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Amine terminated G-6 PAMAM dendrimer and its interaction with DNA probed by Hoechst 33258

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Abstract

Fluorescence characteristics of Hoechst 33258 bound to G-6 dendrimer, to the DNA–G-6 dendrimer complex, and to DNA were compared with that in an aqueous solution. The spectral properties including fluorescence emission spectrum, accessibility of anionic quencher, as well as the fluorescence decay time of the Hoechst 33258 are different for all three conditions, indicating that the environments in these conditions are different. Close analysis of the fluorescence properties led us to suggest that Hoechst 33258 located at or near the contact area of the dendrimer and DNA in the DNA–G-6 complex. In the complex, in the absence of Hoechst 33258, the shape of the circular dichroism in the DNA absorption region remained, indicating that DNA is in B form in the complex. On the other hand, the magnitude of linear dichroism (LD) decreased upon DNA–G-6 dendrimer complex formation. The decrease in LD magnitude reflects the shortening of the DNA contour length, which is expected from the fact that a large part of linear DNA is required to wrap the surface of G-6 dendrimer.

Keywords: Dendrimer; DNA; Hoechst; Polyplex; Fluorescence

1. Introduction

Dendrimers are highly symmetric spherical polymers. They possess many interesting physico-chemical and biological properties, including high uniformity and high loading capacity for a wide range of molecules, and low toxicity and immunogenicity. Such properties of dendrimers provide a wide range of biological applications. One of the most important possibilities for the usage of dendrimer is as a gene carrier [1–10]. For this possibility, the interaction of various dendrimers and DNA has been widely studied [11–15]. It has been known that DNA condenses or assembles on the surface of various dendrimers by the electrostatic interaction between them.

Although dendrimers are spectroscopically inactive in the UV/vis region, investigations on the physical properties of dendrimers using chemical probes, namely, phenol blue, various azo dyes, and ethidium have been reported in order to understand the mechanism of interaction between dendrimers and DNA [16–18]. Amine-terminated polyamidoamine dendrimers were shown to form a tight, nonpolar association with phenol blue within the dendrimer's interior, probably near its core [17]. On the other hand, azo dyes were shown to aggregate on the surface of dendrimers. Both J-(side-by-side orientations of the aromatic rings) and H-aggregations (face-to-face aggregations of the aromatic rings) were observed depending on the chemical structure of azo compounds [18]. Ethidium bromide exhibited mainly one binding mode for the dendrimer–DNA complex and the dendrimer DNA complex is sufficiently stable that they cannot be displaced with ethidium [16].

Hoechst 33258 is a bis-benzimidazole derivative (Fig. 1) that has been known to bind preferentially at the minor groove of double helical B-DNA at 5 contiguous AT bases, resulting in a significant enhancement of its fluorescence intensity. Its interaction with DNA has been extensively studied by various methods, including X-ray techniques [19–25]. Substantial enhancement of its fluorescence upon binding to DNA, that due to the change of its polar to a non-polar micro-environment and due to the conformation of the dye itself, made this

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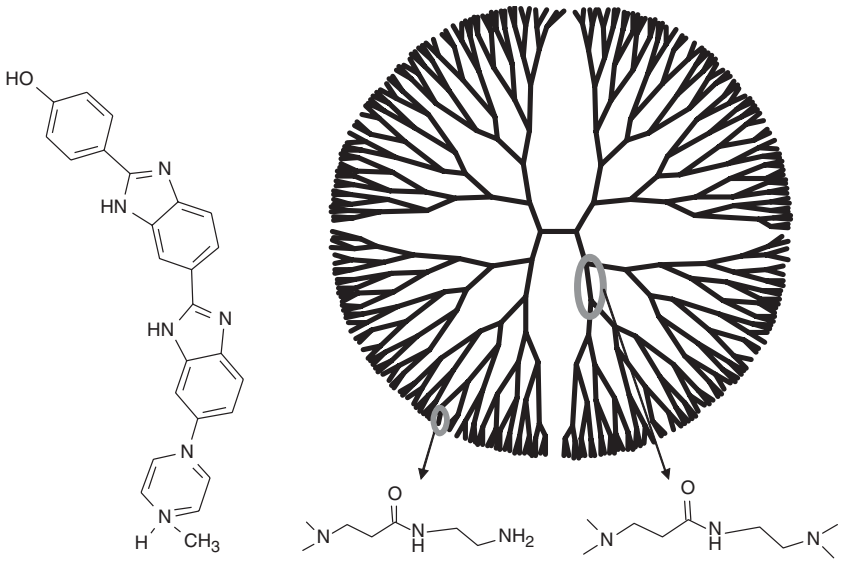

Fig. 1. Molecular structure of G-6 dendrimer and Hoechst 33258.

compound a useful probe for DNA study. In this study, using Hoechst 33258 as a probe, we characterize the physico-chemical property of the generation 6 amine terminated polyamidoamine dendrimer (referred to as G-6 dendrimer, Fig. 1), whose inside consists of tertiary amines and outside consists of primary amines, and its complex formed with calf thymus DNA (referred to as DNA), using various fluorescence techniques and polarized spectroscopy.

