Structural Determination and Interior Polarity of Self-Aggregates Prepared from Deoxycholic Acid-Modified Chitosan in Water

Kuen Yong Lee and Won Ho Jo

Department of Fiber and Polymer Science, Seoul National University, Seoul 151-742, Korea

Ick Chan Kwon, Yong-Hee Kim, and Seo Young Jeong*

Biomedical Research Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea

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ABSTRACT: Hydrophobically modified chitosan derivatives containing 0.6−5.1 deoxycholic acid groups per 100 anhydroglucose units of chitosan were synthesized by an EDC-mediated coupling reaction. Self-aggregates of the chitosan derivatives in aqueous media were formed by sonication with a probe-type sonifier. The mean diameter of self-aggregates determined by dynamic light scattering decreased slightly with an increasing degree of substitution (DS) by hydrophobic groups. Photophysical and photochemical characteristics of self-aggregates were examined by fluorescence probe techniques. Measurement of the binding equilibrium constant (K_v) for pyrene and lifetime (τ) for 1,6-diphenyl-1,3,5-hexatriene (DPH) showed that the interior of self-aggregates became nonpolar as the DS was increased. Microviscosity of the interior of self-aggregates determined by measuring an anisotropy value for DPH was not significantly affected by the DS of hydrophobic groups. The aggregation number of deoxycholic acid groups per one hydrophobic microdomain (n_{DCA}) was estimated by the steady-state fluorescence quenching method with 1-dodecylpyridinium chloride and was almost independent of the DS. From the results of the fluorescence quenching experiment, it was conjectured that there may be multiple (rather than one) hydrophobic microdomains in a self-aggregate.

Introduction

Recently a number of studies have focused on the formation of artificial self-assemblies in aqueous media. The artificial self-assemblies act as host systems for many hydrophobic molecules.[1−3] Owing to preference for the formation of free energy-minimized structure, low-molecular-weight amphiphiles such as surfactants or lipids spontaneously form micelles or vesicles in aqueous media. Since the formation of self-assemblies from polymeric amphiphiles resembles that of low-molecular-weight amphiphiles, polymeric amphiphiles form micelles consisting of the inner core of hydrophobic segments and the outer shell of hydrophilic segments.[4,5] Solution properties of hydrophobically associating polymers in aqueous media have been represented by unusual rheological features arising from the inter- and/or intramolecular interaction among hydrophobic groups.[6,7] Self-assemblies of block copolymer micelles[8−10] or self-aggregates of hydrophobically modified polymers[11,12] have been investigated with respect to theoretical approaches of their formation[13,14] or their biotechnological and pharmaceutical applications.[15−17]

Though various polymeric amphiphiles have been synthesized and their intra- and/or intermolecular associating behavior has been extensively studied, there are a few reports on self-assemblies of naturally occurring polymers and their derivatives. Sunamoto and his co-workers[18−22] have intensively studied cholesterol-bearing pullulans, which formed monodisperse self-aggregates by intra- and/or intermolecular association in a dilute aqueous solution. They reported that the cholesterol groups associated each other to provide noncovalent cross-linking points to form a hydrogel nanoparticle which had a polycore structure.[22] This microscopic structure increased the thermal stability of the proteins that were complexed with self-aggregates.[20,21]

Chitosan, next to cellulose, is the second most plentiful biomass and has a repeated structure of (1,4)-linked 2-amino-2-deoxy-β-D-glucan (Figure 1a). Since chitosan is already known as a biocompatible, biodegradable, and low-toxicity material, there have been many biomedical applications.[23−25] In this paper, chitosan was hydrophobically modified by deoxycholic acid, a main component of bile acid (Figure 1b), to yield self-aggregates in aqueous media. Bile acid is the main product of cholesterol metabolism and biologically the most detergent-like molecules in the body.[26] Since bile acid can self-associate in water and form micelles, it is expected that the chitosan modified by bile acid also self-associates to form self-aggregates. In this context, we report here the effect of degree of substitution (DS) of deoxycholic acid moieties on the formation and the interior structure of self-aggregates prepared from the hydrophobically modified chitosans.

