Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA

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Abstract

We synthesised silica nanoparticles (SiNP) with covalently linked cationic surface modifications and demonstrated their ability to electrostatically bind, condense and protect plasmid DNA. These particles might be utilised as DNA carriers for gene delivery. All nanoparticles were sized between 10 and 100 nm and displayed surface charge potentials from +7 to +31mV at pH 7.4. They were produced by modification of commercially available (IPAST) or in-house synthesised silica particles with either *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane or *N*-(6-aminohexyl)-3-aminopropyltrimethoxysilane. All particles formed complexes with pCMVbeta plasmid DNA as evidenced by ratio dependend retardation of DNA in the agarosegel and co-sedimentation of soluble DNA with nanoparticles. High salt and alkaline pH did inhibit complex formation. Absorption onto the particles also decreased the hydrodynamic dimensions of plasmid DNA as shown by photon correlation spectroscopy. Complexes formed in water at a w/w ratio of Si26H:DNA (pCMVbeta) of 300 were smallest with a mean hydrodynamic diameter of 83 nm. For effective condensation a w/w ratio of Si26H:DNA of 30 was sufficient. Further, the absorbed DNA was protected from enzymatic degradation by DNase I.

Keywords: Silica; Nanoparticle; DNA carrier; Gene delivery

1. Introduction

To date, a number of non-viral transfection agents exist. Most of them can be classified as polycations, including polylysine (Wagner et al., 1990), polyethylenimine (Boussif et al., 1995), dendrimers (Haensler et al., 1993) and chitosan (Erbacher et al., 1998) or liposomal preparations containing cationic lipids (Zelphati et al., 1998; Lee and Huang, 1997). Micellaneous DNA carriers include tensides that can be stabilised by cross-linking (Blessing et al., 1998), organic nanoparticles based on cyanoacrylates (Fattal et al., 1998) or PLA (Maruyama et al., 1997). The

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Table 1
Physicochemical parameters of the synthesised particles

Particle	Size [nm]	IEP [pH]	$\zeta_{pH7.4}$ [mV]	ζ_{max} [mV] (fitted)	Spacer in aminosilane
Si10E	10	8.1	+17	+44	Ethyl
Si22E	22	8.6	+26	+48	Ethyl
Si100E	100	7.7	+7	+56	Ethyl
Si26H	26	8.6	+31	+57	Hexyl

use of anorganic nanoparticles for DNA delivery has not been reported so far. However, these might have a number of advantages over existing vectors. Anorganic nanoparticles of low polydispersity can be reproducibly synthesised (Stoeber et al., 1968). There is a choice between biodegradable, such as hydroxyapatite, and inert matrix materials, e.g. silica. Compared to polycations, there is little concern about toxicity. In contrast to liposomes, the anorganic nanoparticles will be resistant to bile salts and lipases encountered in the gastrointestinal tract, physical stress during aerosolisation and should also, in principle, withstand autoclaving.

We therefore sythesised and evaluated biologically inert silica particles specifically modified to electrostatically bind DNA.

2. Materials and methods

Nanoparticle batches Si10E, Si22E and Si26H were synthesised by modification of commercially available silica particles (IPAST, Nissan Chemical Industries, Tokyo, Japan) with *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) for Si10E and Si22E or *N*-(6-aminohexyl)-3-aminopropyltrimethoxysilane (AHAPS) for Si26H as follows. Twenty gram particle suspension, corresponding to 6 g particle dry weight, 20 g water and 12 ml concentrated acetic acid were stirred for 16 h at 80°C with 7.2 g AEAPS (Si10E), 18 g AEAPS (Si22E) or 6 g AHAPS (Si26H). The product was chilled, mixed with 25 ml ethylene glycol, rotated for 2 h under vacuum at 50°C and dialysed 5 times for 24 h against 20 l of deionized water. For the synthesis of Si100E, silica particles were generated by the method of (Stoeber et al., 1968) and subsequently modified with 1 weight equivalent of AEAPS. Briefly, a mixture of 8 g of triethoxysilane (TEOS) and 13 g ammonium in 250 ml ethanol was subjected to a controlled hydrolysis and condensation reaction for 16 h at room temperature. The particles were collected by centrifugation, washed five times and modified with 4.36 g AEAPS as described above. AEAPS, AHAPS and TEOS were purchased from ABCR (Karlsruhe, Germany).

Particle size was determined by photon correlation spectroscopy on a ALV 5000 at a scattering angle of 90° (sampling time 300 s). Zeta potential titration curves were generated using a Malvern Zetasizer 4 (Malvern, UK). The instrument was calibrated routinely with a −55 mV standard.

Plasmid pCMVbeta was obtained from ATCC (Manassas, VA, USA) and transformed into *Escherichia coli DH5α*. For DNA isolation, a standard Gigaprep (Qiagen, Hilden, Germany) was performed according to the manufacturers instructions. The DNA was precipitated in 70% ethanol, washed and reconstituted in water.

