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Deletion of the Hoc and Soc capsid proteins affects the surface and cellular uptake properties of bacteriophage T4 derived nanoparticles

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ABSTRACT

Recently the use of engineered viral scaffolds in biotechnology and medical applications has been increasing dramatically. T4 phage capsid derived nanoparticles (NPs) have potential advantages as sensors and in biotechnology. These applications require that the physical properties and cellular uptake of these NPs be understood. In this study we used a T4 deletion mutant to investigate the effects of removing both the Hoc and Soc proteins from the capsid surface on T4 tailless NPs. The surface charge, zeta potential, size, and cellular uptake efficiencies for both the T4 NP and T4ΔHocΔSoc NP mutant were measured and compared using dynamic light scattering and flow cytometry and significant differences were detected.

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1. Introduction

Viruses are widely used scaffolds for biotechnology applications due to their diverse sizes, shapes, and versatile surface functional groups allowing chemical modification. Such chemically modified viral based nanoparticles (VNPs) are biocompatible, have low cytotoxicity, and are suitable for various biotechnology and sensor applications [1–3]. However, variability among VNPs resulting from their intrinsic chemical and physical properties define the limitations of their bio-applications, and therefore it is necessary to understand the role of these variables on the cellular uptake effects in order to fine-tune the function of VNPs for various bio-applications.

Among VNPs, phages are preferred due to their robustness and easy accessibility as materials. Based on their shapes, phages can be divided into icosahedral and filamentous types. Filamentous phages, such as M13 and fd, which can be micrometers in length, and icosahedral phage heads often less than 100 nm diameter, are extensively used as scaffolds for chemical modifications for cellular imaging [3,4] and drug delivery [5,6]. In particular, the icosahedral phage based NPs are very similar in many respects to other icosahedral viruses, such as Cowpea Mosaic virus (CPMV) and Cowpea chlorotic mottle virus (CCMV), that are used more often in many biotechnology applications. However by taking advantage of the power of phage genetics and the ability to obtain large quantities of phages from bacterial hosts, icosahedral phages are now being utilized more frequently for imaging and bio-medical applications.

The *Myoviridae* bacteriophage T4 particle consists of an icosahedral capsid containing a 170 kb dsDNA genome and a ~100 nm long contractile tail. T4 has one of the largest head surface areas among the bacteriophage capsids(~75 nm in width and ~100 nm in length), which accommodates more than 1×10^5 functional groups suitable for chemical modification [3]. Moreover, it has a flexible genetic display system allowing peptide display [7,8]. More remarkably, the head can also package more than 100 copies of foreign protein inside the capsid with the DNA [9]. Therefore T4 is an ideal scaffold with broad bio-applications. While the T4 phage head has no bacterial infectivity, the capsid may determine its biological properties related to binding to mammalian cells. Thus mutation of the Lambda (λ) phage capsid protein resulted in escape from entrapment by the mammalian reticuloendothelium [10], suggesting the head protein controls the interaction with the eukaryotic cells by an unknown mechanism. In addition, one T4 head surface protein is known to be highly immunogenic [11]. T4 capsids consist of two major essential capsid proteins, gp23 at the hexamer and gp24 at the pentamer positions, and two non-essential capsid surface proteins that bind to the mature capsid lattice, Hoc (highly immunogenic outer capsid protein) and Soc (small outer capsid protein) [12,13]. It is known that the deletion of Hoc and Soc does not affect the overall structure and assembly of the T4 capsid [14], but the alteration of the physical properties and the effect on cell interaction is unknown. To investigate the effects of the protein content on the physical properties and interaction

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with mammalian cells, a Hoc-Soc mutant (T4ΔHocΔSoc) NP was compared to T4 NP. Dynamic light scattering was used to investigate the size and zeta-potential of the T4 derived NPs and flow cytometry was used to measure their uptake efficiency into tumor cells.

2. Materials and methods

2.1. Preparation of T4 derived NPs

T4 mutantsK10 (38$^-$ 51$^-$*denA*$^-$ *den*B$^-$) and K10ΔHocΔSoc (38$^-$ 51$^-$*denA*$^-$ *den*B$^-$*Hoc*$^-$*Soc*$^-$), were grown in the non-suppressor *Escherichia coli Rosetta,* to make tailless T4 NPs according to our previously established procedure [2,15]. In brief, Rosetta were grown in M9S supplemented with 1/3 volume of Luria Broth, then the cells were infected with phage, followed by centrifugation and cell lysis. Potassium phosphate buffer (50 KP/10 $MgCl_2$) containing 50 mM potassium phosphate (pH 7.5) supplemented with 10 mM $MgCl_2$ and 2 mM $CaCl_2$, $CHCl_3$ (1/20 of total volume), DNase I (40 μg/mL), RNase I (50 μg/mL), and 0.4 μg/mL of PMSF (phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride) was used for resuspending the resulting T4 NPs. The cell debris was removed by spinning and the cell lysate containing the head scaffolds was concentrated through a Microcon YM-100 membrane according to the manufacturer's procedure (Millipore Corp., MA). The T4 NPs were then purified by gel filtration with Superose 6 (GE Healthcare Biosciences, NJ). The flow-through and eluted fractions were then assessed using a 1.2% Tris–acetate agarose gel stained with either ethidium bromide or Coomassie blue.

