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Double-shell gold nanoparticle-based DNA-carriers with poly-L-lysine binding surface

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ABSTRACT

In view of the prospective applications of polyamine coatings in functional gold nanoparticles for use as carriers in gene delivery systems, in tissue repair and as bactericidal and virucidal non-toxic vehicle, we have investigated the interactions of poly-L-lysine (PLL) with gold nanoparticles (AuNP). Since direct binding of PLL to AuNP is not strong at neutral pH, we have focused on PLL interactions with carboxylated self-assembled monolayers (SAM) on AuNP, such as the citrate-capped AuNP. The double-shell nanoparticles AuNP@Cit/PLL thus produced do not contain any toxic thiols. We have observed strong electrostatic interactions between polycationic chains of PLL and AuNP@Cit in weakly acidic to weakly alkaline solutions (pH 5–9), as evidenced by the bathochromic shift of the local surface plasmon (SP) band and strong increase in resonance elastic light scattering (RELS) intensity. The stoichiometry of interactions evaluated on the basis of RELS data indicates on a hyper-Langmuirian type of interactions with stoichiometric coefficient $n = 1.35$ (PLL : AuNP@Cit). From the RELS titration data, a shift of the deprotonation constant for the bound PLL has been determined ($pK_a = 11.6$ for the bound PLL *vs.* 10.48 for the free PLL). The deprotonation of PLL leads to AuNP aggregate disassembly, evidenced by sharp RELS decline and hypsochromic shift of SP band. We have found that under these conditions, a residual aggregation due to the interparticle interactions between β-sheets of PLL overcoat become predominant. The molecular dynamics simulations indicate that multiple hydrogen bonds can also be formed between the PLL linker and the shell molecules of AuNP@Cit. The double-shell nanoparticles, AuNP@Cit/PLL, have been shown to attract DNA molecules using highly sensitive RELS measurements presenting the proof-of-concept for the suitability of this non-toxic nanostructured material for gene delivery applications. The advantage of the proposed material is no toxicity related to the ligand release in gene delivery processes in contrast to the thiol-functionalized AuNP.

1. Introduction

The explorations of non-toxic coatings play a key role in the development of implants, tissue engineering, and in the preparation of microbicidal surfaces to prevent spreading of infectious diseases [1]. With the emergence of nanoparticle probes and nanocarriers in medical diagnostics and therapy [2], the coating functionalization has opened a new world of applications being now actively endeavored [3–7]. In this, some of the nature-developed materials (e.g. polyamines) can be utilized and perfected as coatings with the full biocompatibility and no side-effects.

The cationic polyelectrolytes participate in several processes in living cells. Perhaps the most pronounced activity is shown by poly-L-lysine (PLL) which interacts with DNA and cell membranes. Polycations, including PLL, exhibit stimulatory activity towards production of immunoglobulin and their presence in higher organisms is necessary. The PLL has also been found to increase the production of interferon-beta without influencing the cell proliferation [8]. Furthermore, the PLL shows a strong anti-proliferative properties [9] on several tumors (including erythroleukemia, L1210 lymphoid leukemia, Lewis lung tumor, P388 tumors, Ehrlich carcinoma, and P38 macrophage derived tumor). The synthetic isomeric αPLL enhances the proliferation of astrocytes [10]. The biological activity of peptide-polylysine dendrimers in the cyclase signaling (AC system) in the myocardium and the brain of rats, as well as in functional activity of biogenic amine-sensitive adenylyl, have been investigated [11]. The PLL derivatives offer a high potential as the polycationic polymer carriers for biologically active substances. Both

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αPLL and εPLL have been advised for use as anti viral agents to protect plants against tobacco mosaic virus infecting eggplant family plants, potato virus, and cucumber mosaic virus [12].

