pH-sensitive Assembly of Light-Harvesting Dendrimer Zinc Porphyrin Bearing Peripheral Groups of Primary Amine with Poly(ethylene glycol)-*b*-poly(aspartic acid) in Aqueous Solution

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Received October 13, 2002; Revised Manuscript Received December 17, 2002

ABSTRACT: The light-harvesting dendrimer zinc porphyrin $[NH_2CH_2CH_2NHCO]_{32}$DPZn, a potential photosensitizer in photodynamic therapy, was synthesized and successfully assembled with poly(ethylene glycol)-*b*-poly(aspartic acid) to form micelles in PBS buffer (10 mM) following the concept of polyion complex micelle. The coupling reaction between the $[CO_2H]_{32}$DPZn and *N*-trifluoroacetylethylene-1,2-diamine using the DCC/HOBt method afforded the protected dendrimer $[CF_3CONHCH_2CH_2NHCO]_{32}$DPZn, which was then mildly hydrolyzed to remove all the trifluoroacetyl protecting groups. Light-scattering measurements showed that the $[NH_2CH_2CH_2NHCO]_{32}$DPZn has the ability to assemble with poly(ethylene glycol)-*b*-poly(aspartic acid) to form a spherical micelle with a diameter of about 55 nm, having a narrow size polydispersity in PBS. The micelles were stable both in size and composition up to a 0.90 M (NaCl) salt concentration. The high salt stability is associated with a hydrogen-bonding network formed in the micellar core among the amide groups of the dendrimers and those of poly(aspartic acid) moieties, which could be destroyed by urea, a typical hydrogen-bonding cleaver. Furthermore, the pH range for the stable micelles in physiological saline (0.15 M NaCl) is from 6.2 to 7.4, suggesting the potential of the pH-triggered release of the entrapped dendrimers in the acidic pH environment (pH ∼ 5.0) of the intracellular endosomal compartment.

Introduction

Imparting specific functions to dendrimer is an attractive topic in materials science.[1] Due to the complicated preparation procedure, regardless of the convergent and divergent methods, only distinguished functions can give the dendrimer the ability to be further developed, compared with the conventional functional polymer and hyperbranched polymer.[2,3] As for functional materials, such as catalysts and biomaterials, dendritic structure exhibits at least two advantages. First, a dendrimer usually possesses the well-defined structure, because of the step-by-step synthesis, especially when the convergent method is employed. Second, the peripheral groups are spatially arranged in a three-dimensional structure, and the size is controlled by generation and/or dendron structures. To date, some distinctive features related to the dendritic structures have already been explored.[4,5] For example, polyamidoamine dendrimers appear to be highly efficient nonviral gene vectors with less cytotoxicity for in vitro transfection, the high-generation polycationic dendrimer is shown to be unusually effective at disrupting anionic vesicles (membrane bending model), and the poly(benzyl ether) dendrimers have an unuaual properties in viscosity and melt viscosity. Among functional dendrimers, dendrimer porphyrins exhibit the ability to transfer excitation energy from dendron to the porphyrin core.[6,7] These dendrimer porphyrins usually consist of a porphyrin core and Fréchet's aryl ether dendron,[8] where the periphery could be further modified to bear ester, carboxylic acid, or quaternary ammonium groups.

Photodynamic therapy (PDT) is a topical and promising method for the localized treatment of solid tumors, and an increasing number of the photosensitizers are presently being explored in preclinical and clinical studies, especially for increasing the selectivity or formulation of the photosensitizers.[9,10,11] On the basis of the concept of the enhanced permeability and retention (EPR) effect[12] regarding the accumulation of macromolecular compound in a solid tumor and possible tuning of the globular surface properties of the dendrimer porphyrin as a photosensitizer to form a supramolecular assembly with a biocompatible block copolymer, we investigated the PDT efficacy of ionic dendrimer porphyrins,[13] including $[Me_3N^+]_{32}$DPZn having 32 quaternary ammonium groups and $[CO_2H]_{32}$DPZn having 32 carboxylic groups on their peripheries, in which the former shows a remarkably higher $^1O_2$-induced cytotoxicity against LLC cells compared to the conventional porphyrin compound PIX (protoporphyrin IX).

The self-assembly of the amphiphilic block copolymers has recently received considerable attention due to their ability to form a well-defined nanostructure and potential applications as nanoreactors, drug and gene delivery devices, and structure directive templates.[14] In 1995, Kataoka et al. created a novel class of micelles that are

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10.1021/ma025735y CCC: $25.00
Published on Web 01/29/2003

formed through the electrostatic interaction between two oppositely charged segments,[15] known as polyion complex (PIC) micelles. These micelles can be formed by a charged block copolymer having PEG as one segment and an oppositely charged homopolymer,[16,17] DNA,[18] oligodeoxynucleotide,[19,20] or enzyme.[21] Recently, Stapert et al. successfully encapsulated the ionic dendrimer zinc porphyrins in the micellar core following the PIC concept.[22] Worth noticing is that the micelle from the pair of $[CO_2H]_{32}$DPZn/PEG-*b*-poly(L-lysine) shows an appreciably higher stability against the sodium chloride concentration beyond the physiological condition probably due to the formation of intermolecular hydrogen bonding in the core. No such salt stabilization was observed for the micelle from the pair of $[Me_3N^+]_{32}$DPZn/PEG-*b*-poly(α,*β*-aspartic acid) (PEG-*b*-poly(Asp)).

