Self-Assembled Ternary Complex of Cationic Dendrimer, Cucurbituril, and DNA: Noncovalent Strategy in Developing a Gene Delivery Carrier

ARTICLE *in* BIOCONJUGATE CHEMISTRY · OCTOBER 2002

Impact Factor: 4.51 · DOI: 10.1021/bc025581r · Source: PubMed

7 AUTHORS, INCLUDING:

Yong-beom Lim
Yonsei University
59 PUBLICATIONS **1,983** CITATIONS
SEE PROFILE

Tae-Il Kim
Seoul National University
34 PUBLICATIONS **1,286** CITATIONS
SEE PROFILE

Kimoon Kim
Pohang University of Science and Technology
298 PUBLICATIONS **19,422** CITATIONS
SEE PROFILE

Self-Assembled Ternary Complex of Cationic Dendrimer, Cucurbituril, and DNA: Noncovalent Strategy in Developing a Gene Delivery Carrier

Yong-beom Lim,[†] Taeil Kim,[†] Jae Wook Lee,[‡] Seon-mi Kim,[†] Hee-Joon Kim,[‡] Kimoon Kim,*[,‡] and Jong-sang Park*[,†]

School of Chemistry & Molecular Engineering, Seoul National University, Seoul 151-742, Korea, and Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Korea.
Received July 22, 2002

A ternary complex of PPI-DAB dendrimer [(1,4-diaminobutane); Gen = *N*; *dendri*-poly(propyleneimine); -[NHC(=O)$CH_2NH_2^+(CH_2)_4NH_3^+$]$_z$], DNA, and cucurbituril (CB) was evaluated as an example of a totally self-assembled gene delivery carrier. The complex was formed in a noncovalent way in which DNA interacts with PPI-DAB electrostatistically and CB with PPI-DAB through multiple noncovalent interactions. Dynamic light scattering data indicated that the diameter and size distributions of the complexes were dependent upon the sequence of mixing of each component with unimodal distribution ranging from 150.8 to 210.2 nm under favorable conditions. Fluorescence studies showed the quantitative binding of CB to PPI-DAB after ternary complex formation. The complex was able to transfect mammalian cells with high efficiency and the cytotoxicity of the PPI-DAB/CB complex was relatively low.

Spontaneously assembled supramacromolecular complexes have been the subject of considerable interest, with the noncovalent self-assembly process between biological macromolecules being mimicked in a chemical approach (*1*−*8*). The study of nonviral gene delivery vectors is one such approach in which virus corelike particles are assembled through electrostatic interactions, e.g., between cationic polymer and negatively charged DNA (*9*). A number of cationic polymers, such as poly-L-lysine (*10*), poly(ethylenimine) (PEI) (*11*), PAMAM dendrimer (*12*), and hyperbranched poly(amino ester) (*13*), were able to compact DNA into submicron-sized water-soluble particles (polyplexes) and carry DNA into the cell nucleus. The polyplexes were made to have specialized functional moieties such as targeting ligands, endosome disruptive functions, and nucleus localization signals by directly (covalently) attaching them to the cationic polymer (*14*−*17*). This covalent strategy has worked well; however, it would be more interesting if we were able to produce similar or even more advanced systems by the use of a noncovalent approach.

The packaging of viruses is also an elaborate multistep noncovalent self-assembly process between the viral genome (DNA or RNA) and a number of proteins (*18*). In this regard, a synthetic functional module that recognizes a specific moiety in the cationic polymer in a noncovalent manner would be useful in the realization of synthetic viruslike particles.

In this paper we report on the development of a noncovalently assembled ternary gene delivery complex based on cationic dendrimer, DNA, and CB reminiscent of virus particles (Scheme 1). CB was regarded as a functional module. CB itself has no biological activity; however, this system can be applied to other noncovalent molecular recognitions, and it would be possible to create a really functional polyplex if the functionalized-CB were synthesized, which we are currently investigating. For example, peptide ligand−CB conjugate could easily be made if amine (NH_2)-, alcohol (OH)-, or sulfhydryl (SH)-functionalized CB was synthesized. The purpose of this work is to evaluate the possibility of generating a three-component gene delivery system consisting of cationic polymer, DNA, and noncovalent ligand equivalent. CB is a large-cage compound composed of glycoluril units interconnected with methylene bridges and is able to form stable pseudorotaxanes with strings derived from diaminoalkanes through multiple noncovalent interactions (*19*, *20*).