Experimental Section

Materials. Chitosan with biomedical-grade purity (M_v = 7.0×10^4; degree of deacetylation, 80%) was supplied from Samchully Pharm. Co., Seoul, Korea. Deoxycholic acid with >99% purity and 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC) were purchased from Sigma Co. Pyrene, 1,6-diphenyl-1,3,5-hexatriene (DPH), and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) as fluorescence probes were purchased from Aldrich Co. and used without further purification. 1-Dodecylpyridinium chloride (DPC) was purchased from Aldrich Co. and purified by double recrystallization from absolute ethanol. The water was purified by distillation, deionization, and reverse osmosis (MilliQ Plus).

* To whom correspondence should be addressed: (tel) +82-2-958-5911; (fax) +82-2-958-5909; (e-mail) syjeong@kistmail.kist.re.kr.

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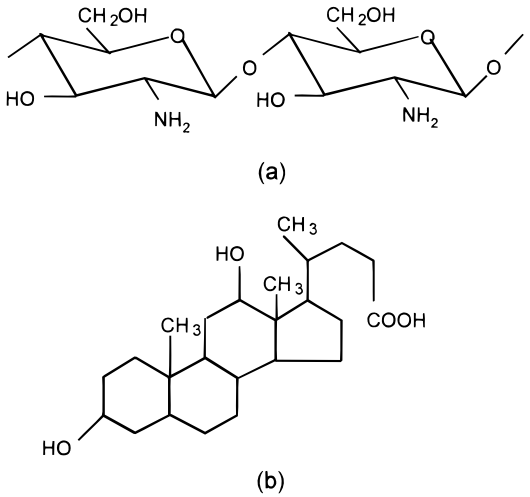

Figure 1. Chemical structure of (a) chitosan and (b) deoxycholic acid.

Synthesis of Chitosan Derivatives. Deoxycholic acid was coupled to chitosan by an EDC-mediated reaction through the formation of amide linkages.[27,28] Chitosan (1 g) was dissolved in a 1% aqueous acetic acid solution (100 mL) and diluted with 100 mL of methanol. Different amounts of deoxycholic acid per glucosamine residues of chitosan (0.09−0.34, mol/mol) were added to the chitosan solution and followed by the dropwise addition of EDC under stirring at room temperature. The mole ratio of EDC per deoxycholic acid used in this study was kept constant. After 24 h, the reaction mixture was poured into 200 mL of methanol/ammonia solution (7/3, v/v). The precipitated chitosan was filtered off and washed thoroughly with distilled water, methanol, and ether, followed by drying in a vacuum at room temperature. The degree of substitution (DS), defined as the number of deoxycholic acid groups per 100 anhydroglucose units of chitosan, was determined by elemental analysis. The DS of deoxycholic acid-modified chitosan (DC) was in the range of 0.6−5.1. The number behind the sample code DC in Table 1 indicates the DS of the sample. The increase of the amide I band at 1655 cm^{-1} in the FTIR spectra of the modified chitosans confirmed the formation of amide linkages between amino groups of chitosan and carboxyl groups of deoxycholic acid.

Sample Preparation. Hydrophobically modified chitosan was suspended in phosphate-buffered saline (PBS) solution (pH 7.2) under gentle shaking at 37 °C for 48 h, followed by sonication using a probe-type sonifier (Sigma Ultrasonic Processor, GEX-600) at 30 W for 2 min. The sonication was repeated 3 times to obtain an optically clear solution. To prevent a sample solution from the heat buildup during sonication, the pulse function was used (pulse on, 5.0 s; pulse off, 1.0 s). The solution of self-aggregates was filtered through a 0.45-μm filter (Millipore) and stored at room temperature.