2. Materials and methods

2.1. Materials

Calf thymus DNA and Hoechst 33258 were purchased from Sigma. DNA was dissolved in a pH 7.0, 5 mM cacodylate buffer containing 100 mM NaCl and 1 mM EDTA by exhaustive stirring at 4 °C. Dissolved DNA was dialyzed several round against 5 mM cacodylate buffer pH 7.0 at 4 °C. This buffer was used in this work. The concentration of DNA and Hoechst 33258 were determined using extinction coefficients $\varepsilon_{260\ nm}$= 6700 cm^{-1} M^{-1} and $\varepsilon_{338\ nm}$=42000 cm^{-1} M^{-1}, respectively. Generation 6 dendrimer was purchased from Aldrich (5 wt.%, in methanol) and used without further purification. The concentration of G-6 dendrimer in this work is in the unit of macro molecule, i.e., 1 μM G-6 dendrimer is equivalent to 256 μM terminal primary amines.

2.2. Instruments

The absorption spectrum was recorded on a Jasco V550 spectrophotometer and the steady state fluorescence on a Perkin Elmer LS50B spectrofluorometer. The slit widths for both excitation and emission were 3 nm. Fluorescence decay was measured on an IBH 5000U Fluorescence Life Time system. For the time-resolved fluorescence measurement, DNA- and G-6 dendrimer bound Hoechst 33258 was excited using the nano-LED 03 source, which provides the excitation beam at 370 nm. Slit widths for excitation and emission were 8 and 12 nm, respectively, for fluorescence decay time measurement. CD and LD spectra were recorded on a Jasco J715 spectropolarimeter.

2.3. Linear dichroism

For the LD measurements, the sample was placed in the inner-rotating Couette cell and was oriented by flow. The magnitude of LD is affected by two factors, namely orientation and optical factors [26,27]. When the optical factor remains constant, it is the orientation factor that depends on the contour length and flexibility of DNA, the flow rate, and the temperature and viscosity of the sample affects the LD magnitude. The orientation factor ranges from 0, for a randomly oriented sample, to a theoretical maximum of +1 for a perfectly aligned sample. In this work, the flow rate, temperature and viscosity of the sample were not changed, therefore, the only factor that LD magnitude reflects is the contour length and flexibility of DNA. Although a calibration is required to use of a CD instrument for LD measurement [28], it would not be essential for the qualitative measurement on the orientation factor, i.e., change in the relative LD magnitude at a fixed wavelength (260 nm).

2.4. Fluorescence quenching

The fluorescence intensity of Hoechst 33258 decreases upon the addition of NaI. If the fluorescence quenching occurs through either a simple dynamic process or a static mechanism, the ratio of the fluorescence intensity in the absence of quencher (F) to its presence (F_0) is expected to be a straight line in the Stern–Volmer plot [29].

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where [Q] denotes quencher concentration, the [NaI] in the current case, and the constant K_{SV} represents the accessibility of

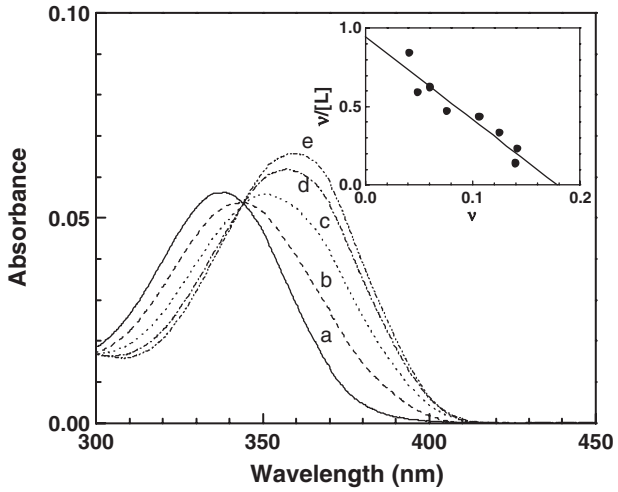

Fig. 2. Representative absorption spectra of Hoechst 33258 in the presence of various concentrations of G-6 dendrimer. [Hoechst 33258]=1.4 μM. a: 0 μM, b: 2.75 μM, c: 5.48 μM, d: 10.87 μM and e: 26.54 μM dendrimer. Insertion: Scatchard plot for the association of Hoechst 33258 to G-6 dendrimer.