The ternary complex was prepared by the sequential treatment of PPI-DAB (G4) with CB and DNA (PPI-DAB/CB/DNA), or DNA and CB (PPI-DAB/DNA/CB). Synthetic details for the preparation of PPI-DAB have been described previously (*21*). The hydrodynamic radii (R_h) of the two preparations were investigated by dynamic light scattering (DLS)[1] as shown in Figure 1. The measured average R_h of PPI-DAB/CB/DNA was 150.8

* Corresponding authors: Jong-sang Park. Phone: +82-2-880-6641. Fax: +82-2-877-5110. E-mail: pfjspark@plaza.snu.ac.kr. Kimoon Kim. Phone: +82-54-279-2113. Fax: +82-54-279-8129. E-mail: kkim@postech.ac.kr.

[†] Seoul National University.

[‡] Pohang University of Science and Technology (POSTECH).

[1] Light scattering experiments were performed at 25 °C with BI-200SM Goniometer (Brookhaven Instruments Corporation) using a Lexel laser model 95 argon laser (100 mW output power at a wavelength of 514.5 nm). Correlator was PD2000 (Precision Detectors), and the scattering angle was 90°. Data were analyzed using the CONTIN algorithm. The sample was prepared by directly mixing each solution of polymer and DNA in water. The final DNA concentration was adjusted to 5 μg/mL.

[2] pEGFPLuc (Clontech) encodes a luciferase from the firefly *Photinus pyralis* under the control of the immediate early promoter of CMV.

10.1021/bc025581r CCC: $22.00
Published on Web 10/18/2002

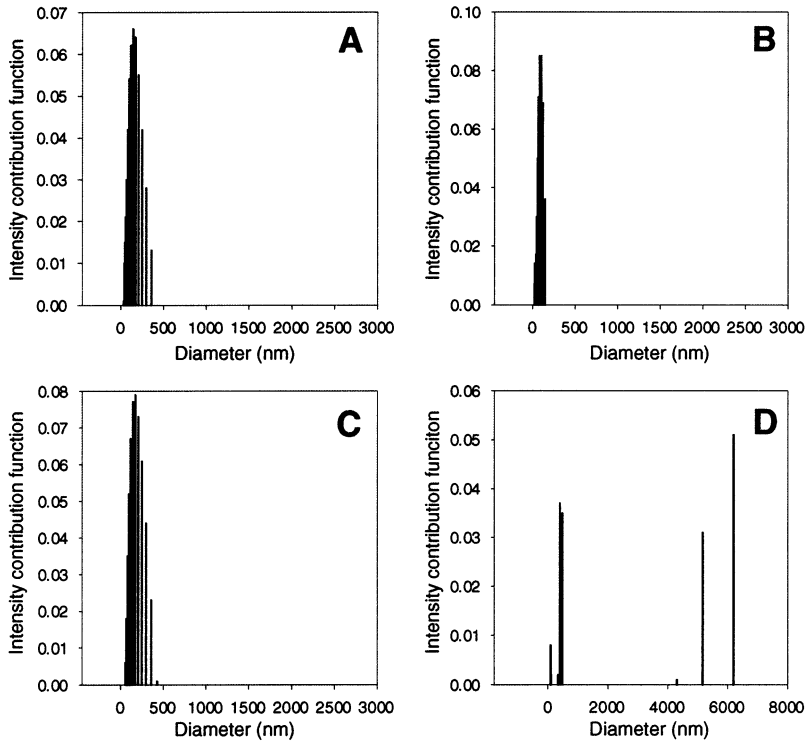
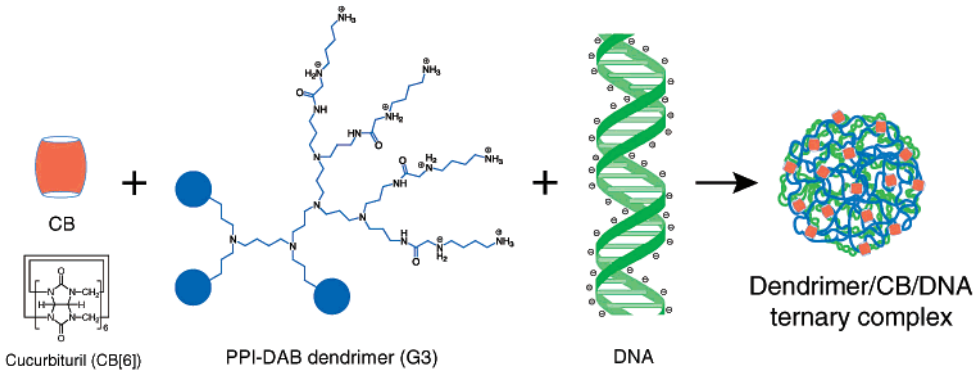


