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Promoting DNA loading on magnetic nanoparticles using a DNA condensation strategy

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ABSTRACT

Maximizing DNA loading on magnetic nanoparticles (MNPs) is crucial for their successful utilization in gene transfer, DNA isolation, and bio-analytical applications. This enhancement is typically achieved by altering particle size and surfaces as well as charge density and ionic strength. We demonstrate a novel route for promoting DNA loading on amino-modified silica-coated magnetic nanoparticles (ASMNPs) by prior condensation of elongated DNA to a compact globule before adsorption. The enhanced DNA-loading capacity, as demonstrated by a reduction in the number of ASMNPs needed to achieve complexation, was presumably due to the elimination of DNA wrapping around nanoparticles and substantially reduced electrostatic interactions of DNA with nanoparticles because the compacted DNA globule conformation decreases its exposed surface charge. The maximum loading capacity of ASMNPs for condensed DNA was 4.4 times greater than that for elongated coiled DNA, achieving the highest ever reported value of 385 μg mg^{-1}. Practical applications for plasmid DNA isolation from cleared lysate confirmed the reliability of the proposed method.

1. Introduction

In the past decade, magnetic nanoparticles (MNPs) with varying magnetic properties, sizes, shapes, and surface chemistries have been widely employed for numerous biomedical and biological applications. A variety of technologies including magnetic resonance imaging, magnetic hyperthermia, drug delivery, and bioseparation, have benefited from the development of MNPs [1–4]. The complexation of nanoparticles with DNA to form magnetoplexes is even more attractive in view of the potential applications in gene magnetofection, DNA isolation, bio-analysis, and the directed assembly of nanostructures [5–7]. When the surface is modified with positively charged ligands, MNPs can adsorb negatively charged nucleic acids through electrostatic interactions. Modification of MNPs with cationic materials is thus necessary for DNA adsorption. Dendrimers, chitosan, lipids, poly(L-lysine), and polyethylenimine (PEI) have all proven to be effective candidates for this purpose [8–13].

The complexation of DNA with positively charged nanoparticles is currently receiving much research attention, focusing on the effect of the DNA length, salt concentration, particle size, surface modification, and charge density [14,15]. However, the influence of the spatial conformation of DNA on its' adsorption to nanoparticles has rarely been reported, and the unique conformational properties modifying adsorption are yet to be elucidated. It is known that DNA molecules adopt an elongated coil conformation in aqueous solutions due to the strong repulsion between negatively charged phosphate groups. The length of the commonly used vector DNA (e.g., plasmid) and genomic DNA is at least several hundred nanometres long, while the diameters of nanoparticles are generally below 100 nm. Consequently, when mixing plasmid or genomic DNA with MNPs, freely dispersed DNA molecules may wrap on the MNP surface and occupy most of the surface binding sites on a single nanoparticle or even associate with several nanoparticles. As a result, the number of DNA molecules potentially absorbed per nanoparticle is limited. This is a concern when nanoparticles are used for transfection and bio-separation applications, where loading capacity is a critical performance factor.

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In this study, we propose a novel and efficient means of enhancing DNA loading capacity during complexation on positively charged MNPs utilizing well-established approaches such as DNA condensation with polyethylene glycol (PEG) and efficient elution with phosphate anions. Herein, DNA molecules are first mixed with condensing agents (e.g., PEG) and NaCl [16] prior to interacting with MNPs. Such pre-treatment of DNA molecules induces a phase transition from an elongated coil conformation to a very dense compact state, which is typically globular in shape [16–18]. The negative phosphate groups on the DNA backbone are thus substantially shielded with fewer available for binding the positively charged nanoparticles, eliminating DNA coils wrapping around the nanoparticles surface. Only a portion of the DNA globule surface phosphate groups participate in electrostatic interactions, resulting in a smaller DNA footprint on each nanoparticle, which can potentially adsorb several DNA globules, enhancing DNA loading levels. Previously, carboxyl-coated magnetic particles were employed to bind DNA in the presence of polyethylene glycol (PEG) and salt (known as SPRI) [19,20]. Once dispersed in aqueous solution, DNA is readily desorbed due to strong charge repulsion between negatively charged carboxyl groups of magnetic particles and phosphate groups of DNA. This is a concern when magnetic nanoparticles are used as solid phase carriers for bio-analytical applications, or for controlled release of DNA within *in vivo* biomedical applications. In this context, positively charged magnetic particles will no doubt provide for much stronger electrostatic interactions. Herein, we studied the interaction between amino-modified silica-coated magnetic nanoparticles (ASMNPs) and condensed double-stranded DNA using PEG/NaCl as a compaction agent, with potential factors for effective adsorption and desorption investigated, such as PEG molecular weight and concentration, ionic strength, incubation time, and DNA concentration. Phosphate ions were used to elute adsorbed DNA from ASMNPs due to high replacement efficiency [21]. Both adsorption and desorption experiments support our proposed model for DNA complexation with oppositely charged nanoparticles. The practical application of isolating plasmid DNA (pDNA) from cleared bacterial lysate confirmed the feasibility of the developed method.