the quencher to the fluorophore. If there are two populations of fluorophore, one being accessible to quenchers and the other being inaccessible, the Stern–Volmer plot displays a downward bending curve. In this case, the Stern–Volmer equation can be easily modified to

$$\frac{F_0}{F_0-F}=\frac{1}{f_a K_a[Q]}+\frac{1}{f_a}$$

where f_a is the fraction of the initial fluorescence which is accessible to quencher and the constant K_a is a measure of the accessibility.

3. Results

3.1. Association of Hoechst 33258 with G-6 dendrimer

The absorption spectrum of Hoechst 33258 above 300 nm in the presence of various G-6 dendrimer concentration is shown in Fig. 2. In the absence of dendrimer, Hoechst 33258 exhibited an absorption maximum at 337 nm, which started to decrease with increasing G-6 dendrimer concentration. A new band with its maximum at 360 nm appeared. The absorbance of the G-6 dendrimer-bound Hoechst 33258 increased by 12.3% compared to that in the absence of dendrimer. In addition to the red-shift and hyperchromism, a clear isosbestic point at 346 nm was observed, suggesting that the changes occurred between two states, namely bound and free Hoechst 33258. It can be further suggested that the environment of all bound Hoechst 33258 is identical. Therefore, it is easy to estimate the concentrations of dendrimer-bound and -free Hoechst 33258. Assuming independent, identical binding sites of G-6 dendrimer for Hoechst 33258, the Scatchard plot can be reduced to

$$\frac{v}{[L]}=\frac{n}{k}-\frac{v}{k}$$

where v is the moles of bound ligand per mole of macromolecule, $[L]$ is the concentration of the free ligand, and k is the dissociation constant [30]. From the slope, the association constant can be calculated. As it is shown in the insertion of Fig. 2, a straight line in the Scatchard plot was observed, suggesting that the Hoechst 33258 binding sites are identical and independent. The association constant was calculated as $1.9\times10^5\ M^{-1}$ with the γ_{max} value of 0.18.

Upon binding to G-6 dendrimer, the fluorescence intensity of Hoechst 33258 significantly increased (Fig. 3a) although the shape of the emission spectrum with its maximum at 475 nm is almost identical in the presence and in the absence of dendrimer. The fluorescence intensity as well as absorbance at 375 nm reached the maximum near the dendrimer concentration of

