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Coiled-coil forming peptides for the induction of silver nanoparticles

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ABSTRACT

Biopolymers with defined sequence patterns offer an attractive alternative for the formation of silver nanoparticle (AgNP). A set of coiled-coil dimer forming peptides was tested for their AgNP formation ability. Seventeen of those peptides mediated the formation of AgNPs in aqueous solution at neutral pH, while the formation of a coiled-coil dimer inhibited the nanoparticle generation. A QSAR regression model on the relationship between sequence and function suggests that in this peptide type the patterns KXQQ and KXEE are favorable, whereas Ala residues appear to have an inhibitory effect. UV–VIS spectra of the obtained nanoparticles gave a peak at around 420 nm, typical for AgNPs in the size range around 40 nm, which was confirmed by dynamic light scattering and transmission electron microscopy. Peptide-induced AgNPs exhibited good antibacterial activity, even after a 15 min contact time, while they had low toxicity to human cells at the same concentrations. These results show that our designed peptides generate AgNPs with antibacterial activity at mild conditions and might be used for antibacterial coatings.

1. Introduction

Silver nanoparticles (AgNP) have already achieved a widespread use, primarily due to their antimicrobial activity. Besides chemical formation an alternative method for their production is the use of molecules that induce generation of homogeneous size nanoparticles, such as peptides with a specified sequence [1–4]. The advantage of peptides as AgNP inducing molecules is that peptides may be attached to the selected surfaces or fused to other functional proteins, such as antibodies, enabling production of smart materials.

In the recent years coiled-coils proved to be one of the most promising designed protein nanostructure building modules [5–7]. This protein structural motif is characterized by a specific seven amino acid repeat denoted *abcdefg* (heptad). The rules by which structure, oligomerization and partner specificity occur are very well understood and relatively straightforward; specific di(oligo) merization is guided by hydrophobic interactions between amino acid residues at positions *a* and *d* and electrostatic interactions between positions *e* and *g* of the heptad repeat. This knowledge has been exploited in the past for *de novo* design of coiled-coil peptides to generate sets of interacting peptides [8–10]. Much effort has been put into the design of a molecular toolbox for protein nanostructure design [11]. One of the advantages of a protein based platform, compared to the DNA nanostructures, is the chemical diversity of amino acid functional groups. Several interesting functionalities have been explored in the context of coiled-coils, such as triggered oligomerization, conformational change [12] or quantum dot binding [13,14].

Recently a great deal of effort has been put into investigating peptides for their silver nanoparticle formation abilities. This is mainly due to the fact that formation of metal nanoparticles via peptides occurs in an aqueous environment, at room temperature without the need for toxic chemicals in contrast to the conventional AgNP production methods. One of the great potentials of silver nanoparticles is their broad antimicrobial activity [15–17]. Despite importance of silver nanoparticle formation, to our knowledge, coiled-coil forming peptides have not been tested for the growth of

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metal nanoparticles. This comes somewhat as a surprise given the vast number of such peptides available. Silver coordinating residues, tyrosine and tryptophan, which have been shown to reduce silver ions [18,19], could be introduced into coiled-coil forming peptides at positions *b*, *c* and *f* without interfering with oligomerization, specificity or stability. Previous silver binding and AgNP forming peptides were found via the phage display method in which a large number of randomly generated peptides were expressed at the surface of bacteriophages and selected for their silver affinity [20]. Here we demonstrate results of the analysis of the set of peptides designed for their coiled-coil dimer forming propensities which were tested for silver nanoparticle formation ability and antimicrobial activity of the resulting nanoparticles. This study provides clues to the residues and sequence motifs important for AgNP formation.

2. Materials and methods

2.1. Nanoparticle formation

For the preparation of nanoparticles, synthetic peptides in aqueous solution were used (purchased from China peptides and Genscript) at a concentration of 0.2 mg/ml. $AgNO_3$ was added to peptides to a final concentration of 100 mM. Solutions were held overnight at room temperature and ambient fluorescent light. The solutions were then dialyzed against 5 mM sodium citrate for stabilization of nanoparticles and remaining $AgNO_3$ removal.

2.2. Nanoparticle characterization

UV–VIS spectrometry was performed on Agilent Carry 8454 UV–VIS spectrophotometer. Absorbance was measured in the range from 200 nm to 800 nm. Molar concentrations of silver nanoparticles were calculated using extinction coefficients from the literature ($\varepsilon = 537 \times 10^8\ M^{-1}\ cm^{-1}$ [21]) and are shown in Table 1. Mass concentrations of peptide generated AgNP were estimated from UV–VIS spectra in comparison with commercially available 40 nm silver nanoparticles (purchased from SIGMA ALDRICH cat. no. 730807-25 ML). Particle size was determined by dynamic light scattering (DLS) obtained with Malvern Zetasizer DLS (Malvern, UK) at 20 °C using an angle of 173° and 633 nm laser. For transmission electron microscopy (TEM) 5 μl of silver nanoparticle suspension was put on a copper carbon coated Holey grid and left to dry. Visualization and elemental analysis was performed on Jeol ARM 200 CF probe Cs corrected TEM/STEM, equipped with Centurio 100 mm^2 energy-dispersive X-ray spectrometer (EDXS), at 120 kV accelerating voltage and 50.000x magnification.

Table 1
Absorbance at 420 nm of peptide induced nanoparticles with respective AgNP molar concentrations.