PLL can assume different conformations of its secondary structure dependent on temperature and pH, including random coil, α-helix, and β-sheet [13,14]. At low temperature (4 °C), the β-sheet structures are not present as they require heating to 25 °C to form. In general, at neutral pH, PLL forms an extended random coil due to repulsions between protonated $-NH_3^+$ groups and changes conformation at higher pH (pH > 11) to α-helix and β-sheet [15–17]. On the basis of CD data, Guo et al. [18] have estimated that at pH = 6.5 the content of random coil is 62.9% and the content of β-turn is 37.1%, while at pH = 11, the contents of α-helix is 40.9% and that of β-sheet is 21.0% and only 30.3% of PLL remains in the form of the random coil and 7.8% in the form of β-turn. The transition to β-sheet, either from random coil at neutral pH or from α-helix at higher pH, can be induced by suspending lipid vesicles (dilauroylphosphatidic acid, DLPA) [19].

Direct interaction of lysine residues with Au surface stabilizes the gold nanoparticles (AuNP) in solution [20]. However, it has been shown by Guo et al. [18] that there is apparently no interparticle interaction between PLL-capped gold nanoparticles in the pH range 6.5–10.5 since only a very small shift of SP absorbance band, from 522 to 526 nm is observed. However, at pH 11.5, a clear aggregation of PLL-capped AuNP has been observed concomitant with a shift in the SP band maximum to $\lambda_{max} = 538$ nm. The aggregation has been attributed to the interparticle β-sheet Van der Waals attractive forces.

According to Joshi et al. [21], binding of lysine at neutral pH to AuNP is fairly weak though it increases at pH = 11 concomitant with deprotonation of $-NH_3^+$ groups (above the p*I* of lysine; p*I* = 9.4 [21]). The assembly of 3D nanoparticle networks from lysine-capped $AuNP_{13\ nm}$ induced by condensing agent EDC (ethyl-3-(dimethylaminopropyl) carbodiimide) have been described [22]. The efficient AuNP binding through a dipeptide cross-linker has been achieved. Other groups advocate binding amine groups directly to gold [23–25]. Theoretical studies of polylysine dendrimers have been carried out by Maiti et al. [26], Ouyang et al. [27], and Manisto et al. [28].

The interactions of PLL with a variety of biomolecules have been reported, including such molecules as DNA [29,30], lipids [19], cyclodextrin [31], etc. The immobilization of PLL onto the probe surface for molecular adsorption-based endotoxin-detection system has been reported [32]. PLL has also been considered as a biosorbent for removal of Cr(VI) species from industrial waste water. The hexavalent chromium which causes oxidative damage to DNA [33], interacts strongly with PLL and can be readily removed from the waste waters.

The growing interest in applications of AuNP in nanomedicine and bioassays stems from the simple functionalization and possibility of designing complex nanoarchitectures with precision recognition of DNA strands, antibody–antigen interactions, and other biorecognition systems [34–41]. There are several important prospective applications of PLL-coated nanoparticles. They include preparation of bactericidal and virucidal injections, use as the stem cell carriers and scaffolds in tissue repair, as well as in gene delivery system where DNA fragments can be attached electrostatically to PLL-coated AuNP carriers. The key feature of a PLL coating is its non-toxicity to the eukaryotic cells.

In this work, we have investigated interactions of PLL with citrate-capped gold nanoparticles in view of the much weaker direct interactions of PLL with a gold surface, for possible application of PLL-functionalized AuNP in gene delivery systems and antimicrobial injections. The gene delivery concept has been pioneered by Rotello et al. [2,42,43] using gold nanoparticles capped with amino acid-modified alkane thiols. The AuNP with lysine residue-modified thiols have been shown to interact with DNA fragments and were able to penetrate eukaryotic membranes [2]. Since the DNA unloading effected by glutathione derivatives [43] is more difficult when thiolate AuNP coating is used (as shown for instance for the ligand exchange homocysteine-glutathione [44]), in this work we have utilized AuNPCit/PLL as the carrier instead. We have explored the utility of the resonance elastic light scattering (RELS) spectroscopy to monitor PLL-induced AuNP assembly. The RELS technique is based on the absorption of photons by molecules and nanoparticles in solution followed by an immediate coherent re-emission of light in all directions without any energy loss [45]. We have recently applied RELS to study nanoparticle assembly [36,44–46] and found it to be very sensitive to supramolecular ensemble formation. The high sensitivity of RELS to AuNP assembly is due to the excitation of the surface plasmon (SP) in gold nanoparticles and coupling of local SP oscillations during the close approach of particles in the supramolecular structure formation. In this paper, we describe the interactions of PLL with AuNP and elucidate the mechanism of the PLL-linked citrate-capped AuNP assembly. For the model system, a hyper-Langmuirian electrosorption kinetics has been found. The interactions of PLL-coated AuNP with dsDNA have been tested to demonstrate the activity of PLL amine functionalities in the nanoparticle shell environment.