Stock solutions of pyrene (3.0×10^{-2} M) or DPH (2.1×10^{-3} M) were prepared in tetrahydrofuran (THF) and stored in a refrigerator. For the measurement of steady-state fluorescence spectra, the pyrene solution in THF was added to the PBS solution to give a pyrene concentration of 12.0×10^{-7} M and then the solution was distilled under vacuum at 60 °C for 1 h to remove THF from the solution. The THF-free pyrene solution was mixed together with a solution of self-aggregates to give concentrations of polymeric amphiphiles from 2.5×10^{-4} to 1.0 mg/mL. The final concentration of pyrene in a sample solution was 6.0×10^{-7} M, which is nearly equal to the solubility in water at 22 °C.[29] Sample solutions for the measurement of fluorescence anisotropy and lifetime were prepared by adding a stock solution (2 μL) of DPH to a solution of polymeric amphiphiles (2 mL). The solution was degassed by gentle bubbling of nitrogen gas for 10 min.

Dynamic Light Scattering (DLS) Measurements. Dynamic light scattering experiments were performed with an argon ion laser system (Lexel Laser model 95) tuned at a wavelength of 488 nm. The sample solution was filtered through a 0.45-μm filter (Millipore) directly into the precleaned 10-mm-diameter cylindrical cell. The intensity autocorrelation was measured at scattering angle (θ) of 90° with a Brookhaven BI-9000AT digital autocorrelator at 25 ± 0.1 °C. When the difference between the measured and the calculated baselines was less than 0.1%, the correlation function was accepted. A nonlinear regularized inverse Laplace transformation technique (CONTIN)[30] was used to obtain the distribution of decay constant. Mean diameter (d) was evaluated by the Stokes−Einstein equation.

Fluorescence Measurement and Data Analysis. Steady-state fluorescence spectra were recorded on an ISS K2 multifrequency phase and modulation fluorometer (ISS, Champaign, IL). Samples were excited using a 300-W xenon arc lamp (ILC Technology, Sunnyvale, CA). For the measurement of the intensity ratio in the excitation spectra of pyrene, the slit openings for excitation and emission were set at 1.0 and 2.0 mm, respectively. The emission wavelength (λ_{em}) was 390 nm. For the measurement of the emission intensity of pyrene, the slit openings for excitation and emission were set at 1.0 and 0.5 mm, respectively. The excitation wavelength (λ_{ex}) was 336 nm, and the spectra were accumulated with an integration time of 5 s/nm.

The aggregation number of deoxycholic acid groups was estimated by the steady-state fluorescence quenching method. DPC was used as a fluorescence quencher for quenching of pyrene fluorescence. The steady-state quenching data do not fit in simple Stern−Volmer kinetics, but fit in quenching kinetics:[11,31]

$$\ln(I_0/I) = [Q]/[M] \tag{1}$$

where I_0 and I are the fluorescence emission intensity in the absence and presence of a quencher, respectively, [Q] is the concentration of the quencher, and [M] is the concentration of hydrophobic microdomains in self-aggregates. Thus, the aggregation number of deoxycholic acid groups per one hydrophobic microdomain (n_{DCA}) can be calculated by eq 2.

$$n_{DCA} = [\text{deoxycholic acid}]/[M] \tag{2}$$

Fluorescence lifetimes for DPH were measured using an ISS K2 fluorometer equipped with a frequency synthesizer (Marconi Instruments, Alendale, NJ) and an ISS-ADC interface for data collection and analysis. The phase shifts and modulation ratios were determined at 10 different modulation frequencies, logarithmically spaced (2.0, 3.3, 5.6, 9.3, 15.5, 25.8, 43.1, 71.9, 119.9, and 200.0 MHz) and corrected for the reference fluorescence lifetime of POPOP/ethanol, 1.35 ns.[32] The fluorescence was measured at 25 °C through a 408-nm cut-on filter. Fluorescence lifetimes were determined using a nonlinear least-squares program (ISS187) from ISS which minimized the reduced χ^2 (square deviations between the observed and the calculated values for phase shifts and modulation ratios) for a goodness-of-fit.