Nanoparticle-DNA complexes were prepared by mixing in water at a fixed DNA concentration of 10 μg ml^{-1}. Agarosegel electrophoresis was

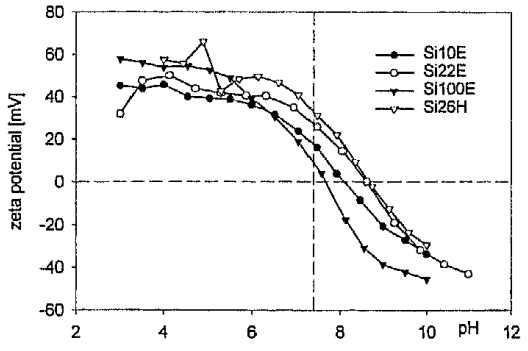

Fig. 1. Zeta potential titration of all synthesised SiNPs.

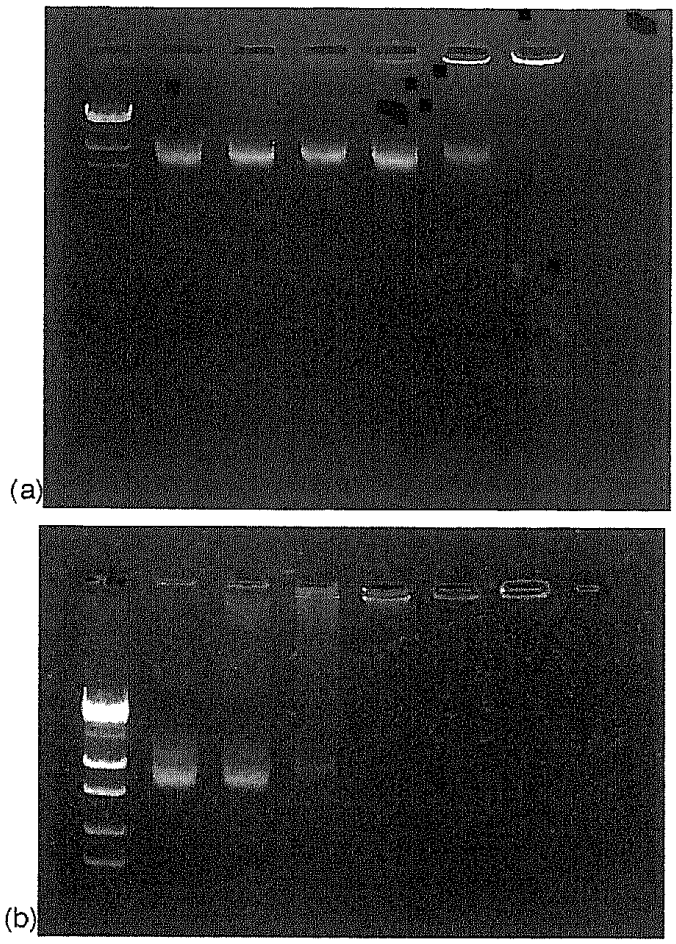

Fig. 2. Agarosegel electrophoresis of SiNP-DNA complexes. (a) Si100E, lanes from left: marker; uncomplexed DNA (10μg ml^{-1}); SiNP-DNA complexes prepared at w/w ratios of 1, 3, 10, 30, 100; Si100E only. (b) Si26H, lanes from left: marker; uncomplexed DNA (10 μg ml^{-1}); Si26H-DNA complexes prepared at w/w ratios of 3, 10, 30, 100, 300; Si26H only.

performed in a 1% (w/v) gel, ethidium bromide included for visualisation, for 4 h at 60 V. For sedimentation analysis, DNA (10 μg ml^{-1}) and Si26H were mixed, incubated for 15 min at room temperature and centrifuged for 15min at 21 000 × *g*. The DNA concentration in the supernatant was determined with Hoechst 33258 dye (Araki et al., 1987).

To assess stability against nuclease digestion, uncomplexed plasmid DNA (10 μg ml^{-1}) and DNA complexed with 10 or 30 weight equivalents of Si26H were incubated with 1 mU DNaseI per μg of DNA for 1 h at 37°C. The reaction was stopped by addition of EDTA, the complexes were dissociated with 0.1 N NaOH and the particles were removed by centrifugation. The released DNA was subjected to agarosegel electrophoresis as described above.

3. Results and discussion

Four batches of modified silica particles termed Si10E, Si22E, Si100E and Si26H were synthesised. The physicochemical properties are summarised in Table 1. The modification with aminosilanes was found sufficient to produce a net positive charge at neutral pH in all cases. At pH 10 and above, where > 90% of the amino groups are not protonated, the particles possessed zeta potentials of − 30 mV or below. When titrated to lower pH, the SiNPs showed an increase in zeta potential to at least + 30 mV, corresponding to the protonation of the amino-modification compensating the negative charge of the silanol groups (Fig. 1). To prevent particle aggregation during storage, the pH of the suspension had to be adjusted to pH 4.