To confirm the formation and the role of the hydrogen bonding between the oppositely charged segments in the core, and to explore a new photosensitizer with a high efficacy for PDT, we synthesized a new dendrimer porphyrin bearing 32 primary amine groups on periphery ($[NH_2CH_2CH_2NHCO]_{32}$DPZn) and investigated its behavior in the assembly with PEG-*b*-poly(Asp) in aqueous solution.

Experimental Section

Materials. *β*-Benzyl L-aspartate (BLA) and bis(trichloromethyl) carbonate (triphosgene) were purchased from Tokyo Kasei Kogyo Co., Ltd. α-Methoxy-*ω*-amino poly(ethylene glycol) (M_w = 12 kg/mol) was a kind gift from Nippon Oil and Fats Co., Ltd. The polymer was precipitated in diethyl ether from chloroform, dried under reduced pressure and subsequently freeze-dried from benzene prior to use in the block copolymer synthesis. 3,5-Dihydroxybenzyl alcohol and methyl 4-(bromomethyl)-benzoate were purchased from Aldrich and used without further purification.

Synthesis of Poly(ethylene glycol)−Poly(α,*β*-aspartic acid) Block Copolymer. Poly(ethylene glycol)−poly(α,*β*-aspartic acid) block copolymer [PEG−P(Asp)] was prepared by a previously reported procedure.[15] Briefly, PEG−P(Asp) was synthesized by alkali hydrolysis of benzyl groups at the side chain of the poly(ethylene glycol)−poly(*β*-benzyl-L-aspartate) block copolymer (PEG−PBLA, M_w/M_n = 1.07), which was synthesized by the ring-opening polymerization of BLA−NCA initiated by the terminal primary amino group of α-methoxy-*ω*-amino poly(ethylene glycol) ($M_n = 1.219 \times 10^3$, DP = 275) under an argon atmosphere in dimethylformamide (DMF).[22] From the ^{1}H NMR spectrum in D_2O, the polymerization degree of poly(aspartic acid) segment was determined to be 28.

$[MeO_2C]_{32}$DPZn. Methyl ester $[MeO_2C]_{32}$DPZn was synthesized by a previously reported procedure.[6] Briefly, alkaline-mediated coupling of 5,10,15,20-tetrakis(3′,5′-dihydroxyphenyl) porphyrin with the methoxycarbonyl-terminated aryl ether dendritic bromide gave $[MeO_2C]_{32}PH_2$, which was metalated with $Zn(OAc)_2$ and gave the titled dendrimer.

$[H_2OC]_{32}$DPZn. $[MeO_2C]_{32}$DPZn was hydrolyzed with KOH in THF/water for 4 h and then in water for 24 h. The reaction mixture was poured into acetic acid, and the resulted precipitate was collected by centrifugation, washed with dilute aqueous HCl, and freeze-dried to give $[HO_2C]_{32}$DPZn, yield (76%). MALDI−TOF−MS (3-indolacrylic acid matrix): M/z = 8030 (calculatedd 8028). ^{1}H NMR (DMSO-d_6, 25 °C): δ = 8.81 (s, 8H, pyrrole-*β*), 7.81, 7.33 (both d, 128H, Ar−*o*-H, Ar−*p*-H), 7.48 (s, 8H, Ar−*o*-H),7.14 (s, 4H, Ar−*p*-H), 6.76 (d, 16H, Ar−*o*-H), 6.61 (d, 32H, Ar−*o*-H), 6.55 (s, 8H, Ar−*p*-H), 6.51 (s, 16H, Ar−*p*-H), 5.17 (s, 16H, OCH_2), 4.96 (s, 64H, OCH_2), 4.91 (s, 32H, OCH_2). IR ν_{max} (KBr pellet): 1695.60 cm^{-1}.