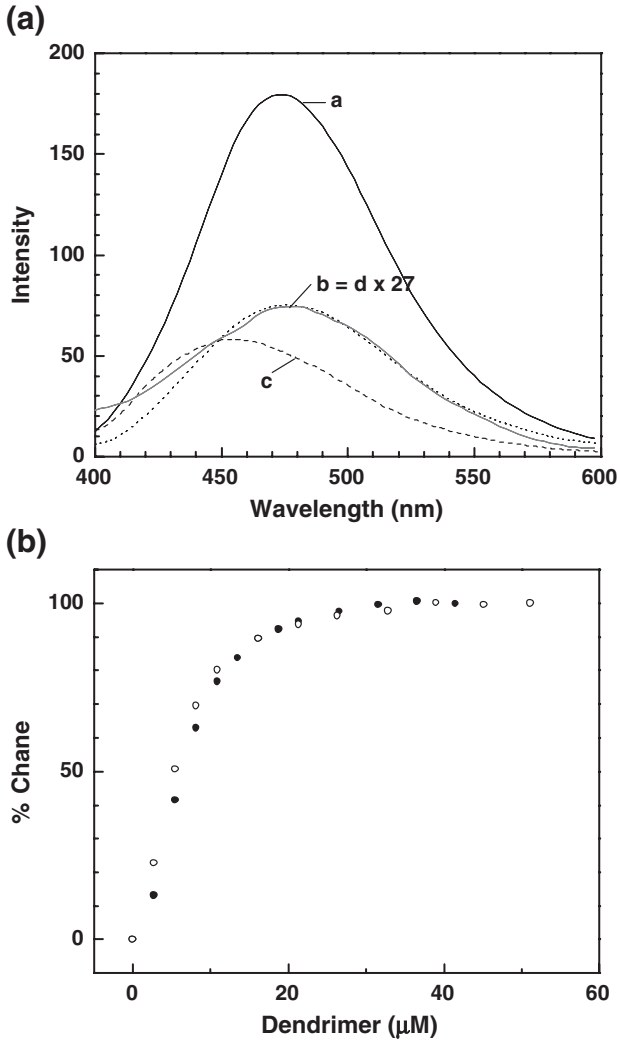

Fig. 3. (a) Fluorescence emission spectrum of Hoechst 33258 in the presence of G-6 dendrimer (curve b: dotted curve, ex: 367 nm), DNA (curve a: solid curve, ex: 360 nm) and DNA–G-6 dendrimer complex (curve c: dashed curve, 354 nm). That of Hoechst 33258 in the absence of DNA and dendrimer is enlarged by 27 times and compared (curve d, solid curve, 331 nm). [Hoechst 33258]=1.4 μM, [DNA]=100 μM, and [dendrimer]=31.6 μM. The sample was excited at the corresponding excitation maximum which is denoted in the parenthesis). (b) Change in the fluorescence intensity (closed circles, ex: 367 nm, em: 475 nm) and absorbance (opened circles, at 375 nm) of Hoechst 33258 with increasing G-6 concentration.

Table 1
Fluorescence decay times of Hoechst 33258 in the presence of DNA, G-6 dendrimer and DNA–G-6 dendrimer complex

	Decay times and amplitudes			
Hoechst	a_1=0.55	τ_1=0.40	a_2=0.45	τ_2=3.88
Hoechst+G-6 dendrimer	a_1=0.26	τ_1=0.80	a_2=0.74	τ_2=2.32
Hoechst+DNA+G-6 dendrimer	a_1=0.58	τ_1=1.09	a_2=0.42	τ_2=2.08

[Hoechst 33258]=1.4 μM, [G-6 dendrimer]=41.5 μM and [DNA]=100 μM.

30 μM (Fig. 3b), which corresponds to one Hoechst molecule per 21.4 molecules of dendrimer or ~ 5500 molecules of surface amine. Upon binding to G-6 dendrimer, fluorescence decay time also changed (Table 1, Fig. 4). In the absence of dendrimer, Hoechst 33258 exhibited two decay components of 0.40 and 3.88 ns with their relative amplitude of 0.55 and 0.45, respectively, which is in agreement with reported values [31–34]. In the presence of G-6 dendrimer, two decay times of 0.80 and 2.32 ns with their relative amplitudes of 0.26 and 0.74, respectively, were observed.

3.2. Complex formation between G-6 dendrimer and DNA

G-6 dendrimer neither absorbs radiation nor fluorescence in the UV-visible region. However, DNA exhibits a bisignate CD spectrum and a negative LD signal at the wavelength below 300 nm and this signal may provide some indirect information on the DNA–G-6 dendrimer interaction. The CD spectrum of DNA with increasing dendrimer concentration is depicted in Fig. 5, reflecting a change in the secondary structure of DNA. Although some decreases in both the positive and negative bands are noticed, DNA remains in its B form even when it forms a complex with dendrimer. The change in the CD intensity at 276 nm upon DNA and G-6 dendrimer complex formation is inserted in Fig. 4a. As the concentration of dendrimer increased, the CD intensity proportionally decreased up to the dendrimer concentration of ~0.15 μM. This concentration corresponds to 666 DNA bases per dendrimer molecule or per 2.6 surface amines. Formation of the complex between DNA and G-6 dendrimer can be monitored more clearly by LD. Upon complexation, the magnitude of LD decreased almost proportionally with G-6 dendrimer concentration. Above a dendrimer concentration of ~0.15 μM, the LD signal completely disappeared. Amongst two factors, namely orientation and optical factors, that affect the magnitude of LD, decrease in orientability is the cause of the decreasing LD of the DNA–G-6 dendrimer complex because the optical factor was constant in our system.

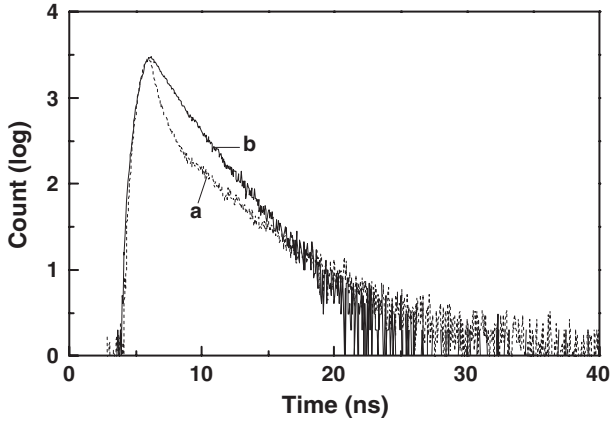

Fig. 4. Fluorescence decay profile of Hoechst 33258 in the presence and absence of G-6 dendrimer.

