the electrical interface of AuNPs [21] towards the formation of bioconjugates in the presence of DXM, with the aim of evaluating their efficiency in the drug loading and release. In particular, AuNPs were prepared and stabilized by sodium 3-mercapto-1-propanesulfonate (3MPS) with different Au/S molar ratios. The functionalized gold nanoparticles (Au-3MPS), after a careful purification procedure, have been characterized by means of UV–Vis, IR, NMR spectroscopies and by dynamic light scattering (DLS) measurements, to investigate their size and size distribution. The bioconjugated materials were widely characterized; XPS and NMR results suggest that the formation of the Au-3MPS/DXM bioconjugate occurs through a F—Au interaction. FESEM and AFM microscopies prove that Au-3MPS nanoparticles change their self-assembly after drug loading.

2. Experimental section

2.1. Chemicals and materials

All chemicals used were commercially available or prepared as reported in the supporting information, together with spectroscopic data. The synthesis of the AuNPs was performed through reduction in aqueous phase of $HAuCl_4{\cdot}3H_2O$ with $NaBH_4$ and using 3MPS as capping agent, according to literature report [21,22]. The DXM loading was carried out on AuNPs, following the procedure of Singh et al. [23]. In the typical loading protocol, a mixture of nanoparticles and DXM (2.0 mg solved in 0.200 μL CH_3OH, mass ratio Au/DXM = 5/1) was dispersed in water under vigorous stirring at room temperature, for 4 h. Afterward, the solution has been centrifuged in order to separate the supernatant, from the solid residue (AuNPs loaded with DXM, after referred as Au-3MPS/DXM). DXM release was investigated in PBS buffer solution at pH = 7.4. The DXM-loaded nanoparticles were dispersed into 100 mL of buffer solution in a thermostatic-bath at 37 °C. Fixed aliquots were removed at set time intervals (24 h, 48 h, 72 h up to 5 days) from the medium and replaced in by fresh buffer solution. From the withdrawn buffers, the amount of drug released in the buffer solution was evaluated by means of a calibration line and following the absorption of DXM (at 242 nm) in UV–vis spectra. For each sample, three independent measurements have been carried out and the mean value and the standard deviation are reported.

2.2. Instruments

Absorption spectra of gold nanoparticles dispersed in deionized water were measured in 1.00 cm optical path quartz cells by using a Cary 100 Varian spectrophotometer. FT-IR and FIR spectra were recorded with a Bruker Vertex 70 instrument using KRS-5 cells, in the range 4000–400 cm^{-1} and 400–200 cm^{-1}, respectively; the samples have been prepared as cast films or from Nujol mulls. NMR spectra were carried out by using a Bruker Avance 400 spectrometer operating at a frequency of 400.13 MHz for the proton. The compounds were suspended in 0.6 ml of deuterium oxide with 3-(Trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP) at a concentration of 2 mM as chemical shift and concentration reference. Bidimensional ^1H homonuclear NOESY experiments [24] were acquired with a mixing time of 150 ms at 298 K, a spectral width of 15 ppm in both dimensions employing a matrix of 4 k × 256 data points, a repetition time of 2 s and 16 scans. FESEM images has been obtained using a Zeiss Auriga 405, adopting a voltage level of 7.5 keV and a working distance of 2.5 mm, on freshly prepared films drop casted from deionized water solutions (c = 1 mg/mL) directly on the metallic sample holder. Atomic Force Microscopy (AFM) images have been acquired in tapping mode in air by using a DIMENSION ICON with a Nanoscope V Controller (Brucker AXS, Germany). High resolution Rotated-Tapping mode etched silicon probes (RTESP) with nominal tip radius of 8 nm and resonant frequency around 300 kHz were used. Samples have been deposited on a silicon substrate, by drop casting the sample solution (c = 1 mg/mL in deionized water) incubated for 10 min, then rinsed with deionized water and gently flushed with a stream of nitrogen for drying. Samples were stored in vacuum for 12 h and analyzed after 24 h. XPS analysis was performed in an instrument of our own design and construction, consisting of a preparation and an analysis UHV chamber, (resolution of 1.0 eV as measured at the Ag $3d_{5/2}$ core level). Mg Kα non-monochromatised X-ray radiation (1253.6 eV) was used for acquiring core level spectra of all samples (C1s, F1s, Au4f, S2p, Na2p and O1s). The spectra were energy referenced to the C1s signal of aliphatic C atoms having a binding energy BE = 285.00 eV. Atomic ratios were calculated from peak intensities by using Scofield's cross section values and calculated λ factors [25]. Curve-fitting analysis of the C1s, F1s, Au4f, S2p, Na2p and O1s spectra was performed using Voigt profiles as fitting functions, after subtraction of a Shirley-type background [26]. Dynamic light scattering (DLS) measurements were carried out on the nanoparticle aqueous suspensions (0.01–0.20 mg/mL), using a Brookhaven instrument (Brookhaven, NY) equipped with a 10 mW HeNe laser at a 632.8 nm wavelength at a temperature of (25.0 ± 0.2) °C [27,28]. The dielectric and conductometric properties of thiol-coated metal NPs in aqueous solution have been measured in the frequency range of 10 kHz to 2 GHz by means of frequency-domain dielectric spectroscopy, using two Precision RF Impedance Analyzers, Hewlett–Packard model 4294A (in the frequency range of 40 Hz to 110 MHz) and model 4291A (in the frequency range of 1 MHz to 2 GHz). Dielectric cell and calibration procedure are described elsewhere [21,29,30].

3. Results and discussion

3.1. Synthesis and characterization of Au-3MPS nanoparticles

The synthesis of AuNPs by chemical reduction is the most convenient method to achieve spherical nanoparticles with tuned sizes and stabilizing ligands [31,32].

In our work, Au-3MPS have been prepared by the reduction in aqueous solution of $HAuCl_4$ with sodium borohydride as strong reducing agent. Sodium-3-mercapto-1-propansulfonate (3MPS) was chosen because it is essential to induce a high solubility of AuNPs in water; their solubility, which is mandatory for biomedical applications, is mainly due to the presence of the charged terminal groups of the thiol.

In order to modulate the functionalization degree of the nanoparticles and the drug loading efficiency on the gold nanoparticle surface, the synthesis was carried out by varying the Au/S molar ratio in the range 1/4, 1/10, 1/20 (Au-3MPS-1, -2 and -3, respectively). The reaction scheme is shown in Fig. 1.

Gold nanoparticles have been characterized by means of IR, UV–vis, NMR and XPS spectroscopies. FIR spectroscopy supports the presence of the covalent Au—S bond [33], with a band at 225 cm^{-1}. FTIR shows the presence of the sulphonic group with a peak at 660 cm^{-1} (νSO_3^-) and the absence of the vibrational band at 2560 cm^{-1}, typical for the S—H stretching mode of free thiol, and confirms that the functionalization occurred. After careful purification, by means of dialysis (6 days in deionized water), UV–vis spectra have been carried out together with DLS measurements on the Au-3MPS samples. The UV–Vis spectrum, Fig. 2a, shows the typical Surface Plasmon Resonance band, at 520 nm.