Peptides	Sequence	$A_{420\ nm}$	c (pmol/L)
P4C2	KIEELKQKIEQLKQENQQLEEENSQLEYGC	0.2478	4.615
P5SC23	ENQSLESKISQLKRKNEELKQEISQLEYGC	0.2201	4.099
P4C1	KISQLKQKIQQLKQENQRLEEENRRLEYGC	0.211	3.93
P3sneg8B	DEIEKLEREIEKLEEKNEELKEKNEELKYG	0.1877	3.496
P6SC2R	KNERLKEEIQRLEQENQQLEEKIQRLKYGC	0.1844	3.434
P3SM	EIQQLEEEISQLEQKNSQLKEKNQQLKYG	0.1831	3.41
P4SNEG8	DKIEELKQKIEELKQENEELEEENEELEYG	0.1408	2.621
P3C1	EIQQLEEEISQLEYKNYALKAKNEELKRGC	0.1226	2.283
P5SN	ENSQLEEKISQLKQKNSELKEEIQQLEYG	0.1205	2.245
P6SCW1	KNSQLKEEIQQLEEENQSLEQKIQQLKYGC	0.1027	1.912
P3C2	EIQQLEEEIRQLEQKNSQLKEKNQQLKYGC	0.08	1.49
P6SC23	KNSQLKQEIQSLEEENRRLESKISQLKYGC	0.0783	1.457
P8SM	KISQLKEENQQLEQKIQQLKEENSQLEYG	0.0724	1.349
P5SCW1	ENSQLEEKISQLKQKNSQLKQEIRRLEYGC	0.0723	1.347
P4SM	KISQLKQKIQQLKQENQQLEEENSQLEYG	0.0586	1.091
P3CP	EIQRLEQEIQRLEQKNRRLKQKNRRLKYG	0.0554	1.031
P7SM	EIQSLEEKNSQLKQEISQLEEKNQQLKYG	0.0526	0.98
P3SNEG8	DEIEELEEEIEELEQKNEELKEKNEELKYG	0.0479	0.891
P6SN	KNSELKEEIQQLEEENQQLEEKISELKYG	0.0433	0.806
P5SC2	ENARLEEKIRQLKQKNSQLKEEIQQLEYGC	0.0323	0.601
P5SM	ENAALEQKIAQLKQKNAALKQEIQALEYG	0.0316	0.588
P4CP	KIRRLKEKIRRLKQENRRLEQENRRLEYG	0.0308	0.573
P5SC1	ENSQLEEKISQLKQKNSRLKEEIARLEYGC	0.0291	0.542
P6SC2	KNERLKEEIQRLEQENQQLEEKISQLKYGC	0.0255	0.475
P6SC1	KNSQLKEEIQQLEEENQQLEEKIRQLKYGC	0.0243	0.452
P3	SPEDEIQQLEEEIAQLEQKNAALKEKNQALKYG	0.0148	0.275
P4	SPEDKIAQLKQKIQALKQENQQLEEENAALEYG	0.0084	0.156
P7	SPEDEIQALEEKNAQLKQEIAALEEKNQALKYG	0.0031	0.057
P2	SPEDKIAQLKEKNAALKEKNQQLKEKIQALKYG	0.0029	0.053
P8	SPEDKIAQLKEENQQLEQKIQALKEENAALEYG	0.0008	0.014

2.3. Antibacterial activity

Antimicrobial activity was evaluated against *Escherichia coli* ATCC 11775. The cells were grown overnight at 37 °C and 160 rpm in LB (Luria–Bertani medium). Afterwards they were inoculated at $OD_{600} = 0.1$ and grown to reach the exponential growth phase at approximately $OD_{600} = 0.8$. Bacteria were subsequently diluted to $OD_{600} = 0.01$ and incubated with 2 μg/ml peptide-generated nanoparticles for 1 h at room temperature with shaking at 700 rpm. Concentration dependence of antimicrobial activity was evaluated by varying the concentrations of peptide generated silver nanoparticles. 2 μg/ml, 1 μg/ml, 0.5 μg/ml, 0.25 μg/ml or 0.125 μg/ml of AgNP solutions were added to the same amount of cells and incubated with shaking for 1 h. The effect of the contact duration was tested by plating cells after 15 min, 30 min, 1 h or 2 h incubation with peptide induced AgNPs. In all experiments aqueous solution of 5 mM sodium citrate was used as a control.

2.4. Mammalian cell viability test

Silver nanoparticles were tested for their cytotoxicity against mammalian cells. HEK293T cells were seeded in 96-well plates and exposed to different concentrations of peptide-induced AgNP overnight in triplicates. Cell proliferation was determined by XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate)) assay as described in literature [22]. Cells without nanoparticles were used as negative control and cells that were exposed overnight to 0.001% Triton-X 100 as positive control. Cells were cultured in DMEM (Dulbecco/Vogt modified Eagle's minimal essential medium) medium with 10% FBS (Gibco, Invivogen) at 5% CO_2 and 37 °C.

2.5. QSAR model

In order to identify the underlying sequence motifs of inspected peptides facilitating production of silver nanoparticles a QSAR analysis was performed using experimentally determined absorbance values at 420 nm and a set of descriptors based solely on the peptide sequence. For the calculation of physicochemical descriptors in-house PEDES (peptide descriptors from sequence) computer software was used [23]. Reduction capabilities of peptides were modeled as a linear combination of selected descriptors:

$$log(A_{420nm}(j)) = \sum_{i=1}^{N} a_i P_i(j) + C,$$

where N is the number of descriptors, $P_i(j)$ is the value of the *i*-th descriptor for peptide *j* and a_i are model coefficients.