Journal of Colloid and Interface Science 377 (2012) 469–475

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Characterization of magnetic nanoparticles modified with thiol functionalized PAMAM dendron for DNA recovery

Tsuyoshi Tanaka [a,*], Keiyu Shibata [a], Masahito Hosokawa [a], Keiichi Hatakeyama [a], Atsushi Arakaki [a], Hideyuki Gomyo [a], Takeyuki Mogi [b], Tomoyuki Taguchi [b], Hitoshi Wake [b], Takeo Tanaami [b], Tadashi Matsunaga [a]

[a] Division of Biotechnology and Life Science, Institute of Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo 184-8588, Japan
[b] Yokogawa Electric Corporation, 2-9-32, Naka-cho, Musashino-shi, Tokyo 180-8750, Japan

ARTICLE INFO

Article history:
Received 17 December 2011
Accepted 14 March 2012
Available online 28 March 2012

Keywords:
Magnetic nanoparticle
Dendrimer
Polyamidoamine
Phospholipid bilayer
DNA carrier

ABSTRACT

Magnetic nanoparticles (MNPs) modified with the thiol functionalized polyamidoamine (PAMAM) dendron were synthesized to estimate their DNA recovery capabilities. Aminosilane-modified MNPs and MNPs surrounded by a phospholipid (distearoylphosphatidylethanolamine (DSPE)) bilayer were used as core particles. Cystamine-core PAMAM dendrimers were reduced by dithiothreitol to dendron thiols and chemically conjugated to the core particles. Characterization of the synthesis revealed an increase of the surface amine charge from generation 1 (G1) to G6, starting with an aminosilane initiator. Particle size distribution analysis indicated that G6 PAMAM-modified MNPs exhibited monodispersity in an aqueous solution. G6 PAMAM–MNPs and G6 PAMAM–PE–MNPs synthesized by the proposed method have equivalent DNA recovery abilities to PAMAM–MNPs prepared by the conventional divergent synthesis method. In optimized conditions, 96% of λDNA was recovered using G6 PAMAM–PE–MNPs. Therefore, the method for preparing PAMAM–MNPs and PAMAM–PE–MNPs proposed in this study will be a novel approach for producing DNA carriers for efficient DNA purification by magnetic separation.

1. Introduction

Solid-phase DNA purification has been commonly used in molecular biology and biotechnology due to its quick processing time, low chemical requirements, and ease of implementation. Silica-based DNA purification was first established in 1979 [1]. The addition of chaotropic ions promotes the binding of DNA to silica [2,3]. Solid-phase reversible immobilization technology using carboxyl group-coated solid support has been also proposed [4–6]. PEG–DNA precipitates under high NaCl conditions and reversibly binds to these supports. These approaches circumvent the use of organic solvents that may influence subsequent applications, such as gene amplification and enzymatic digestion of DNA. Recently, our research group developed a novel DNA extraction method using aminosilane-modified solid supports based on electrostatic interactions between the amino groups on the solid supports and nucleic acids, as well as subsequent DNA release with changing ionic strength [7–10]. These approaches have minimized the effect of organic solvents. Improved DNA recovery was attained by using magnetic nanoparticles (MNPs) modified with a polyamidoamine (PAMAM) dendron (PAMAM–MNPs) to generate a colloidal suspension. PAMAM coating enables the reduction of particle agglomeration, and the terminal groups on the periphery can be tailored to control composite solubility [11]. A PAMAM dendrimer can introduce a dense outer amine shell through cascade-type generation.

In general, there are two methods for dendrimer preparation: divergent synthesis and convergent synthesis. The former grows a dendron (molecular tree) from a core site (root) [12]. The latter links beforehand prepared dendron molecular surfaces to the core site [13]. The divergent synthesis method has been also employed to prepare PAMAM–MNPs, which have been applied for protein immobilization [14] and gene delivery [15], as well as DNA extraction. The amino groups on their surface were alkylated using methyl acrylate and then amidated using ethylenediamine for each generation. Stepwise growth using methyl acrylate and ethylenediamine was repeated until the desired number of generations was achieved. However, the particle concentration used in the reaction step was limited, because highly dispersed conditions for MNPs were required for efficient chemical reactions on their surfaces. Conversely, cystamine-core PAMAM dendrimers, which exhibit self-assembled dendronization, have been developed and used for the surface modification of gold nanoparticles and CdSe/CdS (core–shell) quantum dots [16,17]. The synthesis method using the thiol core, functionalized PAMAM dendrons allows the direct dendronization of a wide variety of nanosubstrates.