Fluorescence anisotropy of a sample solution containing DPH (2.1×10^{-6} M) was measured by using L-format geometry of detection. Fluorescence anisotropy (r) was calculated from the following relationship:

$$r = \frac{(I_{VV} - I_{VV}^{s}) - G(I_{VH} - I_{VH}^{s})}{(I_{VV} - I_{VV}^{s}) + 2G(I_{VH} - I_{VH}^{s})} \tag{3}$$

where I^s is the contribution of scattered light from a sample solution in the absence of DPH, G ($= I_{VH}/I_{HH}$) is an instrumental correction factor, and I_{VV}, I_{VH}, I_{HV}, and I_{HH} refer to the resultant emission intensity polarized in the vertical or the horizontal detection planes (second subindex) when excited with vertically or horizontally polarized light (first subindex).[33] The excitation wavelength was 360 nm, and the emission was measured at 430 nm.

Table 1. Properties of Self-Aggregates in PBS Solution (pH 7.2)

sample	DS[a]	x[b]	d[c] (nm)	cac[d] $\times 10^2$ (mg/mL)	K_v[e] $\times 10^{-5}$	r[f]
DC2.8	2.8	0.061	180	4.47	0.16	0.300 ± 0.005
DC4.2	4.2	0.089	159	3.02	1.41	0.312 ± 0.005
DC5.1	5.1	0.106	161	1.32	5.20	0.314 ± 0.003

[a] Degree of substitution of deoxycholic acid per 100 anhydroglucose units of chitosan. [b] Weight fraction of deoxycholic acid. [c] Mean diameter measured by dynamic light scattering. [d] Critical aggregation concentration determined from I_{336}/I_{333} data. [e] Binding equilibrium constant of pyrene calculated from eqs 5 and 6. [f] Fluorescence anisotropy value of DPH calculated from eq 3.

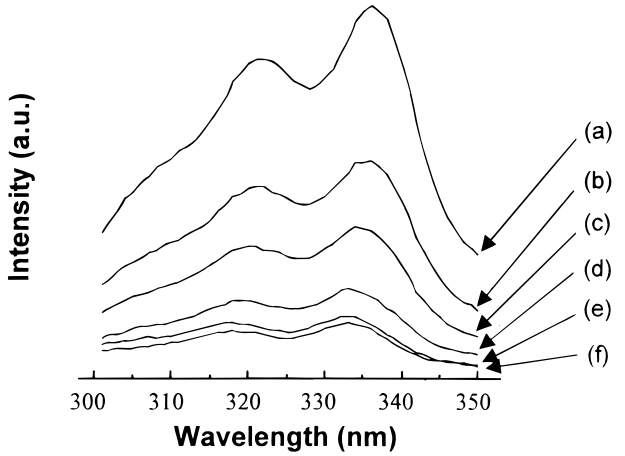

Figure 2. Excitation spectra of pyrene (6.0×10^{-7} M) in PBS solution in the presence of DC5.1. [DC5.1] = (a) 1, (b) 0.5, (c) 0.25, (d) 0.1, (e) 0.01, and (f) 0.001 mg/mL. The emission wavelength was 390 nm, and the spectra were accumulated with an integration time of 5 s/nm.

Results and Discussion

Formation of Self-Aggregates. In general, the formation of micelles or aggregates from polymeric amphiphiles in aqueous media resembles that of low-molecular-weight amphiphiles.[4,5] Deoxycholic acid-modified chitosan forms self-aggregates in aqueous media whose mean diameter (d) is less than 180 nm, depending on the degree of substitution of hydrophobic groups (Table 1). Laplace inversion of the autocorrelation function indicates an unimodal size distribution. Since the unmodified chitosan does not form self-aggregates, it cannot be used as a control. Fluorescence excitation spectra of DC5.1 at various concentrations in the presence of 6.0×10^{-7} M pyrene are shown in Figure 2. At low concentrations of polymeric amphiphiles, changes in the total fluorescence intensity and in the shift of the (0,0) band at 333 nm in PBS solution are negligible. As the concentrations of polymeric amphiphiles increase, however, the increase of the total fluorescence intensity and the red shift of (0,0) band are clearly observed. Since pyrene in a polar environment shows only a small fluorescence intensity, the increase of the total fluorescence intensity with the addition of DC5.1 indicates that the probe is transferred from aqueous media to the less polar microdomains such as the interior of self-aggregates. The (0,0) band for pyrene at 333 nm in PBS solution shifts to 336 nm by the addition of DC5.1.

