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Short communication

The fate of differently functionalized gold nanorods in human serum: A response from capillary electrophoresis–inductively coupled plasma mass spectrometry

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ABSTRACT

A highly selective and sensitive method was developed to characterize intrinsic intramolecular interactions between potential theranostic agents, gold nanorods (AuNRs), and plasma proteins. The method is based on a hyphenation of capillary electrophoresis (CE) with inductively coupled plasma mass spectrometry (ICP-MS), which enables monitoring the speciation changes of AuNRs under physiologically compatible conditions. To improve the separation resolution between the intact nanorods and different gold–protein conjugates, the CE system was optimized by varying the type and concentration of background electrolyte, applied voltage, and sample loading. Optimization allowed also for acquiring the acceptable figures of merit such as migration time and peak area precision of 4.7–8.2% and 5.1–6.3%, respectively, detection limits in the range of 5.5–5.7 $\mu g\,L^{-1}$ Au, and recoveries on the order of 91–99%. With the developed method the metal-specific profiles were recorded for differently functionalized AuNPs in combinations with individual serum proteins and in human serum. In case of carboxy-modified AuNPs, proteinization in real-serum environment occurs without albumin participation, apo-transferrin dominating the protein corona under equilibrium conditions. On the contrary, the AuNRs with surface amino-groups first form the albumin conjugate but albumin in this "soft" corona becomes slowly replaced by other, less abundant proteins, exhibiting a higher affinity toward the aminated surface.

1. Introduction

Rod-shaped gold nanostructures, or gold nanorods (AuNRs), feature unique optical properties that are highly dependent on their size and aspect ratio (i.e. the ratio between the length and width) [1,2]. In contrast to spherical gold nanoparticles absorbing mainly in the visible range, AuNRs exhibit also intense absorption in the vis-NIR region due to the longitudinal excitation of plasmons [3]. It is this type of absorption that opens a prospect to a range of biomedical applications of AuNRs [4,5], for which intravenous administration is the most plausible route for human use. As is the case for every type of metal-based nanomaterials, in contact with blood the AuNRs encounter high concentrations of proteins that tend to adsorb onto the nanorod surface. The protein corona can completely mask the functionalized surface, hence greatly affecting the biological behavior of AuNRs, including cellular uptake, intracellular interactions, targeting efficiency, etc. [6].

Certain progress has recently been achieved in the characterization of proteinization of AuNRs, using a variety of analytical techniques [7–11], but all being incapable to provide pertinent speciation information. Most probably this is the reason why none of these studies has been advanced to testing interactions between the AuNRs and proteins in plasma (or serum) environment. There is no need to emphasize that only by analyzing such real-life situation we could answer the pertinent questions such as: how fast, at which (equilibrium) extent and into what protein-bound form(s) the AuNRs are converted on the way to the target. It is also quite evident that upon intake the whole proteome, as well as low molecular-mass blood constituents, will compete for binding to the nanorod surface and via encountering an array of binding partners a range of gold biospecies will be generated.

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This work was therefore aimed at developing a practical technique that can distinguish the free and protein-bound AuNRs with high selectivity and sensitivity and shed light – from the kinetic and equilibrium standpoints – on the fate of AuNRs in human serum. The method reported herein exploits an on-line combination of CE – to selectively separate the bio-nano species of interest – with ICP-MS – to specifically detect the separated species. The on-line coupling as the mode of operation provides a closed system with reduced outside exposure of the fragile analytes. Furthermore, from the separation side of this hyphenated technique, mild, biocompatible conditions are ensured which is an asset of CE-ICP-MS in biospeciation analysis [12,13]. A minimal impact of the separation system is crucial for the AuNRs to maintain their stability and to protect their conjugates from breakdown.

2. Experimental

2.1. Chemicals

Sodium borohydride, cetyl trimethylammonium bromide (CTAB), chloroauric acid, silver nitrate, ascorbic acid, alpha methoxy omega mercapto polyethylene glycol (PEG) (molecular weight (MW) 5000), *O*-(3-carboxypropyl)-*O*′′-[2-(3-mercaptopropionylamino)ethyl]-PEG (MW 5000), polysorbate 20, albumin, transferrin (from human serum), holo-transferrin, apo-transferrin, immunoglobulin G, and human serum (from human male AB plasma), as well as all chemicals for preparing the buffer solutions, were purchased from Sigma Aldrich. Thiol-PEG-amine (MW 5000) was obtained from Ibis Technologies.