N-trifluoroacetyl-1, 2-ethylenediamine hydrochloride. Ethylenediamine (57 mmol) was added to cooled trifluoroacetic acid (30 mL) and the mixture was heated to 55 °C. A mixture of trifluoroacetic anhydride (47 mmol; 6.7 mL) and trifloroacetic acid (10 mL) was added dropwise with stirring over 2 h. After standing at room temperature for 4 h, the product was evaporated to dryness. The residue was taken up in water (30 mL), and treated with concentrated hydrochloric acid (50 mL). After being evaporated to dryness, the resulting solid was extracted with portions of 2-propanol (30 mL). The combined alcoholic extracts were evaporated and the residue was successively crystallized from 2-propanol and water, dried at 60 °C and under vacuum to give the titled compound, mp 115.0−117.0 °C, yield (62%). IR ν_{max} (KBr pellet)/cm^{-1}: 1712. ^{1}H NMR (D_2O, 25 °C): δ = 3.53−3.48 (t, 2H, −CONHCH_2−), 3.06−3.18 (t, 2H, $CH_2NH_3^+$).

Synthesis of $[CF_3CONHCH_2CH_2NHCO]_{32}$DPZn. Triethylamine ($2.28 \times 10^{-3}$ mol) was added to trifluoroacetyl-1,2-ethylenediamine hydrochloride (1.14×10^{-3} mol) in 3 mL of anhydrous DMF, stirred for 30 min at room temperature, and then filtered to remove the side product triethylamine hydrochloride. A mixture of $[CO_2H]_{32}$DPZn (2.54×10^{-6} mol), *N*-trifluoroacetylethylene-1,2-diamine (1.14×10^{-3} mol), DCC (4.88×10^{-4} mol), and HOBt (3.25×10^{-4} mol) in DMF (8 mL) was stirred at room temperature under Ar for 7d. The solution was dialyzed against water, then dried in a vacuum and purified by column chromatography gradually eluting with increasing methanol to 20% methanol/$CHCl_3$ to give $[CF_3CONHCH_2CH_2NHCO]_{32}$DPZn, yield (32%). MALDI−TOF−MS (dithranol matrix): M/z = 12 448.6 (calculated 12448.3). ^{1}H NMR (DMSO-d_6, 25 °C): δ = 9.48 (s, 32H, NHCOCF_3), 8.88 (s, 8H, pyrrole-*β*), 8.57 (s, 32H, NHCO), 7.81, 7.33 (both d, 128H, Ar−*o*-H, Ar−*p*-H), 7.48 (s, 8H, Ar−*o*-H),7.14 (s, 4H, Ar−*p*-H), 6.76 (d, 16H, Ar−*o*-H), 6.61 (d, 32H, Ar−*o*-H), 6.55 (s, 8H, Ar−*p*-H), 6.51 (s, 16H, Ar−*p*-H), 5.17 (s, 16H, OCH_2), 4.96 (s, 64H, OCH_2), 4.91 (s, 32H, OCH_2). IR ν_{max} (KBr pellet): 1717.3 cm^{-1} (CF_3CONH−), 1630.0 cm^{-1} (−CONH−).

Synthesis of $[NH_2CH_2CH_2NHCO]_{32}$DPZn. The resulting dendrimer $[CF_3CONHCH_2CH_2NHCO]_{32}$DPZn was dissolved in 13 mL of methanol (105 mg of K_2CO_3 and 0.9 mL of water), and refluxed for 3 h, dialyzed, and lyophilized to provide the title dendrimer, yield (53%). ^{1}H NMR (DMSO-d_6, 25 °C): δ = 8.81 (s, 8H, pyrrole-*β*), 8.50 (s, 32H, NHCO), 7.81, 7.33 (both d, 128H, Ar−*o*-H, Ar−*p*-H), 7.48 (s, 8H, Ar−*o*-H),7.14 (s, 4H, Ar−*p*-H), 6.76 (d, 16H, Ar−*o*-H), 6.61 (d, 32H, Ar−*o*-H), 6.55 (s, 8H, Ar−*p*-H), 6.51 (s, 16H, Ar−*p*-H), 5.17 (s, 16H, OCH_2), 4.96 (s, 64H, OCH_2), 4.91 (s, 32H, OCH_2). IR ν_{max} (KBr pellet): 1630.0 cm^{-1} (−CONH−).

Preparation of PIC Micelles. The given amounts of $[NH_2CH_2CH_2NHCO]_{32}$DPZn and the PEG-*b*-poly(Asp) were separately dissolved in NaH_2PO_4 (10 mM, pH 3.0 by adding 0.01 M HCl) and Na_2HPO_4 (10 mM) solution to prepare the stock solutions and then mixed at a stoichiometric ratio, followed by dialysis against 10 mM PBS until the pH of the micelle solution was 7.4.

Methods. GPC was performed on a Superose 6HR 10/30 column (Pharmacia Biotech, Sweden) using 10 mM PBS (NaCl, 150 mM) as eluent; flow rate = 0.3 mL/min, monitored by UV (220 nm) and fluorescence (λ_{ex} = 432 nm, λ_{em} = 605 nm). The ^{1}H NMR spectra were obtained in DMSO-d_6 on a JEOL type GSX-270 spectrometer operating at 270 MHz. Matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI−TOF−MS) was performed on an Applied Biosystems model Voyager-DE STR Tof mass spectrometer using 3-indolacrylic acid or dithranol as matrix.