Critical aggregation concentration (cac), which is the threshold concentration of self-aggregate formation by intra- and/or intermolecular association, can be determined from the change of intensity ratio (I_{336}/I_{333}) of pyrene in the presence of polymeric amphiphiles. Figure 3 shows the intensity ratio (I_{336}/I_{333}) of DC5.1 and deoxycholic acid in PBS solution as a function of amphiphile concentrations. The cac values, determined from the crossover point at low concentrations, are listed in Table 1. The cac values of deoxycholic acid-modified chitosans are lower than the critical micelle concentration (cmc) of low-molecular-weight surfactants, e.g., 2.3 mg/mL for sodium dodecyl sulfate (SDS) in water[34] and 1.0 mg/mL for deoxycholic acid in water.[35] The lower cac values of the modified chitosans as compared with low-molecular-weight surfactants may be one of the important characteristics of polymeric amphiphiles; i.e., a small amount of the chitosan derivatives can form self-aggregates and maintain the stability in dilute condition. The increasing hydrophobicity of the chitosan derivatives by introduction of large amount of deoxycholic acid moieties further reduces the cac values.

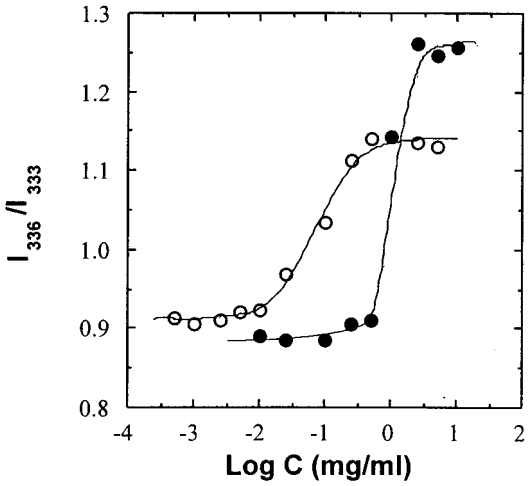

Figure 3. Change of intensity ratio (I_{336}/I_{333}) from excitation spectra of pyrene (6.0×10^{-7} M) with various concentrations of DC5.1 (○) and deoxycholic acid (●) in PBS solution.

Binding Equilibrium of Pyrene. The curves in Figure 3 show a sigmoidal shape when the intensity ratio is semilogarithmically plotted against concentrations of amphiphiles. Above the critical aggregation concentration, the increase in signal due to the binding of pyrene becomes larger than the random error in determining the intensity of the unbound component. This suggests that pyrene interacts with individual polymeric amphiphiles prior to self-aggregation, followed by partitioning into the inner core of the self-aggregates. Assuming a simple partition equilibrium, the equilibrium constant (K_v) for partitioning of pyrene between the water and micellar phases can be calculated according to the method of Wilhelm et al.[29]

$$[\mathrm{Py}]_m/[\mathrm{Py}]_w = K_v V_m/V_w \quad (4)$$

where $[\mathrm{Py}]_m/[\mathrm{Py}]_w$ is the ratio of pyrene in micellar phase to the water phase and V_m and V_w are the volumes of micellar and water phase, respectively. Equation 4 can be written as

$$[\mathrm{Py}]_m/[\mathrm{Py}]_w = K_v xc/1000\rho \quad (5)$$

where x is the weight fraction of deoxycholic acid in polymeric amphiphiles and ρ is the density of the inner core of self-aggregates. ρ can be assumed as the same value of deoxycholic acid in water (= 1.31 g/mL).[26] In the intermediate concentration range of polymeric am-

