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Self-assembly of silver nanoparticles and bacteriophage

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ABSTRACT

Biohybrid nanostructured materials, composed of both inorganic nanoparticles and biomolecules, offer prospects for many new applications in extremely diverse fields such as chemistry, physics, engineering, medicine and nanobiotechnology. In the recent years, Phage display technique has been extensively used to generate phage clones displaying surface peptides with functionality towards organic materials. Screening and selection of phage displayed material binding peptides has attracted great interest because of their use for development of hybrid materials with multiple functionalities. Here, we present a self-assembly approach for the construction of hybrid nanostructured networks consisting of M13 P9b phage clone, specific for *Pseudomonas aeruginosa*, selected by Phage display technology, directly assembled with silver nanoparticles (AgNPs), previously prepared by pulsed laser ablation. These networks are characterized by UV–vis optical spectroscopy, scanning/transmission electron microscopies and Raman spectroscopy. We investigated the influence of different ions and medium pH on self-assembly by evaluating different phage suspension buffers. The assembly of these networks is controlled by electrostatic interactions between the phage pVIII major capsid proteins and the AgNPs. The formation of the AgNPs-phage networks was obtained only in two types of tested buffers at a pH value near the isoelectric point of each pVIII proteins displayed on the surface of the clone. This systematic study allowed to optimize the synthesis procedure to assembly AgNPs and bacteriophage. Such networks find application in the biomedical field of advanced biosensing and targeted gene and drug delivery.

1. Introduction

The controlled assembly of bio-hybrid nanostructured materials is an emerging research area due to their potential applications in bioengineering, biosensing and biomedical research. Among the wide variety of biological scaffolds, filamentous bacteriophages have recently attracted much attention for the development of accurately positioned nano-biotemplates, since the phage particle can be modified to form hetero-complexes with organic or inorganic nanomaterials. Ultimately, filamentous bacteriophage M13 represent attractive alternatives to antibodies or synthetic peptides, for developing new nanobiohybrid materials [1–4], due to their robustness, resistance to heat and to many organic solvent (such as 50% methanol [5] and 30% DMSO [6]) acid and alkali as well as a low-cost production [7,8]. Phage display is a high-throughput biotechnique that allows the presentation of exogenous peptides on the surface of one filamentous phage. This technology involves the introduction of exogenous peptide sequences into a location in the genome of the phage capsid proteins such as pVIII and pIII [9]. Thus, the main advantage of phage display is the enormous diversity of variant peptides that can be represented. Random phage libraries, in fact, include billion phage clones expressing on their surface more than 10^{12}–10^{14} different peptides [10]. The library is used to select specific phage clones that interact with particular targets, generating molecular probes with high affinity and selectivity [11–14].

More recently, novel strategies were developed to functionalize gold and silver nanoparticles with different Raman reporter molecules for targeting specific ligands such as peptides, proteins, antibodies, Deoxyribonucleic acid (DNA) and antibody fragments [15–19]. Nevertheless, some drawback still remain such as the availability to identify selectively and with high reproducibility probes that can act like SERS nanotags for the recognition of target cells. Taking into account the above described properties, phage-metallic nanoparticles networks are considered appropriate systems to integrate the unique signal-reporting properties of the metallic nanoparticles while preserving the biological properties of phages [20]. The surface of each filamentous bacteriophage *M*13 virus consists of about 2700 copies of a major coat protein which package a single-stranded circular viral DNA into a rod with a total length of 880 nm and a diameter of 6.6 nm [21]. This major coat protein is a charged α-helix consisting of 50 residues, and constitutes the bulk of the

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total charge on the virus [22]. Approximately six of the 50 residues (and the amino terminus) are solution accessible and contribute to the surface charge on *M*13. By changing the pH, change the protonation states of the amino acids on the virus major coat protein, thereby modifying the surface charge density [21].

Clearly the coat protein has the ability to adopt its conformation, which allows the protein to exist in distinctly different environments, such as the phage filament, the I form phage, the S-form phage, and the membrane-bound form [23]. This is possible because of the amphipathic nature of the coat protein so that it can have both hydrophobic and hydrophilic interactions with its environment. This property gives the protein a large conformational space that allows very flexible protein aggregational schemes.

In this work, the influence of different phage suspension buffers (i.e. the influence of different ions and medium pH) on the self-assembly of AgNPs and bacteriophage are studied in order to find the appropriate conditions to favor the formation of the AgNPs-phage complex. The silver nanoparticles were prepared using the pulsed laser ablation technique in a confining liquid. This is a chemically simple and clean synthesis method to obtain, in a one step top-down procedure, size controlled AgNPs dispersed in water. The intrinsic ability to produce stable species without the a priori need for any aggressive chemicals, like reducing or capping agents, makes laser ablation in liquids particularly attractive as an biocompatible technique, allowing to obtain AgNPs useful for biomedical applications, as that reported in this paper. On the overall, the results obtained show that an appropriate control of the electrostatic interactions between the phage pVIII major capsid proteins and the Ag nanoparticles determines the AgNPs-phage complex formation.

2. Materials and methods

2.1. Ag nanoparticles preparation

Colloidal solutions of Ag nanoparticles were prepared by pulsed laser ablation of a high purity (99.9%) silver target immersed in distilled water, using the second harmonic (532 nm) of a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (model New Wave Mod. Tempest 300) operating at 10 Hz repetition rate with a pulse width of 5 ns [24,25]. The target was irradiated at the laser fluence of 1 J/cm^2 and for an ablation time of 20 min.