Figure 1. Size distribution of (A) PPI-DAB/CB/DNA (with 100% CB relative to DAB), (B) PPI-DAB/DNA, (C) PPI-DAB/DNA/CB (10% CB), and (D) PPI-DAB/DNA/CB (30% CB) complexes by dynamic light scattering.[1] DNA was pEGFPLuc plasmid DNA.[2] All complexes were formed at a charge ratio (+/−) of 3. Charge ratios were calculated based on the assumption that only primary and secondary amines in DAB are protonated.

Scheme 1. Ternary Complex of PPI-DAB Dendrimer, CB, and DNA



nm. PPI-DAB/CB/DNA was prepared with all the DAB moieties being occupied by CB. The average R_h of PPI-DAB/DNA (83.5 nm) was smaller than that of PPI-DAB/CB/DNA; however, the complex grew larger as CB was added. At 10% and 20% CB concentrations (relative to DAB moieties), the average R_h became 176.2 and 506.1 nm, respectively. Two intensity populations existed above 30% CB concentration, with the smaller sizes presumably due to that of well-defined ternary complexes and the larger sizes probably attributed to aggregates. These results imply differences in tertiary structures between the two preparations. Therefore, further characterization of the ternary complex was conducted with PPI-DAB/CB/DNA for simplification.

The formation of the ternary complex was investigated by observing the shift of DNA electrophoretic migration in agarose gel (Figure 2). All third-generation (G3) polymers and G4 of PPI formed polymer/DNA complexes (polyplexes) at charge ratios (polymer to DNA, +/−) above unity. However, G5 of PPI, G4 and G5 of PPI-DAB, and G4 and G5 of PPI-DAB/CB formed polyplexes at charge ratios above 2. These results indicate that the DNA binding capacity of the three polymers is decreased as the generation number is increased. In addition, the DNA binding capacities were increased in the order, PPI > PPI-DAB ≥ PPI-DAB/CB. Examination of the structural conformations of PPI-DAB and PPI-DAB/CB by molecular dynamic simulation revealed that PPI-DAB/CB adopts a more rigid shell at the exterior than PPI-DAB (*21*). The decreased binding capacity of the higher generation dendrimers and of PPI-DAB/CB and PPI-DAB relative to that of PPI may be related to the formation of a more rigid and compact shell at the exterior where DNA binding amine groups are located, and to the effect of the polymers' different molecular structure and CB on pK_a of amino groups.

To verify the formation of the ternary complex, it was necessary to prove that CB remained associated with PPI-DAB and was not expelled from the polymer in the process of DNA binding. To demonstrate the validity of this assumption, each of CB, PPI-DAB (G4)/CB, and PPI-DAB (G4)/CB/DNA were dialyzed using 3500 M_r cutoff tubing against 0.2 m Na_2SO_4, in which CB dissolves very