2. Materials and methods

2.1. Chemicals

All chemicals used for investigations were of analytical grade purity. Poly-L-lysine (PLL) with molecular mass of 150 kDa (0.1% w/v aqueous solution), deoxyribonucleic acid sodium salt from calf thymus (ctDNA), and tetrachloroauric(III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$), 99.9+% metals basis were obtained from Sigma Aldrich Chemical Company (Atlanta, GA, U.S.A.). Sodium citrate, dihydrate ($Na_3Cit \cdot 2H_2O$) was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ, U.S.A.). Sodium borohydride ($NaBH_4$) was obtained from Fisher Scientific Company (Pittsburgh, PA, U.S.A.). Solutions were prepared using Millipore (Billerica, MA, U.S.A.) Milli-Q deionized water (conductivity $\sigma = 55$ nS/cm). They were deoxygenated by bubbling with purified argon.

2.2. Apparatus

The transmission electron microscopy (TEM) images of Au nanoparticles were obtained using a Jeol Model JEM-2010 HR-TEM instrument (100 kV). The elastic light scattering spectra were recorded using LS55 Spectrometer (Perkin Elmer) equipped with 20 kW Xenon light source operating at 8 μs pulsing mode allowing for the use of monochromatic radiation with wavelength from 200 nm to 800 nm with 1 nm resolution and sharp cut-off filters: 290, 350, 390, 430, 515 nm. The dual detector system consisted of a photomultiplier tube (PMT) and an avalanche photodiode. The RELS (Resonance Elastic Light Scattering) spectra were obtained at 90° angle from the excitation light beam at 516 or 665 nm. The coherent elastic Rayleigh scattering from $AuNP_{5\ nm}$ nanoparticles in solution with Gaussian peak shape centered at $\lambda_{em} = \lambda_{ex} = 516$ or 665 nm results from the absorption of photons followed by secondary emission without any energy loss. The narrow linewidth of $\Delta\lambda = 15$ nm confirms that the effects due to radiation broadening, density fluctuation, fluorescence, and inelastic Raman scattering are negligible. The UV-Vis spectra were recorded using Ocean Optics R4000 Precision Spectrometer in the range from 340 nm to 900 nm. For nanogravimetric measurements, the Electrochemical Quartz Crystal Nanobalance, Model EQCN-700 from Elchema (Potsdam, NY, U.S.A.) with a Data Logger and Control System, Model DAQ-716v, operating under Voltscan 5.0 data acquisition and processing software was employed.

2.3. Procedures

The Au nanoparticles were synthesized according to the published procedure [47]. Briefly, to obtain 5 nm AuNP, a solution of $HAuCl_4$ (10 mM, 2.56 mL) was mixed with a trisodium citrate solution (10 mM, 9.6 mL), ratio 1: 3.75, and poured to distilled water (88 mL). The obtained solution was vigorously stirred and fresh cold $NaBH_4$ solution (5 mM, 8.9 mL) was added dropwise. The solution slowly turned light grey and then ruby red. Stirring was maintained for 30 min. The obtained citrate-capped core-shell Au nanoparticles (AuNP) were stored at 4 °C. Their size was first estimated from UV-Vis surface plasmon absorption band shift and determined more precisely by HR-TEM imaging to be 5.0 ± 0.9 nm ($n = 85$). No larger particle population was present. The concentrations of AuNP's are given in moles of particles per 1 L of solution (usually, in the nM range). The size and distribution of AuNP were also tested using plasmonic absorbance spectra in UV-Vis. For instance,