2.2. Phage clone

P9b phage clone display the foreign peptide QRKLAAKLT [13], that represents a specific and selective probe for *Pseudomonas aeruginosa*, was derived from a M13 pVIII-9aa phage peptide library (kind gift of Prof. F. Felici) through previously described affinity-selection procedures [13]. A total estimated isoelectric point value (p*I*) of 6.3. of P9b phage clone was calculated by using "compute MW/pI," present on the proteomics server of the Swiss Institute of Bioinformatics Expert Protein Analysis System (ExPASy). The phage- displayed peptide P9b was chosen as a prototype for optimize the synthesis procedure to assembly AgNPs and bacteriophage.

2.3. Phage suspension buffers

In order to evaluate the influence of phage suspension buffer on the phage assembly with silver nanoparticles, different pH and ion-type buffers were used:

Phosphate Buffer (*PB**) *0.2 M pH 5.86.* Potassium phosphate monobasic anhydrous (22.4 g/l, Lickson) and sodium phosphate dibasic heptahydrate (3.49 g/l, Sigma-Aldrich) were mixed and solubilized in ultrapure water. The final pH was 5.86.

Phosphate Buffer (*PB**) *0.2 M pH 7.23.* Potassium phosphate monobasic anhydrous (9.36 g/l, Lickson) and sodium phosphate dibasic heptahydrate (32.73 g/l, Sigma-Aldrich) were mixed and solubilized in ultrapure water. The final pH was 7.23.

Phosphate Buffered Saline (*PBS*) *0.01 M pH 7.18.* Potassium phosphate monobasic (0.2 g/l, Lickson), sodium phosphate dibasic (1.15 g/l, Sigma-Aldrich), sodium chloride (8 g/L, Applichem) and potassium chloride (0,2 g/L, AnalytiCals Carlo Erba) were mixed and solubilized in ultrapure water. The final pH was 7.18.

Tris-buffered saline (*TBS*) *pH 5.18 and pH 7.02.* Tris hydrochloride (7.88 g/l, Euroclone) and sodium chloride 140 mM (8.77 g/L, Applichem) were mixed and solubilized in ultrapure water. The pH was adjusted with hydrogen chloride 5 N in order to give the final pH values of 5.18 and 7.02.

2.4. Phage-AgNPs networks preparation

The phage-AgNPs networks were prepared according to the procedure described by Lentini et al. [14]. Silver nanoparticles were incubated with the phage clone resuspended in different buffers (title of $5 \cdot 10^{11}$ pfu/ml) in a 4:1 ratio at 30 °C in orbital shaking at 320 rpm (KS130 Basic IKA) over night. In order to separate the AgNPs-phage network from the unbounded phage and free silver, networks were purified by centrifugation at 20,800 ×g for 30 min and resuspended in 5 ml of their respective buffers. The complexes were stored at 4 °C until utilization.

2.5. Samples characterization

The UV–vis absorption response of the Ag nanostructures was investigated, in the colloidal phase immediately after the ablation process, by means of a Perkin-Elmer Lambda 750 UV–vis spectrometer in the 190–1100 nm range. Further, the Ag sample morphology was investigated by means of Transmission Electron Microscopy (TEM) measurements. The TEM images were taken on appropriately dried solutions by a JEOL JEM-2010 microscope, operating at an acceleration voltage of 200KV and equipped with a Gatan 794 Multi-Scan CCD camera. A fraction of the AgNPs-phage colloidal complex was deposited on carbon substrates to carry out Scanning Electron Microscopy (SEM) characterization. SEM images were taken by a scanning electron microscope (Merlin; model ZEISS-Gemini 2) operating at an accelerating voltage of 5 kV. Micro-Raman spectroscopy measurements were carried out by means of an Horiba XploRa spectrometer equipped with an Olympus BX40 microscope, a Peltier cooled charge coupled device (CCD) sensor

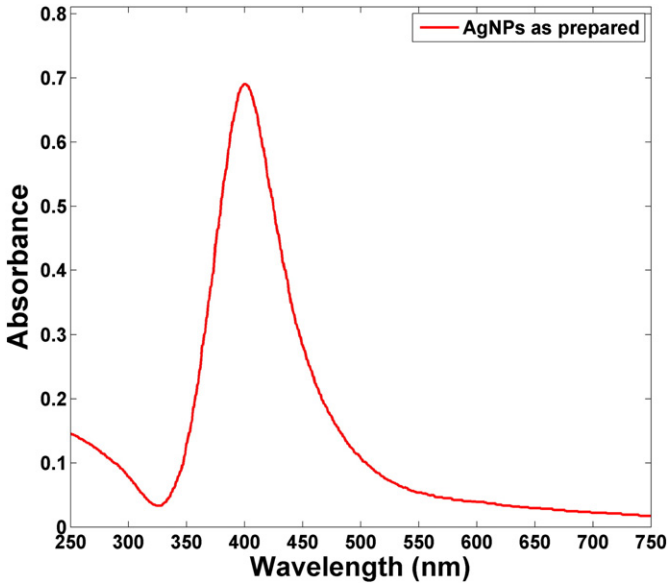

Fig. 1. Optical absorption response of Ag nanoparticles prepared in water.