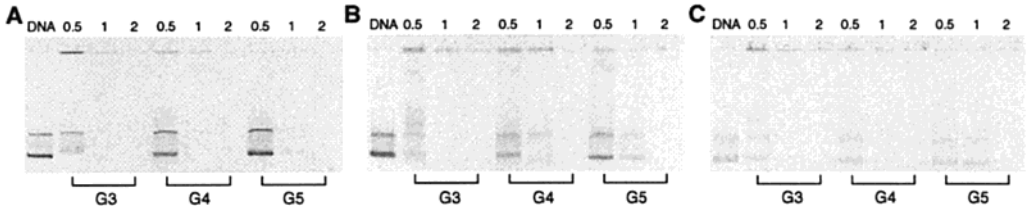


Figure 2. Investigation of polyplex formation by agarose gel band shift assay. Numbers indicate charge ratios (+/−). (A) PPI/DNA polyplex. (B) PPI-DAB/DNA polyplex. (C) PPI-DAB/CB/DNA polyplex. In PPI-DAB/CB/DNA polyplex, all of the DAB moieties are occupied by CB. The original positive image was inverted to make it negative for ease of band discrimination.

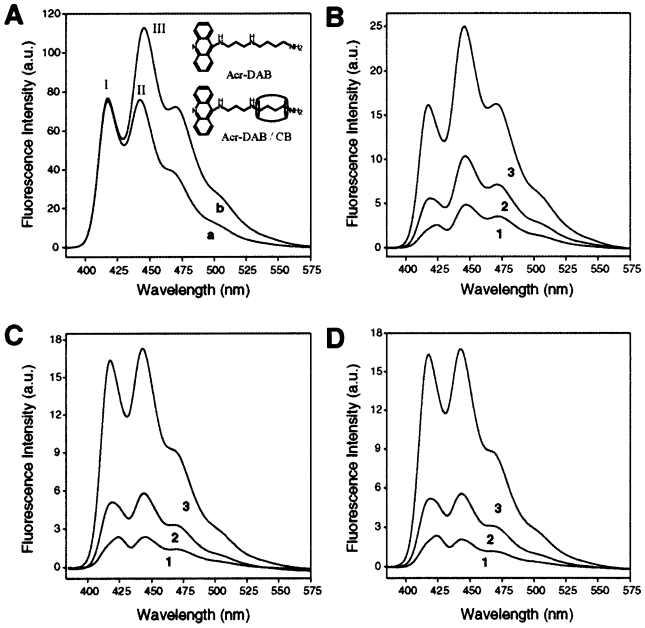


Figure 3. Fluorescence emission spectra of (A) **a**: Acr-DAB; **b**: Acr-DAB/CB and the solution outside the dialysis membrane of (B) CB, (C) PPI-DAB/CB, and (D) PPI-DAB/CB/DNA dialysates after addition of Acr-DAB. **1**: 0.1 μg (1/40 molar equivalent of total CB); **2**: 0.3 μg (3/40); **3**: 1.0 μg (10/40) of Acr-DAB were added to the solution.

well (*20*). In this system, only CB is expected to be freely permeable to the membrane.

The presence of CB in the solution outside the membrane could easily be detected by using DAB-tethered acridine (Acr-DAB) with high sensitivity (Figure 3).[3] The spectrum of Acr-DAB exhibited acridine emission with peaks at 418 (band I) and 442 nm (band II). However, there was a red shift (4 nm) of band II after binding of CB to Acr-DAB with a strong increase in the fluorescence intensity (band III), due to the interaction of CB with the acridine moiety. The shapes of the emission spectra, which were recorded after addition of Acr-DAB to the outside solution of CB dialysate, were identical to that of Acr-DAB/CB (Figure 3B). Varying the amount of added Acr-DAB (1/40, 3/40, and 10/40 molar equivalent of CB) did not influence the shape of the spectrum except in relation to the emission intensity. Identical spectra were obtained in the case where the same amount of Acr-DAB as indicated above was added to an estimated amount of CB, on the assumption that the membrane is freely permeable to CB (data not shown).[4] These results indicate that equilibrium of CB across the membrane had been reached. In contrast, the shape of the emission spectra recorded after addition of varying amounts of Acr-DAB to the outside solution of PPI-DAB/CB or PPI-DAB/CB/DNA dialysate are almost identical to that of Acr-DAB (Figure 3, parts C and D). However, there were slight increases in the fluorescence intensity of band II, indicating that a small amount of CB exists outside the membrane. It has been well established that CB binds tightly to the diaminobutane moiety with high affinity (*19*−*21*). Therefore, the amount of CB present outside the membrane would be minute, and the nearly identically shaped spectra in parts C and D of Figure 3 indicate that there was no change in the CB "beads" threaded on PPI-DAB after the addition of DNA to the PPI-DAB/CB binary complex.