Agarosegel electrophoresis demonstrated the ability of all synthesised particles to immobilise DNA. Fig. 2a and b show the agarosegels for Si100E and Si26H. Addition of increasing amounts of particles lead to an electrophoretic immobilisation of the DNA, implying binding to the particle surface. For > 95% immobilisation, a w/w ratio of particle DNA of 100 or 10 for Si100E or Si26H, respectively, was required. Analysis of Si10E and Si22E revealed similar activities (data not shown).

For Si26H, the binding of DNA to the particle surface was confirmed by co-sedimentation analysis (Fig. 3). This method also allowed to show the dependency of this interaction on pH, reflecting the deprotonation of the modified silica particles at alkaline pH (Fig. 3b). The particle-DNA binding was also inhibited by the presence of high salt concentrations, but not by glucose (Fig. 3a). This suggests that this interaction is based on electrostatic forces, resulting in interpolyelectrolyte complexes. The extent and the kinetics of binding as found in the co-sedimentation analysis correlates well with the results of the agarosegel electrophoresis.

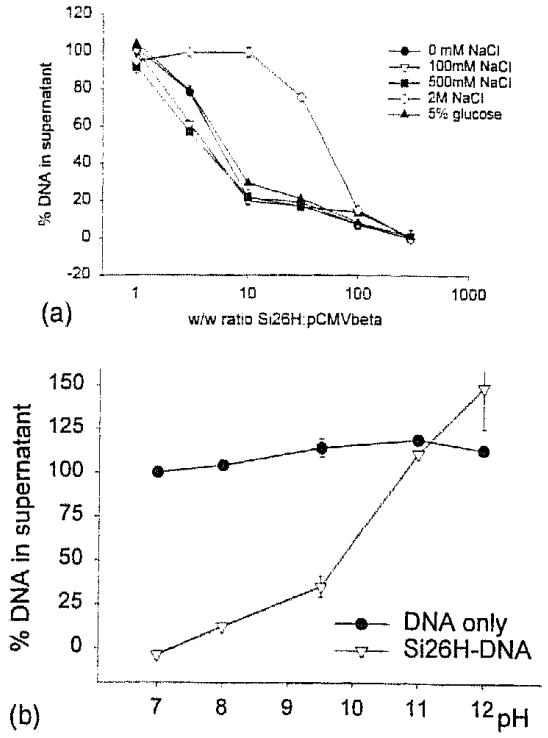

Fig. 3. Co-sedimentation of DNA with Si26H. (a) Dependency on SiNP:DNA ratio and medium. (b) Release of DNA from Si26H-DNA complexes prepared at a w/w ratio of 30 with increasing pH.

The PCS measurements revealed a reduction in size of the plasmid upon binding onto the particles (data not shown). Typical complex sizes for all particles were between 100 and 300 nm.

To investigate whether surface binding is sufficient to provide protection against enzymatic degradation, free and complexed pCMVbeta plasmid DNA was challenged with DNase I. Free plasmid DNA was degraded completely into fragments smaller than 1 kb. Addition of 10 parts Si26H prevented plasmid digestion almost completely. Only a small fraction of the supercoiled DNA was transformed into the nicked cirular form. Thirty parts Si26H protect the DNA entirely — no degradation products were detected. There was no increase in nicked circular or linear DNA. However, the experimental setup failed to release the supercoiled DNA at this Si26H:DNA ratio (Fig. 4).

Further biological characterisation will be required to elucidate the potential of the SiNPs as gene carriers. However, the results obtained so far are very encouraging. The SiNPs can thightly bind and also release intact plasmid DNA. When bound, the DNA is protected from nucleases and is also condensed to a size favourable for cellular uptake.

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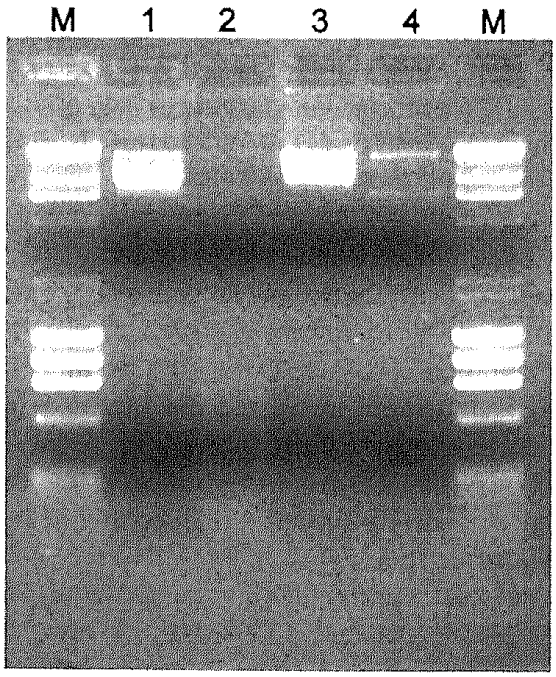

Fig. 4. Resistance of DNA and Si26H-DNA complexes to digestion by DNase I. Lane 1, undigested control DNA; lane 2, digested DNA; lanes 3 and 4, Si26H-DNA complexes prepared at w/w ratios of 10 and 30.

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