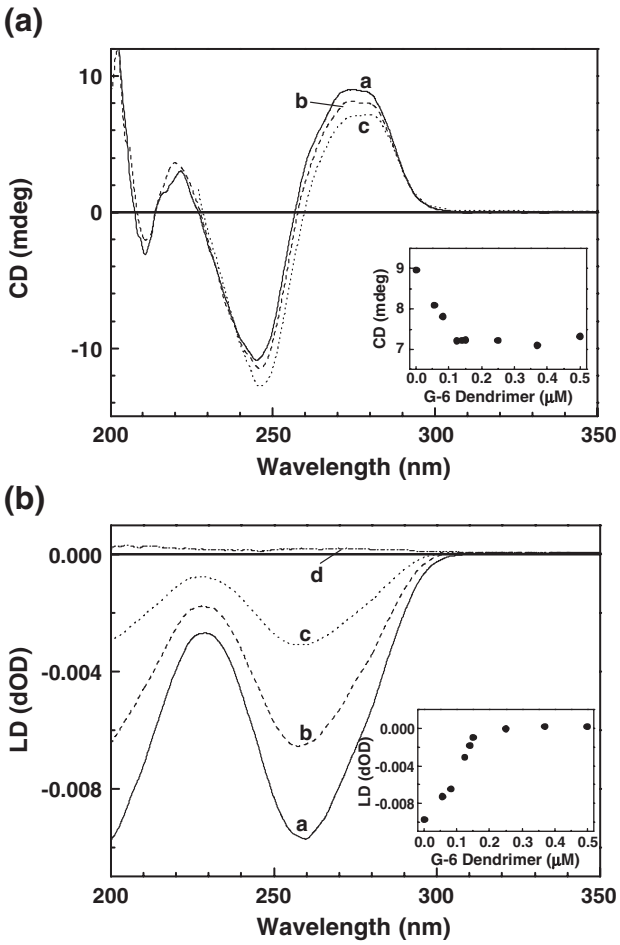

Fig. 5. (a) Representative CD spectrum of DNA in the absence and presence of G-6 dendrimer. [DNA]=100 μM, [G-6 dendrimer]=0 μM (curve a), 0.055 μM (curve b) and 0.50 μM (curve c). Change in CD intensity at 276 nm upon increasing G-6 dendrimer concentration is inserted. (b) Representative LD spectrum of DNA in the absence and presence of G-6 dendrimer. [DNA]=100 μM, [G-6 dendrimer]=0 μM (curve a), 0.082 μM (curve b), 0.124 μM (curve c) and 0.50 μM (curve d). Change in the LD intensity at 260 nm with respect to the G-6 dendrimer concentration is inserted.

3.3. Interaction of G-6 dendrimer, DNA and Hoechst 33258

The fluorescence emission spectrum of Hoechst 33258 in the presence of the DNA–G-6 dendrimer complex is shown in Fig. 3a. The extent of the increase in the fluorescence intensity upon binding to G-6 dendrimer is less than half compared to that in the presence of DNA. Furthermore, the maximum in the excitation and emission in the presence of G-6 dendrimer (ex: 367 nm and em: 475 nm) is different from that in the presence of DNA (ex: 360 nm, em: 471 nm), suggesting that the environment at DNA and G-6 is quite different. When G-6

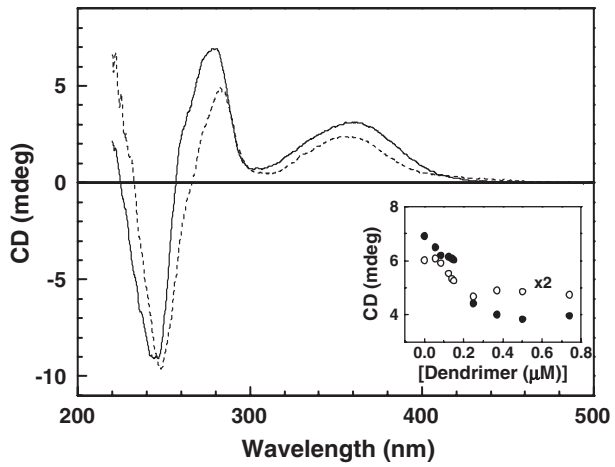

Fig. 6. Representative induced CD spectrum of the DNA-Hoechst 33258 complex in the Hoechst absorption region in the presence of G-6 dendrimer. [Hoechst 33258]=1.4 μM and [DNA]=100 μM. [dendrimer]=0 μM (solid curve) and 0.37 μM (dotted curve). Change in the CD intensity at 353 nm (open circles, twice enlarged) and at 278 nm (closed circles) with respect to the G-6 dendrimer concentration is inserted.