Light-Scattering Measurements. The light-scattering measurements were performed on a Photal dynamic laser scattering DLS-7000DL spectrometer (Otsuka Electronics Co., Ltd.) equipped with an argon laser (λ_0 = 488 nm). All the micelle samples were aged for 24 h before the measurement.

During the dynamic light-scattering measurements, the autocorrelation function, g(τ), was analyzed using the cumulant method in which

$$g(\tau) = \exp[-\Gamma\tau + (\mu_2/2)\tau^2 - (\mu_3/3!)\tau^3 + ...] \qquad (1)$$

yielding an average characteristic line width Γ. The *z*-averaged

diffusion coefficient was obtained from Γ using the following equations:

$$\Gamma = Dq^2 \tag{2}$$

$$q = (4\pi n/\lambda)\sin(\theta/2) \tag{3}$$

Here q is the magnitude of the scattering vector, n is the refractive index of the solvent, and θ is the detection angle. The hydrodynamic diameter, d_h, can be calculated using the Stokes−Einstein equation:

$$d_h = k_B T/(3\pi\eta D) \tag{4}$$

Here k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

For the static light-scattering measurements, the light scattered by a dilute polymer solution may be expressed as

$$Kc/\Delta R(\theta) = 1/M_{w,app}[1 + q^2 R_g{}^2/3] + 2A_2 c \tag{5}$$

$$K = (4\pi^2 n^2 (dn/dc)^2)/(N_A \lambda^4) \tag{6}$$

where $\Delta R(\theta)$ is the difference between the Rayleigh ratio of the solution and that of the solvent, $M_{w,app}$ is the apparent weight-average molecular weight, A_2 is the second virial coefficient, $R_g{}^2$ is the mean square radius of gyration, c is the concentration of the solution, N_A is Avogadro's number, and dn/dc is the refractive index increment with concentration.

The specific refractive index increment (dn/dc) of the dendrimer polyion complex solutions was determined using a DRM-1020 (Otsuka Electronics Co., Ltd.) double beam refractor meter.

Results and Discussion

Dendrimer Synthesis. The dendrimer porphyrin (Figure 1; $[NH_2CH_2CH_2NHCO]_{32}$DPZn) was synthesized by the amidation of the carboxylic acid groups of $[CO_2H]_{32}$DPZn with $NH_2CH_2CH_2NHCOCF_3$, followed by deprotection of the trifluoroacetyl group. Aida et al. have reported the synthesis of $[CO_2H]_{32}$DPZn by an alkaline-mediated coupling of 5,10,15,20-tetrakis(3′,5′-dihydroxyphenyl) porphyrin with the methoxycarbonyl-terminated aryl ether dendritic bromide.[6] Despite many literature methods for the monoprotection of symmetric diamines,[23,24] the monoselective protection of ethylenediamine is seldom mentioned and the most utilized is the Boc-protected one.[9] Taking into account that zinc porphyrin may be demetallized under acidic condition, which, however, is necessary for the deprotection of Boc groups, we designed an alternative method to achieve much milder deprotection process. Referring to the method of the mono-*N*-trifluoroacetyl derivative of 1,4-butylenediamine,[25,26] the modified procedure successfully provided the corresponding desired *N*-trifloroacetylethylenediamine hydrochloride in acceptable yield (62%). The amidation of the peripheral carboxylic acid groups of the dendrimer with the monoprotected ethylenediamine was effective by using the conventional DCC/HOBt catalyst in peptide synthesis. With the reaction monitored by TLC, we found that the degree of amidation did not increase after 7 days. The purification of $[CF_3CONHCH_2CH_2NH]_{32}$DPZn was easily carried out by subject to SiO_2 gel column chromatography with increasing methanol content in chloroform as eluent. The deprotection progressed in a rather mild condition by refluxing in methanol in the presence of K_2CO_3 (MeOH:water = 1:12 in volume). The structure of the resulting dendrimer porphyrin $[CF_3CONHCH_2CH_2NH]_{32}$DPZn was confirmed by ^{1}H NMR (Figure 2).

