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Membrane interactions of mesoporous silica nanoparticles as carriers of antimicrobial peptides

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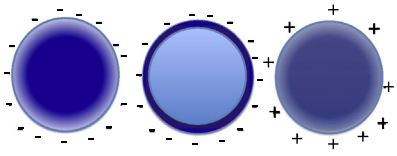
GRAPHICAL ABSTRACT



	MSNc (-)	NSN (-)	MSNa (+)
Peptide load	+++++	+	+
Location	Pore	Surface	Pore
Antimicrobial entity	Peptide	Particle	Particle
Proteolytic stability	+	-	-
Toxicity	-	-	+

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ABSTRACT

Membrane interactions are critical for the successful use of mesoporous silica nanoparticles as delivery systems for antimicrobial peptides (AMPs). In order to elucidate these, we here investigate effects of nanoparticle charge and porosity on AMP loading and release, as well as consequences of this for membrane interactions and antimicrobial effects. Anionic mesoporous silica particles were found to incorporate considerable amounts of the cationic AMP LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES (LL-37), whereas loading is much lower for non-porous or positively charged silica nanoparticles. Due to preferential pore localization, anionic mesoporous particles, but not the other particles, protect LL-37 from degradation by infection-related proteases. For anionic mesoporous nanoparticles, membrane disruption is mediated almost exclusively by peptide release. In contrast, non-porous silica particles build up a resilient LL-37 surface coating due to their higher negative surface charge, and display largely particle-mediated membrane interactions and antimicrobial effects. For positively charged mesoporous silica nanoparticles, LL-37 incorporation promotes the membrane binding and disruption displayed by the particles in the absence of peptide, but also causes toxicity against human erythrocytes. Thus, the use of mesoporous silica nanoparticles as AMP delivery systems requires consideration of membrane interactions and selectivity of both free peptide and the peptide-loaded nanoparticles, the latter critically dependent on nanoparticle properties.

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1. Introduction

Due to increasing problems with resistance development against conventional antibiotics [1], antimicrobial peptides (AMPs) are currently receiving considerable attention as potential therapeutics [2,3]. Through lysis of bacterial membranes, AMPs provide fast and broad-spectrum antimicrobial effects. Key for the successful use of AMPs as therapeutics is their selective lysis of bacterial membranes, without simultaneous rupture of mammalian cells and resulting toxicity. In order to identify selective AMPs, several approaches have been used, including quantitative structure-activity relationship studies, identification of endogenous peptides derived from infection-related proteolysis, and end-tagging with short tryptophan or phenylalanine stretches [4]. In addition to their antimicrobial effects, some AMPs also display other host defense properties, including anti-inflammatory and anticancer effects [5,6], both of which depend on membrane interactions of AMPs.

While there have been considerable efforts to identify potent and selective AMPs, drug delivery aspects of such compounds have been rarely investigated in literature. This is somewhat surprising, considering numerous potential hurdles related to efficient and safe delivery of AMPs. For example, infected tissue is often characterized by high proteolytic activity, mediated both by bacterial proteases and proteases of human defense cells. Thus, unless the peptide has been designed to be proteolytically stable [7,8], administration of AMPs to chronic wounds, infected eyes, or cystic fibrosis lungs is likely to result in rapid degradation of the peptide and in corresponding activity loss. Secondly, since AMPs are amphiphilic and positively charged, they bind to serum proteins, and are rapidly cleared from bloodstream circulation [9,10], which risks translating into reduced efficacy and toxicity effects related to accumulation in the reticuloendothelial system. In addition, some infections, such as tuberculosis, are characterized by intracellular bacteria localization, which poses a challenge in how to reach the intracellular bacteria without lysing and killing the host macrophages [11]. Yet other challenges relate to need for sustained or triggered AMP release, e.g., for implants and recurring infection. In these and other contexts, it would therefore be advantageous to combine AMPs with delivery systems designed for the application at hand.