dendrimer and DNA coexist, the excitation and emission maximum appeared at 354 and 455 nm, respectively. The shape of the emission spectrum of Hoechst 33258 in the presence of the DNA–G-6 dendrimer complex is different from the spectrum of those bound to dendrimer or DNA. This observation indicates that the environment of Hoechst 33258 is different when it bound to DNA, dendrimer and the DNA–dendrimer complex. Difference in the environment can also be noticed from the CD spectrum (Fig. 6). In the absence of G-6 dendrimer, the DNA–Hoechst 33258 complex exhibited a CD maximum at 361 nm, which is in agreement with reported values [25]. Upon increasing the G-6 dendrimer concentration, the induced CD of the DNA–Hoechst 33258 complex shifted to the short wavelength (from 361 to 355 nm upon binding to the G-6 dendrimer) and the magnitude decreased. A small decrease and red-shift in the DNA absorption region was also observed, particularly, near 270 nm. A decrease in the CD intensity of the DNA–Hoechst 33258 complex at 353 nm, representing the change in the environment of the Hoechst 33258, as well as a decrease in CD intensity at 278 nm, representing the conformation of DNA, was plotted with respect to the G-6 dendrimer concentration (Fig. 6, insertion). The magnitude of the CD intensity fell to its minimum in the presence of 0.2 μM G-6 dendrimer, and is slightly higher than that of the DNA–G-6 complex formation in the absent of Hoechst 33258. At low G-6 dendrimer concentrations (<0.1 μM), the CD signal at 278 nm gradually decreases while that at Hoechst 33258 absorption region, a dragging in CD decrease was observed.

The Stern–Volmer plot, representing the accessibility of anionic quencher, I^-, to the polymer-free Hoechst 33258, that associated to G-6 dendrimer and that associated with the DNA–G-6 dendrimer complex is shown in Fig. 7(a). While polymer-free Hoechst 33258 appeared as a straight line in the Stern–Volmer plot, those bound either to G-6 dendrimer or to the DNA–dendrimer complex appeared to be upward bending curves. A straight line in the Stern–Volmer plot for the polymer-free Hoechst 33258 indicates that the fluorescence quenching follows a simple dynamic or static process. The accessibility of the anionic quencher, I^-, to polymer-free Hoechst 33258 was calculated as 4.1 M^{-1}. Two downward bending curves in the Stern–Volmer plot were re-plotted according to the modified Stern–Volmer equation, in which part of the fluorophore is assumed to be accessible to the quencher and the other is not (Fig. 7(b)). As a result, straight lines were obtained for the Hoechst 33258–G-6 dendrimer complex and the Hoechst 33258–DNA–G-6 dendrimer complex. From the slope and *y*-intercept, the accessibility and the initial fraction of the fluorescence intensity that is accessible to the quencher were calculated as 49.7 M^{-1} and 0.13 for the Hoechst 33258–G-6 dendrimer complex. The accessibility of the Hoechst 33258–DNA–G-6 dendrimer complex was 16.1 M^{-1}. The inaccessible fraction of this complex was 0.28. The fraction of the fluorescence of the inaccessible component is not necessarily equivalent to the concentration because the fluorescence quantum yield may vary.