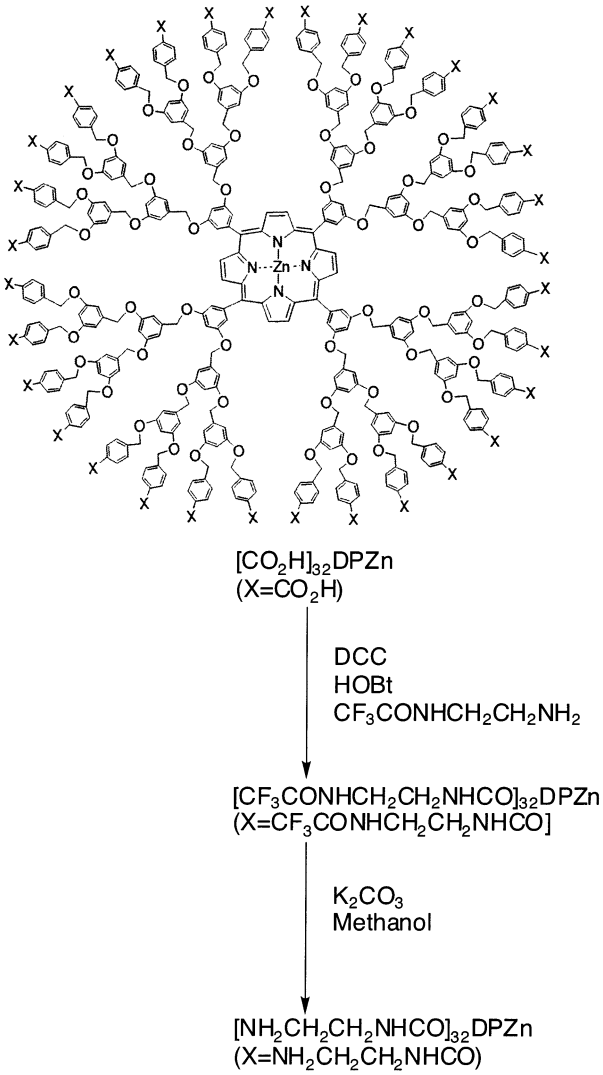

Figure 1. Chemical structure of related porphyrin dendrimers and the synthesis route.

Upon amidation of the peripheral carboxylic acid groups, two signals corresponding to the −NH− group of −ArCONH− and CF_3CONH− group newly appeared at 8.57 and 9.48 ppm, respectively. The ratios of their protons to that of Ar−*o*-H are consistent with the calculated values. Furthermore, the FT-IR and MALDI−TOF−MS spectra also supported the above NMR results. On the other hand, when the reaction was refluxed in water−methanol in the presence of K_2CO_3, the proton signal at 9.48 ppm due to CF_3CONH− groups disappeared completely, indicating a quantitative deprotection of trifluoroacetyl groups.

Preparation of Dendrimer-Entrapped Polyion Complex Micelle. Figure 3 shows the GPC profiles of the micelle detected by fluorescence detector (a) and by UV detector (b), and the copolymer PEG-*b*-P(Asp) by UV detector (c). Clearly, the GPC profiles of the micelles show a single, narrow, and symmetrical peak at 24.53 min, suggesting the formation of micelles with narrow size distribution. Under the same conditions, the peak from the PEG-*b*-P(Asp) appears at 44.07 min.

To further verify the characteristics of these micelles, we investigated the size, the size distribution, and the angular dependence of these micelles at different temperatures and salt concentrations by DLS: 25 °C, 0 mM NaCl; 25 °C, 150 mM NaCl; 37 °C, 150 mM NaCl

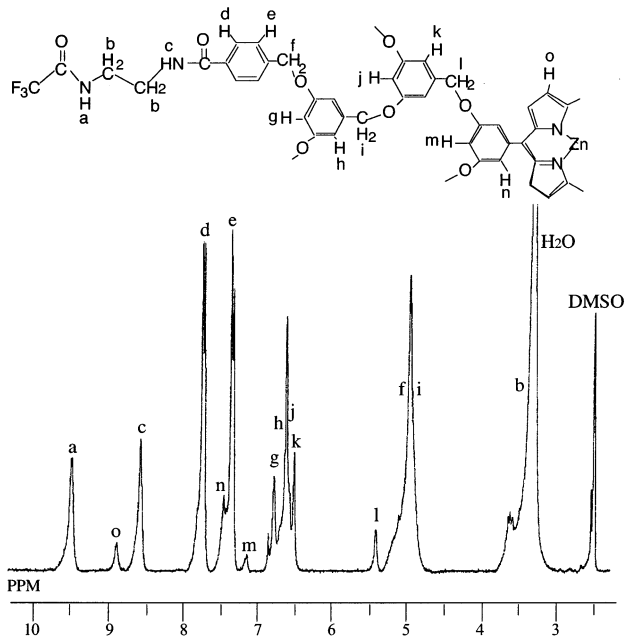

Figure 2. ^{1}H NMR spectrum of the porphyrin dendrimer $[CF_3CONHCH_2CH_2NHCO]_{32}$DPZn in DMSO-$d_6$.