* Corresponding author. Fax: +81 42 385 7713.
E-mail address: tsuyo@cc.tuat.ac.jp (T. Tanaka).

0021-9797/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2012.03.039

In this study, PAMAM–MNPs were developed by the self-assembly dendronization method using thiol functionalized PAMAM dendrons to estimate their abilities to recover DNA compared with those of PAMAM–MNPs prepared by the divergent synthesis method. Aminosilane-modified MNPs and MNPs surrounded by a phospholipid (distearoylphosphatidylethanolamine; DSPE) bilayer were used as the core particles. The properties of the resulting PAMAM–MNPs were characterized by determining the amine numbers, zeta potentials, particle dispersibility, magnetic behaviors, and DNA recovery ratio.

2. Materials and methods

2.1. Materials

PAMAM dendrimers with cystamine cores (generations 1–6 (G1–G6 dendrimers)) were obtained from Sigma–Aldrich (St. Louis, MO). *N*-(4-maleimidobutyryloxy)succinimide (GMBS) and dithiothreitol (DTT) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 3-[2-(2-Aminoethylamino)-ethylamino]-propyltrimethoxysilane (AEEA) was obtained from Fluka Chemical (Buchs SG, Switzerland). *N*-(3-maleimido-1-oxpropyl)-l-α-phosphatidylethanolamine, distearoyl (DSPE-MAL) and *N*-(succinimidyl-glutaryl)-l-α-phosphatidylethanolamine, distearoyl (DSPE-NHS) were purchased from NOF Corporation (Tokyo, Japan). Sulfosuccinimidyl 6-[3′-(2-pyridyldithio)-propionamido]hexanoate (Sulfo-LC-SPDP) was purchased from Pierce Chemical (Rockford, IL). The Pico Green (λ_{ex} = 502 nm, λ_{em} = 523 nm) dsDNA assay kit was purchased from Invitrogen (Carlsbad, CA). MNPs purified from a magnetotactic bacterium, *Magnetospirillum magneticum* AMB-1 (ATCC 700264), were used as solid supports for silanization as previously described [18]. Electron microscopy revealed that the MNPs consisted of a single crystal of magnetite (Fe_3O_4) with a single-domain magnetic structure. The magnetite crystals were 50–100 nm in size (average diameter: 80 nm) and exhibited a cuboctahedral morphology. Other commercially available reagents were either of analytical or laboratory grade.

2.2. Preparation of AEEA-modified MNPs (AEEA–MNPs)

MNPs (10 mg) were incubated with 20 mL of 2% AEEA solution in EtOH at room temperature for 10 min (Fig. 1A). After dimethylformamide (DMF) washing, aminosilane-modified MNPs (AEEA–MNPs) were baked at 120 °C in 20 mL of DMF for 30 min with sonication every 10 min. After this treatment, the particles were washed with MeOH three times and stored in MeOH at 4 °C. The concentration of MNPs in suspension was determined by measuring the optical density of the solution at 660 nm using a spectrophotometer (UV-2200; Shimadzu, Kyoto, Japan). A value of 1.0 corresponded to 213 μg (dry weight) MNPs/mL.