To evaluate the ternary complex as a potential gene delivery vector, the cytotoxicity of the polymers and gene transfer mediated by the complexes were investigated (Figure 4). The results indicated that PPI-DAB/CB polymer had relatively low toxicity with about 50% cells remaining viable even at high concentrations of 100 μg/mL (Figure 4A). Transfection efficiencies (TE) were dependent on the cell type, usually 300−500 times higher in 293 cells than in Vero 76 cells for all polyplexes (PEI, PPI, PPI-DAB, and PPI-DAB/CB polyplexes) tested (Figure 4B). It is well-known that 293 cells are very susceptible to transfection. However, the relative TEs among the polyplexes were similar between the two cell lines. Transfection mediated by PPI-DAB/CB polyplexes was relatively high and was only about 10 times lower than that of PEI, which is one of the most efficient gene carriers hitherto reported (*9*). Szoka and colleagues have shown that fractured PAMAM dendrimers are more efficient in transfection than intact PAMAMs due to the increased flexibility of the fractured dendrimers that enables them to be compact when complexed with DNA and swell when released from DNA (*22*). The lower TEs of PPI, PPI-DAB, and PPI-DAB/CB polyplexes than that of PEI polyplexes may be related to the same mechanism. The TEs of PPI, PPI-DAB, and PPI-DAB/CB polyplexes were increased as the generation number of dendrimers became higher (Figure 4C). Taken together, these results indicated that CB had no negative influence on the TE of polymer/DNA binary complex and the ternary complex is a highly active form of gene delivery vector. This prototype of supramacromolecular ternary complex can

[3] PPI-DAB (G4)/CB or PPI-DAB (G4)/CB/DNA (+/−, 2) each containing 60.4 μg of CB was dissolved in 2 mL of 0.2 M Na_2SO_4. Each of the two solutions and 2 mL of 0.2 M Na_2SO_4 solution containing 60.4 μg of CB were dialyzed against 8 mL of 0.2 M Na_2SO_4 using 3500 molecular weight cutoff tubing (Spectrum Laboratories). After extensive dialysis, 1 mL of the solution outside the tubing was taken. Varying amounts of Acr-DAB (0.1 to 1.0 μg) were added to the solution, and then fluorescence measurements were taken. Fluorescence emission spectra were recorded at room temperature on a Jasco FP-750 spectrofluorometer. The emission bandwidth was set at 5 nm. The excitation wavelength was 370 nm.

[4] For the spectra measurements in Figure 2B, 60.4 μg of CB in 2 mL of 0.2 M Na_2SO_4 was dialyzed against 8 mL of the same solution and 1 mL of the solution outside the membrane was taken for the measurement (final 10-fold dilution). Therefore, 6.04 μg of CB was used for this control.