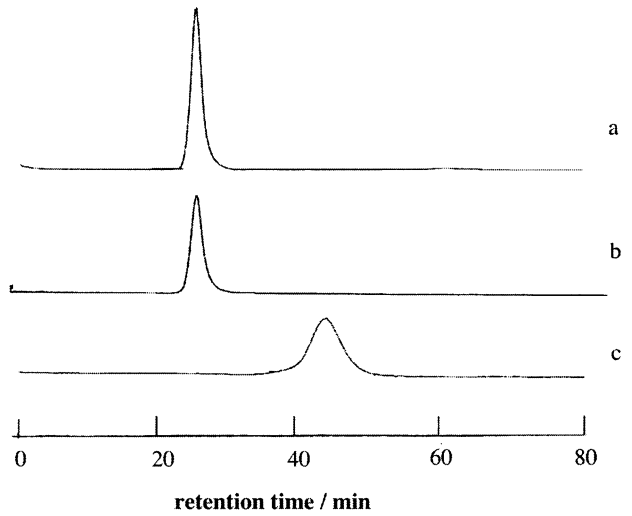

Figure 3. GPC profiles of $[NH_2CH_2CH_2NHCO]_{32}$DPZn/PEG-*b*-P(Asp) dendrimer polyion complex system (a, fluorescence detector; b, UV detector) and PEG-*b*-P(Asp) alone (c, UV detector).

(similar to the physiological condition). The histograms of Γ-size distribution are shown in Figure 4, and the related data are summarized in Table 1. The polydispersity indices (μ_2/Γ^2) were all below 0.09, suggesting that these micelles are essentially narrowly dispersed without any sign for the formation of larger aggregates (clusters of micelles). Figure 5 shows the angular dependence of the diffusion coefficient (D_T). The plots revealed that the Γ/q^2 values were independent of the scattering vector, suggesting that all these micelles may have a spherical shape, even at 37 °C in 150 mM NaCl. The Γ/q^2 values of the spherical particles have been reported to be independent of the scattering vector (detection angle), resulting from the undetectable rotational motion.[27] Thus, it is safe to conclude that these polyion complexes of dendrimer porphyrins with PEG-*b*-P(Asp) are narrowly dispersed spherical micelles, with the ionic dendrimer porphyrin in the core surrounded by PEG chains as a corona.

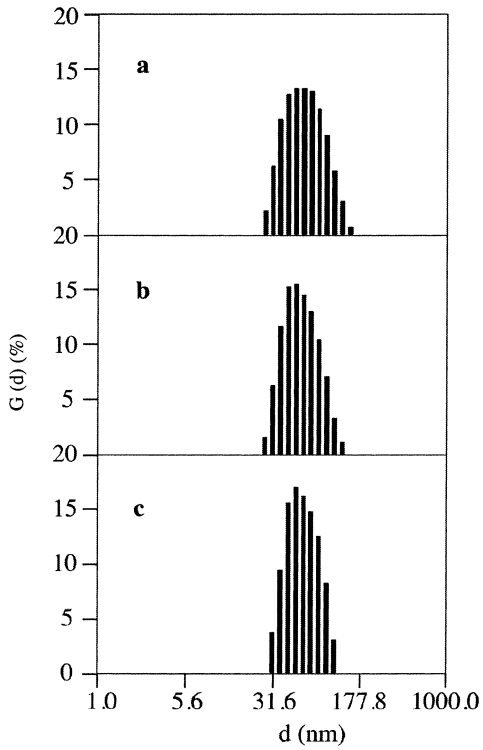

Figure 4. DLS histograms of $[NH_2CH_2CH_2NHCO]_{32}$DPZn/PEG-*b*-P(Asp): (a) 25 °C, 0 mM NaCl; (b) 25 °C, 150 mM NaCl; (c) 37 °C, 150 mM NaCl. Detection angle 90°.

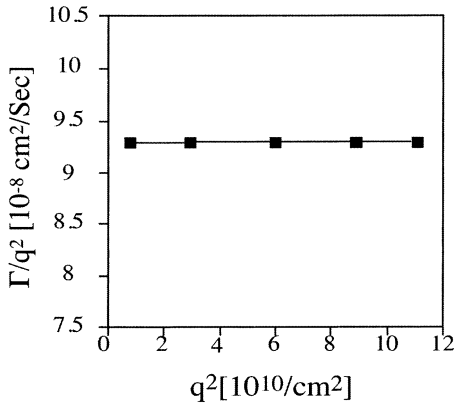

Figure 5. Dependence of diffusion coefficient on detection angle. Detection angles: 30, 60, 90, 120, and 150°: Micelle conentration: 2.93 mg/mL, 150 mM NaCl, 37 °C.