2.2. Dye conjugation

Purified tailless T4 NP solutions in 50 KP/10 $MgCl_2$were reacted with Alexa 488 or Alexa 546 (Invitrogen, Carlsbad, CA) in the same buffer with the addition of 10% DMSO (Sigma–Aldrich, St. Louis, MO) with the ratio of 5 dyes to each amine group and incubated at 22 °C overnight [3]. The dye-labeled T4 NPs were then loaded separately into a Superose™ 6 prep grade (GE Healthcare Biosciences, Pittsburgh, PA) column and the first 1.5 mL fractions were collected and analyzed by gel electrophoresis, and UV–Vis spectroscopy.

2.3. Flow cytometry

A549 cells were treated with dye-conjugated T4 based NPs at 50000 T4/cell according to our previously published protocol [3]. The stained cells were then run on an Accuri C6 flow cytometer using standard lasers and filters for PE (FL-2) and APC (FL-4). For each sample 2×10^4 events were collected in a gate corresponding to the live cell population based on forward vs. side scatter. The percentage of cells positive for the dye-T4 NPs was calculated using the histogram subtraction tool on FSC Express V3.

2.4. Size analysis of suspended particles and aggregates

The size of suspended T4 based NPs was investigated by dynamic light scattering spectroscopy (DLS) using a Malvern Zetasizernano-ZS (Westborough, MA) equipped with MPT-2 titrator at 25 °C. The details of DLS techniques can be found elsewhere [16]. The effect of pH on the sizes of suspended T4 based NPs was determined by the stepwise-addition of 0.1 N NaOH or HCl with the help of MPT-2 titrator and subsequent DLS analysis. After each NaOH or HCl addition, the mixed aqueous solution was stirred for 8 min. After mixing the solution, the pH was determined and the mixed aqueous solution was introduced to the Zetasizer sample cell with the circulation system integrated into the MPT-2 titrator for the DLS size analysis.

2.5. Zeta potential analysis of suspended particles and colloids

The analysis of zeta (z)-potential as a function of pH was conducted by laser Doppler velocimetry (LDV) using a Malvern Zetasizernano-ZS equipped with a MPT-2 titrator at 25 °C according to Furukawa et al. [17]. Each suspension sample, with an appropriate adjustment to pH with either 0.1 N NaOH or HCl, was loaded into a capillary cell with embedded electrodes at either of the two ends using the MPT-2 titrator. Suspended particles move towards the electrode of the opposite charge when the potential is applied, and their velocity is measured and expressed in unit field strength as a function of their mobility. By knowing the physical properties of the suspension medium, the velocity can be converted to the z-potential using the Smolchowski equation [16].

3. Results

Tailless T4 mutants, K10 (T4 NP), and K10 lacking Hoc and Soc capsid proteins (T4ΔHocΔSoc NP) were used to make tailless T4 based NPs for our experiments. There is no Hoc or Soc gene expression in the T4ΔHocΔSoc mutant examined by PCR and Western blotting (data not shown). The T4ΔHocΔSoc NPs were able to withstand the purification procedures and chemical reaction with Alexa 546 (A546) as shown in Fig. 1 without producing lower molecular weight bands. The deletion of Hoc and Soc does not affect the head assembly pathway and does not change the interior head dimension, therefore this mutant will package the same amount of DNA as wild type (wt) T4 head using head-full packaging mechanism [8]. Our results indicated that T4ΔHocΔSoc NPs appeared to be more negative relative to T4 wt NPs and migrated faster on the agarose gel due to the alteration of surface protein content (Lane 2 in Fig. 1). For unknown reasons, we also observed that each T4 based NP ran as doublet bands, although they appeared to be homogenous in size in atomic force microscopy images [2]. Using a dynamic light scattering instrument, the average size for tailless T4 NPs and T4ΔHocΔSoc NPs between pH 5–9 were found to be 125 nm and 116 nm respectively (Fig. 2). Large aggregates appear to form around the capsid isoelectric point at pH 4 (Fig. 2A and B) and could be reversibly dispersed into separated NPs at pH 5 or greater. The average zeta potential for T4 NPs is ~−27 mV and T4ΔHocΔSoc NPs is ~−33 mV at between pH 7–8. This is consistent with the result from electrophoretic mobility measurement in agarose (Figs. 1 and 2).

The Alexa 546 conjugated T4 NPs appeared to be more negative, due to the contribution of one negative charge from Alexa 546 (Fig. 1C). Although there is no measurement of the zeta potential and size for dye-T4 NPs, we can estimate that the zeta potential

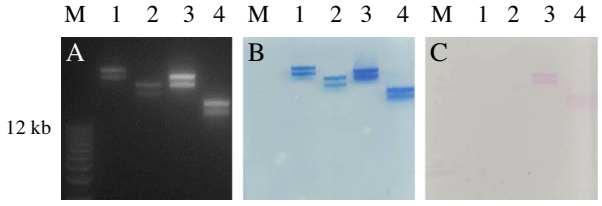

Fig. 1. Electrophoretic mobility on agarose gel for phage T4 derived NPs. Panel A, B and C were derived from the same gel. A is the image of the gel subjected to ethidium bromide staining to visualize DNA bands. B is the image of the gel stained with coomassie blue to visualize the protein bands. C is the image of the gel without any staining. The pink bands represent the T4 NPs cross-linked with Alexa 546. Lanes M, 1, 2, 3 and 4 represent DNA size marker, T4 NPs, T4ΔHocΔSoc NPs, Alexa 546-T4 NPs, and Alexa 546-T4ΔHocΔSoc NPs.