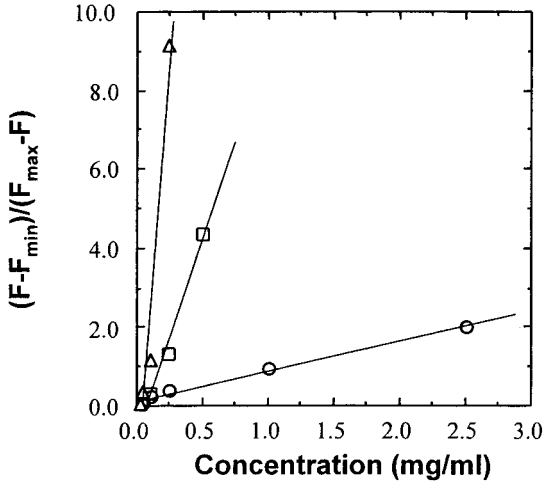

Figure 4. Plots of $(F - F_{min})/(F_{max} - F)$ vs concentration of the hydrophobically modified chitosan in PBS solution. ○, DC2.8; □, DC4.2; △, DC5.1. Solid lines indicate the best fit to the data according to eqs 5 and 6.

phiphiles, $[Py]_m/[Py]_w$ can be obtained from the excitation spectra of pyrene as given by

$$\frac{[Py]_m}{[Py]_w} = \frac{F - F_{min}}{F_{max} - F} \tag{6}$$

where F is the intensity ratio ($= I_{336}/I_{333}$) at the intermediate concentration range of polymeric amphiphiles and F_{min} and F_{max} are the intensity ratios at low concentration and at high concentration, respectively, in Figure 3. Combining eq 5 and eq 6, a plot of $(F - F_{min})/(F_{max} - F)$ versus concentration (c) gives K_v of the pyrene which bound to the inner core of self-aggregates (Figure 4). The K_v value increases with increasing DS of the hydrophobic groups (Table 1), indicating that less polar microdomains may exist in the inner core of self-aggregates. In cases of SDS micelles and poly(styrene−ethylene oxide) (PS−PEO) block copolymer micelles, K_v values were reported as 1.2×10^5 and 3.0×10^5 (dimensionless), respectively.[36] Since the magnitude of K_v of the modified chitosans is larger than the magnitude of K_v of SDS micelles or PS−PEO diblock copolymer micelles, it may be concluded that larger amount of pyrene is partitioned into the inner core of self-aggregates of the modified chitosans. This means that nonpolar hydrophobic microdomains are formed because of the hydrophobic association between deoxycholic acid moieties.

Lifetime Measurements. Though K_v value is a measure of polarity of the interior of self-aggregates, it does not provide site-specific information unless the location of pyrene is known.[37] Site-specific information of polarity can be obtained from the measurement of lifetime. Typical phase and modulation data at various frequencies are plotted in Figure 5. The frequency range and the number of frequencies used in this study are determined according to the complexity of the emission process and the precision of the resolved lifetime.[38] The phase (τ_p) and modulation (τ_m) lifetimes are calculated by

$$\tau_p = \frac{1}{\omega}\tan \phi \tag{7}$$

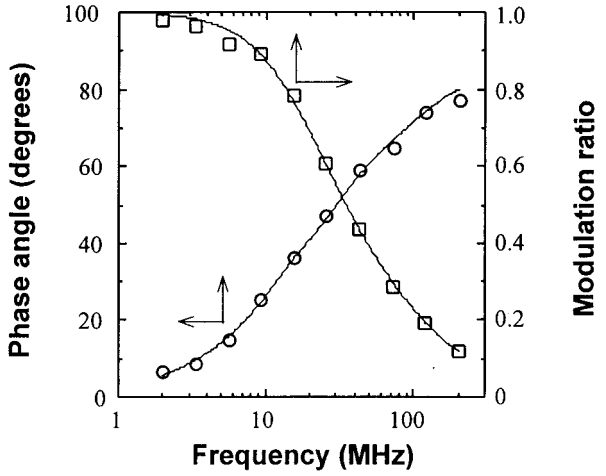

Figure 5. Frequency-dependent phase (○) and modulation (□) data for DPH in PBS solution (pH 7.2) in the presence of DC5.1. Solid lines indicate the best fit to the data ([DC5.1] = 2 mg/mL; [DPH] = 2.1×10^{-6} M; T = 25 °C).