2.2. Preparation and characterization of functionalized AuNRs

The AuNRs (6×29 nm, an aspect ratio of 4.83) were prepared according to the protocol introduced Nikoobakht and El-Sayed [14], with slight modifications. Functionalization with PEG modified by introducing different groups (H_2N-PEG, HOOC-PEG, CH_3O-PEG) was performed following the procedure by Tatini et al. [15]. Briefly, two times centrifuged and decanted particles were transferred into acetate buffer solution (pH 5) containing CTAB and alpha methoxy omega mercapto PEG, *O*-(3-carboxypropyl)-*O*′′-[2-(3-mercaptopropionylamino)ethyl]-PEG or thiol-PEG-amine and left to develop at 37 °C for 2 h, before centrifugation, decantation and resuspension in 1% (v/v) aqueous polysorbate 20. Finally, the mixture was purified by four cycles of centrifugation and decantation and transferred to sterile phosphate-buffered saline.

The structural and optical properties of AuNRs were characterized by transmission electron microscopy (TEM) and UV–vis-NIR analysis. TEM images were acquired on a TEM microscope (Philips CM12, Amsterdam, The Netherlands) operated at 80 kV. Optical extinction spectra were recorded using a double-beam Cary 5 spectrophotometer (Varian, Palo Alto, CA, USA) in the range 1000–350 nm at room temperature with 1-cm path length PE cuvettes and using Cary WinUV Scan software. UV–vis-NIR spectra were recorded after dilution (1:20) of the functionalized AuNRs in Milli-Q water (see Supplementary data, Fig. S-1). The determination of the gold concentration for functionalized AuNRs was performed in triplicate using a Varian 720-ES ICP-atomic emission spectrometer, as described in detail in Supplementary data. The Au concentrations in stock solutions for AuNR-PEG-NH_2, AuNR-PEG-COOH, AuNR-PEG-OCH_3 were 8.85, 8.72, and 14.39 mM, respectively.

2.3. CE and ICP-MS instrumentation

CE-ICP-MS analyses were performed on a HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) coupled to a 7500a ICP mass spectrometer (Agilent Technologies, Tokyo, Japan) through a microconcentric CEI-100 nebulizer (CETAC, Omaha, USA) and a custom-machined low-dead-volume conical spray chamber. Polyimide-coated fused-silica capillaries (I.D. 75 μm; O.D. 375 μm; total length 70 cm) were purchased from CM Scientific Ltd. (Silsden, UK). Further details concerning system operational parameters and procedures of equipment preparation are described in Supplementary data.

3. Results and discussion

3.1. Optimization of CE conditions

With ICP-MS detection, no molecular information on protein conjugates of AuNRs can be acquired. Therefore, prior to serum analysis, individual conjugates have to be identified in mixtures of AuNRs with each serum protein available separately. A prerequisite to carrying out this workflow is quantitative elution of nanorods from the separation capillary, with reproducible migration times and peak areas. In addition, analyte peaks are required to be as high and as narrow as possible. The latter condition is important to cope with real doses of AuNRs in blood (at the tens mg-per-liter level [4,16,17]) and to maintain high resolution in the event of a number of biotransformation products formed in the blood. With this objective, several experimental parameters and capillary pre- and post-run conditioning protocols were optimized (see Supplementary data for detail). As can be seen from Table 1, at optimized conditions (summarized in the footnote) the method recovery, precision, and sensitivity thresholds are satisfactory. The only problem is posed by AuNRs-PEG-OCH_3 that decomposes in the CE system, clogging the nebulizing capillary and precipitating in the spray chamber.

3.2. Interactions with individual plasma proteins

The conjugates of AuNR-PEG-NH_2 and AuNR-PEG-COOH with the most abundant plasma proteins, albumin, transferrin (as individual apo- and holo-forms and their physiological mixture), and immunoglobulin G, were produced by incubating the reactants at 37 °C in physiological buffer medium (10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl). Although the formation of the protein corona is varied as a function of time, the nature of AuNRs and proteins, and protein-to-AuNR ratio, each conjugate can be well characterized by a specific ^{197}Au signal and precise migration time (Table 2). It was also interesting to differentiate kinetically between each nanorod–protein system. In general time required to attain equilibrium binding is very short ($\geq$5 min). Nonetheless, for amino-functionalized AuNRs the formation percent of albumin conjugate was found to be about 5 times higher in comparison to the carboxy-analogue.

3.3. Speciation in human serum

In the blood, the protein corona is a temporarily dynamic complex and for a given sort of nanomaterial, its formation and composition are governed by protein abundance and binding affinity [18–20]. Typical protein-binding profiles acquired after exposure of AuNRs to human serum for different periods of time, shown in Fig. 1, prove that this is the case, particularly for more reactive amino-derivatives (note that before mixing with serum, a signal corresponding to AuNR-PEG-NH_2 had a migration time about 35 min – data not shown. Just after mixing with serum, binding with transferrin went so fast that even after 5 min of observation the AuNR-PEG-NH_2 signal was not present in the electropherogram). With the data on migration times collected in Table 2, the assignment of the protein conjugates is rather straightforward. Starting