Table 1. Translational Diffusion Coefficient, Hydrodynamic Diameter, and Size Distribution of PEG-*b*-P(Asp)/Dendrimer Micelle

	D_T (cm^2/s)	d_h (nm)	μ_2/Γ^2
25 °C, 0 mM NaCl	9.30E−8	53.8	0.087
25 °C, 150 mM NaCl	9.05E−8	55.2	0.077
37 °C, 150 mM NaCl	1.16E−7	56.0	0.063

Stability of Dendrimer-Entrapped Polyion Complex Micelles in Salt Solution. The effects of the added salt on the stability of the polyion complex micelles were investigated by DLS and SLS. Figure 6 shows the change in the translational diffusion coefficient (D_T) and normalized $(KC/\Delta R(0))^{-1}$ (normalized to the micelle at 0 mM NaCl), where D_T is related to the hydrodynamic micellar size based on the Stokes−Einstein equation and normalized $(KC/\Delta R(0))^{-1}$ is related to the change in average apparent molecular weight of the micelles. Before the salt concentration

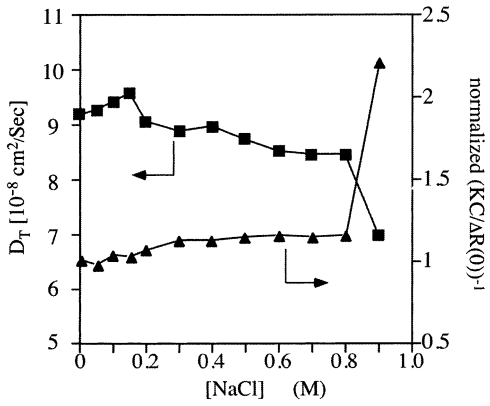

Figure 6. Effect of salt concentration on the translational diffusion coefficients D_T (square, detection angle 90°) and normalized $KC/\Delta R(0)^{-1}$ (triangle) for $[NH_2CH_2CH_2NHCO]_{32}$DPZn/PEG-*b*-P(Asp), $T = 25$ °C, micelle concentration: 2.93 mg/mL (pH 7.4, 10 mM PBS).

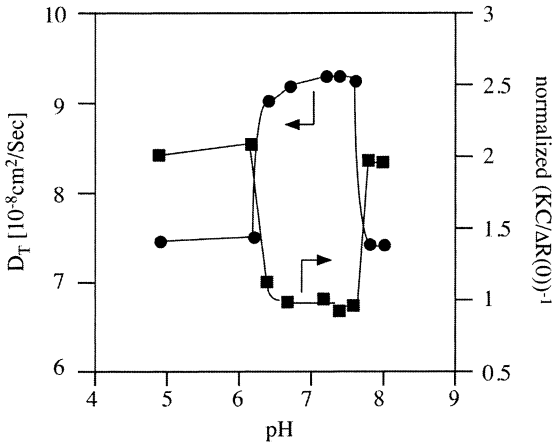

Figure 7. Effect of pH on the translational diffusion coefficients D_T (circle, detection angle 90°) and normalized $KC/\Delta R(0)^{-1}$ (square) for $[NH_2CH_2CH_2NHCO]_{32}$DPZn/PEG-*b*-P(Asp), $T = 25$ °C. Micelle concentration: 2.93 mg/mL.

reached 0.90 M, both parameters were basically constant, although the size did change slightly. At the salt concentration of 0.90 M, the hydrodynamic micellar size and the normalized $(KC/\Delta R(0))^{-1}$ sharply increased, and then precipitation occurred if the salt concentration was elevated to 1.0 M. These results demonstrated that the PIC micelle has an unusual high stability against salt (NaCl), compared with conventional PIC micelles,[28,29] for example, PEG-*b*-P(Lys)/oligonuelectide, and PEG-*b*-P(Asp)/lysozyme, which are destabilized at a low salt concentration of 0.2 M due to electrostatic shielding. Possibly, there should be other noncovalent interactions between the dendrimer and P(Asp) in the core besides the electrostatic interactions, and the network consisting of hydrogen bonding is a reasonable explanation as reported in our previous work.[22] Although some water molecules may be encapsulated in the core,[30] it is still suggested that the microphase of the core should be hydrophobic,[31] which is necessary for hydrogen bonding to exist in aqueous solution.[32] The assembly driven by hydrogen-bonding interactions in the hydrophobic microphase in aqueous solution has been intensively studied by Kawasaki et al. and also extensively exists in nature.[32,33] Furthermore, to confirm the existence of a hydrogen-bonding network and the predominant role to keep the micelle stable, urea was added to the micelle solution (NaCl 0.3 M, Urea 0.2 M). Immediately, the micelle was destabilized and formed a precipitate. On the other hand, a control sample (in 0.5 M NaCl without urea) still maintained the corresponding stability even after 1 month. As for the groups involved in the formation of the hydrogen bonding, Ismail et al. have studied the polyion complex of poly(glutamic acid) and poly(lysine) using IR and CD spectroscopy, indicating the formation of extensive hydrogen bonding among the backbone amide groups, induced by the salt bridge formation between the oppositely charged side chains.[34] Thus, it is postulated that the hydrogen-bonding network mainly results from the backbone amide groups of poly(Asp) and those of the dendrimers. Another possibility is the formation of hydrogen bonds between amine groups of the dendrimer periphery and carboxylic acid groups of the poly(Asp) segment of the copolymer, resulting from proton-transfer at higher salt concentration.[22] In addition, Muthukumar et al. also point out the important role of hydrogen bonding to keep the dendrimer−polyelectrolyte complex driven by electrostatic interaction, according to the simulation studies.[35]