2.3. Surface modification of AEEA–MNPs using PAMAM dendrimers

G1–G6 dendrimers (0.5 mM) in 200 μL of MeOH were mixed with 800 μL of DTT (0.5 mM) in 10 mM phosphate buffer containing 140 mM NaCl (phosphate-buffered saline; PBS, pH 7.4) to produce dendron thiols (Fig. 1B). The dendron thiol solution was stirred at room temperature for 12 h, after which it was diluted 10-fold with PBS. Meanwhile, AEEA–MNPs (10 mg) were incubated with 1 mM GMBS solution in 20 mL of PBS for 1 h to introduce maleimide groups on their surfaces (Fig. 1C). After three washes with PBS, the maleimide-functionalized MNPs (final concentration: 0.5 mg/mL) were reacted with the dendron thiol solution (Fig. 1D); PAMAM–MNPs). The PAMAM–MNP suspension was incubated at room temperature for 1 h with sonication. After three washes with MeOH, the PAMAM–MNPs were stored in MeOH at 4 °C. To quantify free thiols in solution after DTT treatment, the following experiment was performed. G4 cystamine-core PAMAM dendrimers were mixed with a 4-fold molar excess of DTT and stirred for 12–48 h at room temperature. After reduction treatment, Cy3-maleimide solutions were added to 400 μL of the thiol-containing solution. The solutions were allowed to incubate for 60 min in the dark at room temperature and then centrifuged to remove free DTT and excess Cy3-maleimide within the solution using centrifugal filter devices (Amicon Ultra-0.5, 3000 NMWL, Millipore Corp.) before measuring absorbance at 550 nm. A standard curve was generated with different concentrations of Cy3-maleimide.

2.4. Surface modification of AEEA–MNPs using distearoylphosphatidylethanolamine and PAMAM dendrimers

AEEA–MNPs (1 mg) were incubated with 1 mM DSPE-NHS solution in 2 mL of dimethyl sulfoxide at room temperature for 1 h with sonication (Fig. 1E). After three washes with EtOH, the DSPE-modified MNPs were suspended in 1 mM DSPE-MAL solution in 2 mL of EtOH (Fig. 1F). The MNP suspension (in EtOH) was heated at 65 °C under continuous sonication, and then, the suspension was added to 20 mL of PBS (room temperature) with a syringe. After MNPs were washed with PBS, they were suspended in 2 mL of dendron thiol solution (Fig. 1G; PAMAM–PE–MNPs (PAMAM dendron- and DSPE-modified MNPs)) and incubated at room temperature for 1 h with sonication. After three washes with PBS, the PAMAM–PE–MNPs were stored in PBS at 4 °C.

2.5. Determination of surface amines

To determine the number of amine moieties on their surfaces, PAMAM-modified MNPs (250 μg) were incubated in 10 mM Sulfo-LC-SPDP in PBS at room temperature for 30 min. Sulfo-LC-SPDP-conjugated MNPs were washed three times with PBS and incubated in 200 μL of 20 mM DTT in PBS to release 2-pyridylthiol. The absorbance of 2-pyridylthiol at 343 nm was measured using a spectrophotometer. The concentrations of Sulfo-LC-SPDP reacted with primary amine groups on MNPs were determined using a standard curve generated with different concentrations of Sulfo-LC-SPDP in 20 mM DTT [7,8].

2.6. Measurement of MNP size distributions and zeta potentials

The size distributions and zeta potentials of PAMAM-modified MNPs were analyzed using a laser particle analyzer (ELS-8000, Otsuka Electronics, Osaka, Japan). The size distributions of MNPs (10 μg/mL) were measured at 3 min after dispersion using an ultrasonic bath. The zeta potential was calculated from their electrophoretic mobilities.

2.7. Vibrating sample magnetometer (VSM) analysis

PAMAM-modified MNPs (1 mg) were suspended in 60 μL of distilled water. The suspensions were placed in a VSM sample container (Tamagawa Co., Ltd. Sendai, Japan) and dried overnight. Prepolymer solution (poly(ethylene glycol) diacrylate:TE buffer (10 mM Tris-HCl buffer; 1 mM ethylenediaminetetraacetic acid; pH 8.0):2-hydroxy-2-methylpropiophenone = 100:50:1 (vol.); 45 μL) was added into the container and solidified by ultraviolet light exposure for 30 s. The magnetic properties of MNPs were measured using a VSM (TM-VSM101483N7-MRO, Tamagawa Co., Ltd.).