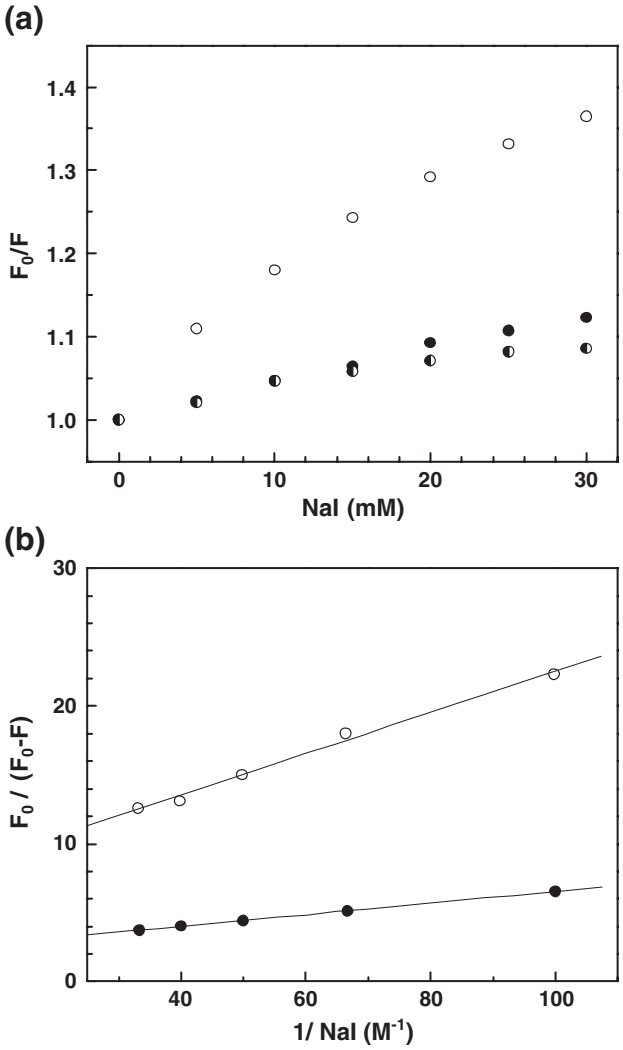

Fig. 7. (a) Quenching of the Hoechst 33258 (half closed circles), that bound to G-6 dendrimer (open circles), and that bound to the DNA–G-6 dendrimer complex (closed circles) by NaI. (b) Modified Stern–Volmer plot of the Hoechst 33258 bound to G-6 dendrimer and the DNA–G-6 dendrimer complex (see text).

4. Discussion

4.1. Property of Hoechst 33258 bound to G-6 dendrimer

The fluorescence decay times and the quantum yield of Hoechst 33258 depend on the protonation states of the molecule [31,33,34]. At a low pH or when bound to DNA, the piperazine units are protonated and a certain degree of charge transfer occurs from the protonated piperazine-bis-benzimidazole subunit to the phenol-bis-imidazole. As a consequence of the charge transfer, the interconnecting bond between the two imidazole units becomes flat and stronger in the excited state [32,34]. At a high pH, the piperazine unit is deprotonated, preventing the charge transfer in the excited state. Consequently, the excited state is deactivated through internal conversion involving the rotation of the intramoleculear bond between the two imidazole units. Various fluorescence decay times in aqueous solution that depend on the condition of the solution have been reported. For instance, the fluorescence decay of the Hoechst 33258 in Tris–EDTA buffer (pH 7.4) was described by a biexponential curve with the decay times of $\tau_1 = 0.18$ ns (83%) and $\tau_2 = 3.33$ ns (17%) [35], and those in phosphate buffer (pH 7.0) were $\tau_1 = 0.10 \sim 0.34$ ns (78%) and $\tau_2 = 3.5$ ns (22%) [34]. Similar results were obtained in this study with $\tau_1 = 0.40$ ns (55%) and $\tau_2 = 3.88$ ns (45%). The smaller amplitude for the short component may be attributed to the difference in the solution condition (cocodylate buffer, pH 7.0 in this study). Upon binding to G-6 dendrimer, the decay time of the short component lengthened becoming $\tau_1 = 0.80$ ns, and the amplitude decreased (26%). The long component shortened to $\tau_2 = 2.32$ ns and this component was dominant in the presence of G-6 dendrimer. This result indicates that the excited states of both the protonated, charge transferred, flat form and the deprotonated, rotation-allowed form of Hoechst 33258 are affected by the presence of dendrimer. It is, therefore, conclusive further that, protonated species in the excited state are dominant. An additional source of the protonation may be the abundant amines of the surface of the G-6 dendrimer. The enhancement in the fluorescence intensity of Hoechst 33258 upon binding to G-6 dendrimer can be described by the same argument. Change in the absorption spectrum of Hoechst 33258 upon binding to G-6 dendrimer is characterized by the increased absorbance and red-shift in the absorption spectrum with an isosbestic point. The equilibrium constant is 1.9×10^5 M, which is far lower compared to the association of Hoechst 33258 to various DNAs [23,24].