pH-Dependent Behavior of Dendrimer-Entrapped Polyion Complex Micelles in Water. The pH-dependent behavior of the dendrimer-entrapped polyion complex micelles in 0.15 M NaCl is shown in Figure 7. Both the hydrodynamic size and normalized $(KC/\Delta R(0))^{-1}$ (normalized to the micelle at pH 7.4 and 150 mM NaCl) basically remained unchanged in the pH range from 6.2 to 7.4, in which the micelles had the size of ca. 55 nm with narrow distribution ($\mu_2/\Gamma^2 < 0.09$). When the pH was outside the above range from 6.2 to 7.4, either from 5.0 to 6.2 or from 7.6 to 8.2, the diameters of micelles increased to ca. 90 nm with higher polydispersity indices ($\mu_2/\Gamma^2 > 0.2$) and an increased normalized $(KC/\Delta R(0))^{-1}$. When the pH is higher than 6.2, the salt bridge between the carboxylic acid of the side chain of poly(Asp) and the primary amine of the dendrimer appears to be predominant, together with the hydrogen-bonding network mentioned above, leading to the stable core−shell micelle with a compact core. At a pH below 6.2, a certain amount of the poly(Asp) appears to be protonized, because the pK_a of PEG-*b*-poly(Asp) (salt free) is about 5.12 and undoubtedly should increase in the 0.15 M salt solution; thus, the polymer linear charge density diminishes. On the other hand, the surplus cationic charges appear on the surface of the dendrimer, in which the globular macroions certainly repulse each other. Thus, the well-defined core−shell structure may become more diffuse in the sense that a broad core−shell transition develops,[30] resulting in a merging of the micelles. Thus, these structure changes of PIC micelles at a lower pH may cause the variations in both parameters of Figure 7. The analogous system, the poly(ethylene glycol)-*b*-poly(Lys) complexes with letinoic acid, exhibit the similar behavior,[30] where the particle sizes significantly increase within a small range of variation of the pH from 6.0 to 5.5. Moreover, the behavior of the micelle at a pH above 7.4 is related to the deprotonation of the dendrimer porphyrin, though its pK_a is not known at present, yet the turbidity of dendrimer solution appeared at pH 6.9 (salt free). To support the above mechanismic interpretations, the measurement of pK_a of $[NH_2CH_2CH_2NHCO]_{32}$DPZn and acid−base titration of the corresponding micelles will be the upcoming research topic.

Importantly, when pH is lower than 6.2, the micelles adopt a substable state (less stable state). Thus, upon accumulating on a solid tumor, where the local pH

should be significantly lower than that of normal tissue,[36,37] or entering the acidic intracellular endosomal compartment (~pH 5.0), the substable structure may be easily broken down in such complex circumstances, due to a variety of charged biopolymers, releasing the encapsulated dendrimer porphyrin and providing a unique photosensitizing effect.

Conclusions

We have described the synthesis of a new dendrimer porphyrin $[NH_2CH_2CH_2NHCO]_{32}$DPZn bearing 32 primary amine groups on the periphery. The stoichiometric mixing of the dendrimer porphyrin $[NH_2CH_2CH_2NHCO]_{32}$DPZn and PEG-*b*-P(Asp) in solutions spontaneously formed water-soluble PIC micelles, driven by electrostatic interactions. The resulting micelle is spherical, with a diameter of ca. 55 nm and narrow size distribution (unimodal, $\mu_2/\Gamma^2 < 0.09$). Interestingly, we found that these spherical micelles have two features. First, the micelles possess extraordinary stability in high salt (NaCl) concentrations (up to 0.90 M). Second, there is a strict pH-dependent stability of the micelles, that is, they can remain stable in the solutions with a pH ranged from 6.2 to 7.4, whereas a sharp structural transition takes place if the pH is outside this region. These two features are related to the globular structure of the dendrimer porphyrin and the unique interactions between the rigid macroion and the poly(aspartic acid) segment of the block copolymer. Overall, both the high stability under physiological conditions (pH 7.4, 0.15 M NaCl) and destabilization under acid circumstances suggest the high potential of the present micelle encapsulated dendrimer porphyrins as tumor environment-sensitive delivery systems of photosensitizers for PDT.

Acknowledgment. The authors acknowledge the Core Research for Evolutional Science and Technology (CREST) by JST. G.-D.Z. also acknowledges financial support from the Japan Society for the Promotion of Science (JSPS).

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MA025735Y