2. Experimental

2.1. Materials

$FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, $NH_3{\cdot}H_2O$ (25%), and tetraethyl orthosilicate (TEOS) were purchased from KeLong Corp. (Chengdu, China). 3-Aminopropyltriethoxysilane (APTES) and polyethylene glycol (PEG 400, 2000, 8000 and 20,000) were obtained from Aladdin (Shanghai, China). Hoechst 33258 used for DNA quantification was purchased from Sigma (St. Louis, MO). DNA solutions were prepared by dissolving DNA (double-stranded DNA of salmon, Sigma) in TE buffer (20 M Tris–HCl, 2 mM EDTA, pH 7.0). Deionized (DI) water was used throughout the study. All other reagents were of analytical grade and used as received.

2.2. Synthesis of ASMNPs

The synthesis of ASMNPs was performed according to previously reported methods with some modifications [22–24]. To prepare the iron oxide nanoparticles, a fresh mixture of 2.592 g $FeCl_3{\cdot}6H_2O$ and 1.034 g $FeCl_2{\cdot}4H_2O$ (in 60 mL water) was added to 20 mL ammonia solution stirred at 80 °C for 30 min. The as-synthesized iron oxide nanoparticles were separated using a magnet, washed twice with water, and then dispersed in 100 mL sodium citrate (62.5 mM, pH 4.8) for 90 min at 90 °C. Iron oxide nanoparticles were recovered by acetone precipitation, dried under vacuum, and dispersed in 200 mL water to form a stable ferrofluid. Before the silica coating process, 20 mL ferrofluid was added to a mixture containing 20 mL water, 140 mL ethanol, and 5 mL $NH_3{\cdot}H_2O$ and sonicated for 2 min. Then 0.6 mL TEOS (in 20 mL ethanol) was added. After stirring for 12 h at room temperature, silica-coated iron oxide MNPs (SMNPs) were prepared. The black SMNPs were magnetically separated, washed with ethanol three times, and dispersed in a solution containing 95 mL ethanol and 5 mL water. After sonicating for 5 min, 0.65 mL APTES was added and reacted for 12 h under vigorous stirring to produce ASMNPs. After washing with ethanol and water, the final product was dispersed in water at a concentration of 10 mg mL^{-1}.

2.3. Characterization of nanoparticles

Samples for transmission electron microscopy (TEM) 100 CX II (JEOL, Japan) were dispersed in water and sonicated for 30 s, and then transferred to carbon-coated copper grids for visualization. The surface charge property of the ASMNPs was investigated by measuring the Zeta potential with a ZEN3600 Nano Zetasizer (Malvern, England). The colloid stability of ASMNPs was evaluated by monitoring absorbance change at 400 nm for 30 min at room temperature. Fluorescence microscopy image of condensed DNA–ASMNPs complexes were recorded with an Olympus fluorescence microscope (BX51) equipped with a 100× oil-immersed lens and selective filters.

2.4. DNA adsorption and desorption experiments

The basic adsorption procedure was as follows. In each experiment, 300 μL salmon DNA and 300 μL PEG/NaCl buffer (molecular weight and concentration are specified in following sections) were mixed and incubated at room temperature for 1 min, before addition and mixing with 15 μL ASMNPs. After incubating for 5 min at room temperature, ASMNPs with adsorbed DNA were removed with a magnet, leaving a transparent supernatant. The amount of DNA adsorbed by ASMNPs was calculated from the depletion of DNA from the supernatant after adsorption and magnetic separation. To study the adsorption of DNA (100 μg mL^{-1}) as influenced by varying binding conditions, the amounts of adsorbed DNA by ASMNPs were determined under different environmental buffer conditions, including water, NaCl (0–2.0 M), and PEG/NaCl (NaCl 1 M; PEG ranged between 0 and 30% (w/v) for PEG 400, 2000 and 8000, 0–10% (w/v) for PEG 20,000). The adsorption isotherms in the above-mentioned buffer conditions were obtained by preparing a series of salmon DNA solutions with final concentrations ranging from 10 to 400 μg mL^{-1}, and the adsorption dynamic curves were obtained through measuring DNA concentration in the supernatant at predetermined time-points (within 3 min) after mixing ASMNPs with DNA (100 μg mL^{-1}).

To investigate DNA desorption behaviour, ASMNP–DNA composite was prepared by first incubating 300 μL DNA (200 μg mL^{-1}) with 300 μL condensing buffer (15% PEG 8 000/2 M NaCl) for 5 min, followed by incubating with ASMNPs for 5 min at 25 °C. The ASMNP–DNA composites were magnetically separated and twice washed with 70% ethanol and rapidly dried. Phosphate buffers (PB, pH 8.0) with concentrations of 0, 0.05, and 1 M were evaluated as elution buffers. Elution experiments were carried out at 25 °C using 300 μL elution buffer for different times (1–90 min). After magnetic separation, the supernatant was measured for DNA concentrations to determine desorption; recovery of eluted DNA was estimated by comparing with the total amount of DNA before adsorption. All of the adsorption and elution experiments were repeated three times.