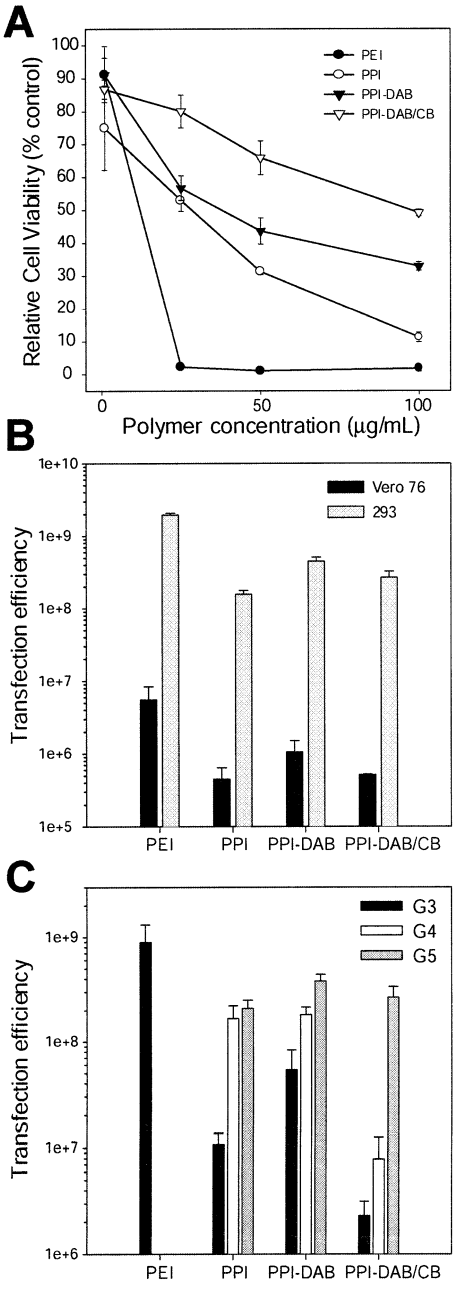


Figure 4. (A) Cytotoxicity in Vero 76 cells by MTT assay. (B) Transfection of Vero 76 and 293 cells with PEI (25 kDa, branched), PPI (G5), PPI-DAB (G5), PPI-DAB (G5)/CB polyplexes. (C) Effects of dendrimer generation on transfection in 293 cells. All polyplexes were formed at charge ratios (+/−) of 2 except PEI polyplex, which was prepared at a weight ratio (polymer/DNA) of 1. TE was expressed as relative light units per mg of cellular protein.

be applied to any type of polymer bearing suitable alkyl diaminobutane moieties and will assist in the further development of artificial viruslike particles.

ACKNOWLEDGMENT

This work was supported by the Korea Research Foundation (DP-0344) and by the Korea Science and Engineering Foundation (R02-2001-000-00031-0 and R11-2000-080-01002-0) for J.-s Park, and the Creative Research Initiative Program of the Korean Ministry of Science and Technology and Brain Korea 21 Program of the Korean Ministry of Education for K. Kim.

LITERATURE CITED

(1) Jenekhe, S. A., and Chen, X. L. (1998) Self-assembled aggregates of rod-coil block copolymers and their solubilization and encapsulation of fullerenes. *Science 279*, 1903−1907.

(2) Ohkita, M., Lehn, J.-M., Baum, G.., and Fenske, D. (1999) Helicity coding: programmed molecular self-organization of achiral nonbiological strands into multiturn helical superstructures: synthesis and characterization of alternating pyridine-pyrimidine oligomers. *Chem. Eur. J. 5*, 3471−3481.

(3) Archut, A., Azzellini, G. C., Balzani, V., De Cola, L., and Vögtle, F. (1998) Toward photoswitchable dendritic hosts. Interaction between azobenzene-functionalized dendrimers and eosin. *J. Am. Chem. Soc. 120*, 12187−12191.

(4) Harada, A., and Kataoka, K. (1999) On−off control of enzymatic activity synchronizing with reversible formation of supramolecular assembly from enzyme and charged block copolymers. *J. Am. Chem. Soc. 121*, 9241−9242.

(5) Prins, L. J., Huskens, J., de Jong, F., Timmerman, P., Reinhoudt, D. N. (1999) Complete asymmetric induction of supramolecular chirality in a hydrogen-bonded assembly. *Nature 398*, 498−502.

(6) Haycock, R. A., Yartsev, A., Michelsen, U., Sundström, V., and Hunter, C. A. (2000) Self-assembly of pentameric porphyrin light-harvesting antennae complexes. *Angew. Chem., Int. Ed. 39*, 3616−3619.

(7) Isobe, H., Tomita, N., Lee, J. W., Kim, H.-J., Kim, K., and Nakamura, E. (2000) Ternary complexes between DNA, polyamine, and cucurbituril: A modular approach to DNA-binding molecules. *Angew. Chem., Int. Ed. 39*, 4257−4260.