The Stern–Volmer plot for the fluorescence quenching by an anionic quencher, I^{-1}, appeared to be a downward bending curve, which is in contrast with that in the absence of the dendrimer. In the latter case, a straight line in the Stern–Volmer plot was observed. The quenching efficiency of the anionic quencher, I^-, was surprisingly high when Hoechst 33258 formed a complex with G-6 dendrimer in spite of that the Hoechst 33258 molecule is expected to be shielded, at least in part, from the quencher. This observation may be attributed to that at least some of the surface of the dendrimer is positively charged in the neutral pH and, thereby, the population of the negatively charged I^- is expected to be high near the surface, resulting in the enhanced quenching efficiency. It is the protonated form of the Hoechst 33258 whose fluorescence is quenched because the decay time of the deprotonated species is very short. However, from the results shown in this work, the possibility of the quenching of both forms of Hoechst 33258, with different accessibilities, cannot be completely ruled out. The quenching result implies that the Hoechst 33258 molecule conceivably locates at the surface of the G-6 dendrimer.

4.2. DNA–G-6 dendrimer association

Complex formation between DNA and G-6 dendrimer can be directly monitored by CD and LD in the absence of Hoechst 33258. For instance, a drastic decrease in the LD magnitude upon formation of a complex between DNA and polyethyleneimine, reflecting the aggregation of DNA with polymer has been reported [36]. In the DNA–polyethyleneimine complex, the CD spectrum of DNA also collapsed, suggesting the secondary structure of DNA in the aggregation is changed. In contrast with polyethyleneimine, the conformation of DNA remained even when it forms a complex with G-6 dendrimer. When a small decrease in the CD intensity at 276 nm of the 100 μM DNA was plotted against the dendrimer concentration, a straight line was observed when the dendrimer concentration was lower than 0.15 μM. Further increase in the dendrimer concentration did not affect the DNA CD spectrum, indicating that DNA was saturated. This concentration corresponds to 2.5 nucleo-bases, or phosphate, per surface amine. However, this ratio suggests only the upper limit because there must be DNA portions that act as a linker between the DNA–G-6 dendrimer complexes. In other word, the number of DNA bases (or phosphate) that contact directly with surface amines may be lower than 2.5. A similar stoichiometry was obtained from the LD measurement. In the absence of Hoechst 33258, the magnitude of LD in the DNA absorption region gradually decreased when G-6 dendrimer concentration increased. When the dendrimer concentration reached 0.15 μM (38.4 μM surface amine), the LD signal completely collapsed. The magnitude of LD signal in the DNA system is affected by two factors, namely optical and orientation factors [26,27]. Since the optical factor, which mainly reflects the concentration of DNA, was kept constant in this study, it is only the orientation factor that affected the LD magnitude. Physical conditions that affect the orientation factor including viscosity and temperature were not changed during the measurement. Therefore, the only factors that affect the LD magnitude at current experimental conditions are contour length and the flexibility of DNA. The flexibility can be changed mainly from the denaturation of DNA. However, no evidence for DNA denaturation was found, therefore, it is the shortening in the contour length of DNA that reduces the LD magnitude. Upon increasing the concentration of the DNA–G-6 dendrimer complex, the linker DNA may be shortening. The DNA in the complex (or aggregation) conceivably does not contribute to the LD signal.

In the presence of Hoechst 33258, the stoichiometry appeared to be somewhat different. The CD intensity reached

a minimum at the G-6 dendrimer concentration of 0.2∼0.3 μM. In the low dendrimer concentration range, the decrease in the CD intensity at 278 nm is linear with respect to the dendrimer concentration while the CD intensity remained the same in the Hoechst 33258 absorption region. This observation conceivably indicates that G-6 dendrimer formed a complex with Hoechst-free region of DNA first and then bound at the Hoechst 33258 bound site of DNA. The shape of the fluorescence emission spectrum and CD spectrum of Hoechst 33258 that bound to the DNA–G-6 dendrimer complex is different from that bound to DNA or that in aqueous solution, indicating that at least part of Hoechst 33258 binds at the DNA–G-6 dendrimer complex. The accessibility of the anionic quencher, I^-, to Hoechst 33258 bound to the DNA–G-6 dendrimer complex is far less compared to that complexed with G-6 dendrimer, but is more accessible than that in the polymer-free Hoechst. The difference in the accessibility can be understood by assuming that, as it was discussed above, the population of anionic quencher increases near the positively charged dendrimer surface. At the surface, Hoechst 33258 is bound in such a way that it is more exposed to the solvent compared to that located in the minor groove of DNA. In the latter case, I^- quencher is not accessible (data not shown). In the DNA–G-6 dendrimer complex case, Hoechst 33258 is protected by DNA, which will reduce the accessibility.

5. Conclusion

Hoechst can probably bind to the surface of G-6 dendrimer with one binding mode. When it binds to the DNA–G-6 dendrimer complex, the environment of Hoechst 33258 differs from that of the dendrimer's surface and the minor groove of DNA, suggesting that the binding site is the contact area of dendrimer and DNA.

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