$$\tau_m = \frac{1}{\omega}\left(\frac{1}{M^2} - 1\right)^{1/2} \tag{8}$$

where ω is the circular frequency ($= 2\pi \times$ modulation frequency in hertz), ϕ is the phase angle (degrees), and M is the modulation ratio. The calculated τ_p and τ_m values are listed in Table 2. As expected, the τ_p value at any given modulation frequency is always less than the τ_m value and both τ_p and τ_m values decrease with increasing the modulation frequency.

For a goodness-of-fit (small reduced χ^2), the measured phase shift and modulation factors are analyzed in terms of a discrete biexponential function. More complex functions are not adequate for our system. Lifetime data for DPH in the presence of the hydrophobically modified chitosans are listed in Table 3. Self-aggregates may have two different microdomains, and the longer lifetime value arises from less polar microdomain. Thus, the increase of average lifetime data with increasing the DS also indicates the existence of less polar microdomains in the inner core of self-aggregates. These results are consistent with the K_v values in Table 1.

Microviscosity of the Inner Core of Self-Aggregates. Microviscosity, the viscosity of the inner core of self-assemblies, can be determined by measuring the molecular anisotropy as a result of molecular rotational diffusion. Because of the restricted rotational motion of DPH, the anisotropy increases with increasing microviscosity of the microenvironment where DPH lies. The anisotropy values (r) of deoxycholic acid-modified chitosans are listed in Table 1. There are few differences of anisotropy in the microenvironment of self-aggregates formed in PBS solution (pH 7.2) because the rigidity of the inner core of self-aggregates is not significantly affected by the DS. But, the anisotropy values of the modified chitosans are somewhat larger than the values for SDS (0.073), poly(ethylene-*co*-maleic acid) (0.187), and poly(1-octadecene-*co*-maleic acid) (0.273).[37] This may indicate that a quite rigid inner core of chitosan self-aggregates is formed in PBS solution.

Aggregation Number of Self-Aggregates. To estimate the aggregation number and to identify the microscopic structure of self-aggregates, a fluorescence quenching method was used. This method has been applied to determine the aggregation number of micelles

Table 2. Lifetime Parameters for DPH in PBS Solution (pH 7.2) in the Presence of DC5.1

frequency (MHz)	phase (deg)	modulation ratio	τ_p (ns)	τ_m (ns)
2.0	6.61	0.977	9.222	17.329
3.3	8.86	0.964	7.812	13.086
5.6	15.14	0.918	7.731	12.318
9.3	25.26	0.890	7.557	8.796
15.5	36.07	0.781	7.489	8.216
25.8	47.41	0.605	6.703	8.111
43.1	59.16	0.436	6.186	7.630
71.9	64.87	0.286	4.720	7.416
119.9	74.32	0.193	4.269	6.748
200.0	77.37	0.121	3.551	6.528

Table 3. Lifetime Data for DPH in PBS Solution (pH 7.2) in the Presence of Hydrophobically Modified Chitosans

sample	τ_1 (ns)	f_1[a]	τ_2 (ns)	f_2	χ^2[b]	$\langle\tau\rangle$[c]
DC2.8	1.00	0.15	6.91	0.85	2.70	6.02
DC4.2	1.48	0.20	8.43	0.80	3.68	7.04
DC5.1	1.93	0.12	8.76	0.88	3.24	7.94

[a] Fractional intensity (f_i) = $\alpha_i\tau_i/\sum\alpha_i\tau_i$ and α_i is the pre-exponential factor. [b] Square deviations between the observed and the calculated values for phase shifts and modulation ratios. [c] Average lifetime ($\langle\tau\rangle$) = $f_1\tau_1 + f_2\tau_2$.