(8) Gibson, H. W., Yamaguchi, N., Hamilton, L., and Jones, J. W. (2002) Cooperative self-assembly of dendrimers via pseudorotaxane formation from a homotritopic guest molecule and complementary monotopic host dendrons. *J. Am. Chem. Soc. 124*, 4653−4665.

(9) Nishikawa, M., and Huang, L. (2001) Nonviral vectors in the new millennium: Delivery barriers in gene transfer. *Hum. Gene Ther. 12*, 861−870.

(10) Wu, G. Y., Wilson, J. M., Shalaby, F., Grossman, M., Shafritz, D. A., and Wu, C. H. (1991) Receptor-mediated gene delivery in vivo. Partial correction of genetic analbuminemia in Nagase rats. *J. Biol. Chem. 266*, 14338−14342.

(11) Boussif, O., Lezoualc'h, F., Zanta, M. A., Mergny, M. D., Scherman, D., Demeneix, B., and Behr, J. (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: Polyethylenimine. *Proc. Natl. Acad. Sci. U.S.A. 92*, 7297−7301.

(12) Kukowska-Latallo, J. F., Bielinska, A. U., Johnson, J., Spindler, R., Tomalia, D. A., and Baker, J. R., Jr. (1996) Efficient transfer of genetic material into mammalian cells using Starburst polyamidoamine dendrimers. *Proc. Natl. Acad. Sci. U.S.A. 93*, 4897−4902.

(13) Lim, Y.-B., Kim, S.-M., Lee, Y., Lee, W.-K., Yang, T.-G., Lee, M.-J., Suh, H., and Park, J.-S. (2001) Cationic hyperbranched poly(amino ester): A novel class of DNA condensing molecule with cationic surface, biodegradable three-dimensional structure, and tertiary amine groups in the interior. *J. Am. Chem. Soc. 123*, 2460−2461.

(14) Eaton, M. A. W., Baker, T. S., Catterall, C. F., Crook, K., Macaulay, G. S., Mason, B., Norman, T. J., Parker, D., Perry, J. J. B., Taylor, R. J., Turner, A., and Weir, A. N. (2000) A new self-assembling system for targeted gene delivery. *Angew. Chem., Int. Ed. 39*, 4063−4067.

(15) Cartier, R.; Reszka, R. (2002) Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. *Gene Ther. 9*, 157−167.

(16) Wagner, E., Plank, C., Zatloukal, K., Cotton, M., and Birnstiel, M. L. (1992) Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: Toward a synthetic virus-like gene-transfer vehicle. *Proc. Natl. Acad. Sci. U.S.A. 89*, 7934−7938.

(17) Wu, G. Y.; Wu, C. H. (1988) Evidence for targeted gene delivery to Hep G2 hepatoma cells in vitro. *Biochemistry 27*, 887−892.
(18) Frankel, A. D., and Young, J. A. (1998) HIV-1: Fifteen proteins and an RNA. *Annu. Rev. Biochem. 67*, 1−25.
(19) Whang, D., Jeon, Y.-M., Heo, J., and Kim, K. (1996) Self-assembly of a polyrotaxane containing a cyclic "bead" in every structural unit in the solid state: Cucurbituril molecules threaded on a one-dimensional coordination polymer. *J. Am. Chem. Soc. 118*, 11333−11334.
(20) Jeon, Y.-M., Kim, J., Whang, D., and Kim, K. (1996) Molecular container assembly capable of controlling binding and release of its guest molecules: Reversible encapsulation of organic molecules in sodium ion complexed cucurbituril. *J. Am. Chem. Soc. 118*, 9790−9791.
(21) Lee, J. W., Ko, Y. H., Park, S.-H., Yamaguchi, K., and Kim, K. (2001) Novel pseudorotaxane-terminated dendrimers: Supramolecular modification of dendrimer periphery. *Angew. Chem., Int. Ed. 40*, 746−749.
(22) Tang, M. X., Redemann, C. T., and Szoka, F. C., Jr. (1996) In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconjugate Chem. 7*, 703−714.

BC025581R