Apart from more traditional drug delivery systems, such as polymer, lipid, and surfactant systems [12], inorganic nanomaterials (e.g., gold and iron oxide nanoparticles, mesoporous silica, layered double hydroxides, hydroxyapatite, graphene, carbon nanodots and nanotubes, quantum dots, and up-conversion particles) have attracted considerable interest during the last few years as delivery systems for biomacromolecular drugs, such as peptides, proteins, siRNA, and DNA [13]. Such nanomaterials may provide key functional advantages, e.g., protection from chemical and enzymatic degradation, conformational stabilization, control of drug release rate, reduction of toxicity, and increased bioavailability. In addition, they offer opportunities related to their interaction with external fields (light, NIR, magnetic fields), e.g., as triggers for drug release, or for theranostic applications combining drug delivery and diagnostics. Among the inorganic nanoparticles, mesoporous silica nanoparticles (MSN) are of primary concern for the present investigation [14]. Due to well-defined pores in the nm range, drug loading and release kinetics is widely controllable, e.g., through surface area, as well as pore size, form, and surface chemistry. Through this, functional advantages can be obtained, e.g., related to increased drug load, sustained drug release, or reduction of burst release. In addition, MSN display good physical and chemical stability, and have been found to be relatively biocompatible, although depending on MSN specifics, dose, and administration route [15,16].

The controlled use of MSN as delivery systems for AMPs requires a basic understanding of the factors determining loading and release of such peptides. In the present study, we therefore address this by investigating the effects of particle porosity and surface charge on the loading and release of the benchmark AMP LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) [17], as well as consequences of this for particle interactions with lipid membranes, as well as for antimicrobial effect, cell toxicity, and proteolytic stabilization.

2. Experimental

2.1. Chemicals

LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) was synthesized by Biopeptide Co. (San Diego, USA), and was of >95% purity, as evidenced by mass spectral analysis (MALDI-TOF Voyager). Tetramethylorthosilicate (TMOS, purum, ⩾98%, Fluka Analytical/Sigma Aldrich), tetraethylorthosilicate (TEOS, purum, ⩾99%, Sigma Aldrich), cetyltrimethylammonium bromide (CTAB; Sigma-Aldrich), (3-aminopropyl)trimethoxysilane (APTMS, 97%, Aldrich Chemistry), methanol (technical, VWR), ethanol (99.5% denatured with 1% MEK, VWR), NaOH (⩾98%, Sigma Aldrich), and ammonium hydroxide solution (28%, VWR) were used without further purification. All other chemicals used were of analytical quality.

2.2. Microorganisms

The bacterial isolate *Escherichia coli* (*E. coli*) ATCC 25922 was obtained from the American Type Culture Collection.

2.3. Silica nanoparticle synthesis

Mesoporous silica particles were synthesized according to the synthesis published by Rosenholm et al. [18]. Briefly, in order to obtain amino-functionalized silica nanoparticles (MSNa) the structure-directing agent CTAB was dissolved in a methanol–water solution under basic conditions. Additionally, tetramethyl orthosilicate, TMOS, and aminopropyltrimethoxysilane, APTMS, were mixed under an inert atmosphere and added rapidly to the alkaline surfactant solution, with a final sol composition of TMOS:APTMS:NaOH:CTAB:methanol:water 1:0.1:1.24:1.36:1.3 × 10^3:3 × 10^3 (molar ratios). The CTAB was subsequently extracted with ammonium nitrate in ethanol. Particles were ultrasonicated in ammonium nitrate solution three times and washed twice in ethanol for purification. The particles were then dried under vacuum. To obtain calcined mesoporous silica particles (MSNc), MSNa particles were calcined at 550 °C for 4 h with a heating rate of 1 °C/min. Finally, a modified synthesis method leading to non-porous (Stöber-type) silica nanoparticles (NSN) was used as described by Sato-Berrú et al. [19]. Ethanol, water, 3 mL of ammonium hydroxide solution (28%), and 1.5 mL of TEOS, corresponding to a molar ratio of TEOS:NH_3:etanol:water 1:4.74:93.05:89.92, were mixed and stirred for 1 h at room temperature. The as obtained particles were washed twice in ethanol and dried under vacuum.

2.4. Nanoparticle characterization

Morphologies and dimensions of the silica nanoparticles were obtained using a Hitachi S-5200 Scanning electron microscope (SEM) (Hitachi, Tokyo, Japan) operated at 20 kV. Microtomed particles were also studied by TEM using a Jeol 1400 transmission electron microscope operated at 80 kV. Nitrogen adsorption and desorption isotherms were measured at −196 °C using a Quadrasorb-SI (Quantachrome Instruments, Boynton Beach, USA),