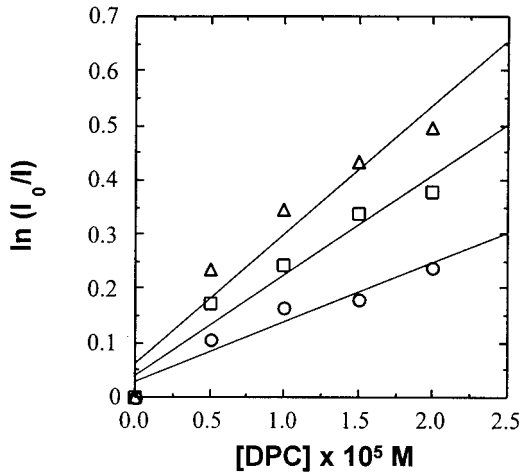

Figure 6. Plots of $\ln(I_0/I)$ of pyrene fluorescence vs DPC concentration in the presence of various concentrations of DC5.1 in PBS solution (pH 7.2). ○, 1; □, 0.75; △, 0.5 mg/mL. Solid lines indicate the best fit to the data according to eq 1.

consisting of low-molecular-weight surfactants[39] or polymeric amphiphiles.[11] Figure 6 shows the plot of $\ln(I_0/I)$ of pyrene fluorescence versus [DPC] in the presence of various concentrations of DC5.1. From the slope of eq 1, we can obtain the concentration of hydrophobic microdomains in self-aggregates. Hence, the aggregation number of deoxycholic acid groups per one hydrophobic microdomain (n_{DCA}) can be calculated from eq 2 and the n_{DCA} values are listed in Table 4. The n_{DCA} value is not significantly affected by the DS and is almost constant at 3.5. The aggregation number of sodium deoxycholate is known as 8 ± 2.[35,40] A small aggregation number in the case of self-aggregates is considered to result from the steric hindrance of deoxycholic acid moieties attached to the chitosan backbone. Considering the number of deoxycholic acid groups in one polymer chain, it may be concluded that there exist several hydrophobic microdomains in a self-aggregate. For example, since DC5.1 carries 21 deoxycholic acid moieties per one polymer chain, one polymer chain of DC5.1 may form at least six independent hydrophobic microdomains in the interior of a self-aggregate. The

Table 4. Results from Quenching Experiments of Pyrene Fluorescence[a] by DPC[b] in the Presence of Self-Aggregates

sample	n_{DCA}[c]	n_{chain}[d]	n_{domain}[e]
DC2.8	3.4 ± 0.2	0.28	3.4
DC4.2	3.4 ± 0.2	0.19	5.1
DC5.1	3.5 ± 0.5	0.16	6.0

[a] [pyrene] = 6.0×10^{-6} M. [b] 1-Dodecylpyridinium chloride. [c] Aggregation number of deoxycholic acid groups per one hydrophobic microdomain. [d] Number of polymer chains required to form one hydrophobic microdomain. [e] Number of hydrophobic microdomains that may be formed by one polymer chain.

multiple hydrophobic microdomains in a self-aggregate were suggested by Sunamoto and his co-workers as a polycore structure.[22] Therefore, the increase of equilibrium binding constant (K_v) for pyrene and of lifetime (τ) for DPH is considered to result from the formation of a large number of hydrophobic microdomains in a self-aggregate with increasing DS.

Conclusions

Chitosan, hydrophobically modified by deoxycholic acid, forms colloidally stable self-aggregates in water above the critical aggregation concentration. The structural determination of self-aggregates and their internal polarity are investigated by fluorescence spectroscopy. The interior polarity of the self-aggregates becomes nonpolar with increasing DS of the hydrophobic groups. It may be concluded that there exist several hydrophobic microdomains in a self-aggregate. Since self-aggregates have positive charges on the outer shell, they can be used as a delivery carrier for DNA, a negatively charged polyelectrolyte.

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