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Optical properties of dye-doped deoxyribonucleic acid films

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Abstract Dye-doped deoxyribonucleic acid–surfactant films have been prepared by an intercalation method and their optical properties have been investigated. Coumarin 1 (C1), known as a neutral laser dye, can be successfully incorporated in deoxyribonucleic acid-surfactant films by immersing them in aqueous dye solution. About 6 wt% C1 can be doped in samples when the immersion time is 86400 s. C1-doped samples show pronounced optical absorption and photoluminescence bands. Not only hydrophobic–hydrophobic interactions but also molecular structures of dyes play prominent roles in the formation of neutral dye-doped deoxyribonucleic acid-surfactant films. It is surmised that C1 stays inside the double helix of deoxyribonucleic acid (DNA) and/or between the alkyl groups of surfactants.

Introduction

In recent years, there has been growing interest in synthesizing dye-functionalized deoxyribonucleic acid (DNA) because of its unique nanostructures [1, 2]. DNA is a biopolymer composed of DNA strands made from deoxyribose and phosphate. Two DNA strands organize a DNA double helix by hydrogen bonds between the bases, and are stabilized by π–π interactions. Owing to DNA double helix structures, interactions between some fluorescent molecules and DNA occur. It is generally accepted that there exists three types of interactions: intercalation, groove binding, and external stacking. To date, interaction between DNA and molecules has been extensively investigated in the biochemistry field [3–5]. These molecules are usually called as a DNA binding molecule and widely used for the visualization and detection of DNA. For instance, DNA binding molecules having planer structures can be intercalated between two base pairs in DNA double helix structures by an electrostatic force. Ethidium bromide, one of the cationic DNA binding molecules, is well known as a typical intercalator [3–5]. By intercalation, one DNA binding molecule stays inside the double helix structure as a monomer. It is very interesting to note that some DNA binding molecules show a pronounced enhancement in their fluorescence intensity by intercalation and groove binding [6].

Many functional dyes, such as luminescent, laser, nonlinear optical, and photochromic dyes, are also composed of the benzene ring and thus have a planer structure. If DNA were modified with functional dyes, dye-doped DNA would offer interesting opportunities for optical applications. However, DNA is soluble in water and decomposed by ultraviolet light irradiation. To overcome these problems, water insoluble DNA–surfactant complexes have been developed by a simple mixing of DNA with cationic amphiphilic surfactants [7, 8]. Recently, cationic laser dye-doped DNA–surfactant films have been prepared by a simple casting from dye and DNA–surfactant co-doped solution onto suitable substrates, and amplified spontaneous emission from doped-dyes has been observed [9, 10]. But the co-presence of dyes and DNA–surfactant in the film does not guarantee that they remain intact with each other. The authors found that some laser dyes were spontaneously incorporated into DNA–surfactant films by immersing the films in dye solution. Not only cationic dyes

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(Rhodamine dyes for example) but also neutral dyes (Coumarin dyes for example) could be doped by this method. In this study, synthesis and optical properties of laser dye-doped DNA–surfactant films prepared by an intercalation method have been investigated.

Experimental procedure

The following raw materials were purchased and used without further purification. Salmon sperm DNA sodium salts (300 base pairs, DNA 87.3%, H_2O 9.2%) and tetradecyltrimethylammonium-chloride [$(CH_3)_3N^+Cl^-(CH_2)_{13}CH_3$, TTA-chloride, 98%] were used for the preparation of DNA–TTA complexes. 2-butanol [$C_2H_5CH(OH)CH_3$, 99.5%] was used as a solvent of DNA–TTA. Poly(methyl methacrylate) [$(C_5H_8O_2)_n$, PMMA, average molecular weight = 100,000] and *N*, *N*-dimethylformamide [$HCON(CH_3)_2$, DMF, 99.5%] were used for the preparation of dye-doped PMMA films. Figure 1 shows molecular structures of Coumarin dyes investigated. As neutral laser dyes, Coumarin 1 ($C_{14}H_{17}NO_2$, C1, 99%), Coumarin 7 ($C_{20}H_{19}N_3O_2$, C7, 99%), Coumarin 106 ($C_{18}H_{19}NO_2$, C106, 99%), and Coumarin 334 ($C_{17}H_{17}NO_3$, C334, 99%) were used.

Solubility of DNA in alcohols drastically improves in the presence of cationic amphiphilic surfactants. First, DNA–TTA complexes were synthesized by mixing aqueous DNA solution with TTA-chloride. The DNA–TTA precipitates were collected by filtration and dried in vacuum at room temperature. Next, DNA–TTA films were prepared by casting DNA–TTA solution (100 μL) on SiO_2 glass substrates, then drying at room temperature for 48 h. The concentration of DNA–TTA in 2-butanol was fixed at 5.0 wt%. The dye-doped DNA–TTA films were then prepared by an intercalation method. The DNA–TTA film was immersed in approximately 3×10^{-4} wt% dye solutions for 10 s to 24 h. Acetonitrile (CH_3CN, 99.8%) and distilled water were used as the dye solvent. For comparison, C1-doped PMMA films were also prepared by a spin-coating method (2000 rpm, 1 min) from their DMF solution. The concentration of C1 in PMMA was varied from 0.06 to 14.3 wt%.

The thickness of samples was measured using a surface profiler (Veeco, Dektak3). Visible–UV optical absorption spectra of the sample were measured using a conventional UV–Vis spectrophotometer (JASCO V-570 Spectrophotometer). Phortoluminescence (PL) and photoluminescence excitation (PLE) spectra of the sample were recorded using a spectrophotometer (Perkin Elmer LS-50B). A monochromatic xenon lamp was used as an excitation source.

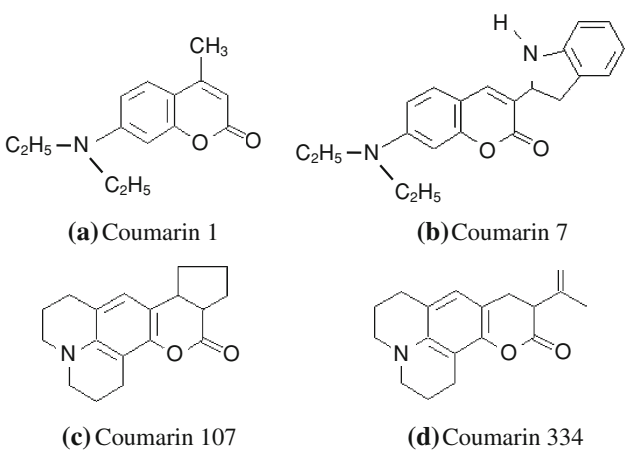

Fig. 1 Molecular structures of Coumarin dyes investigated

Results and discussion

Preliminary experiments showed that DNA–TTA complexes were highly soluble in alcohols such as methanol, ethanol, and 2-butanol but insoluble in water, acetonitrile, tetrahydrofuran and so on. The DNA–TTA films prepared by the casting method were homogeneous, highly transparent, and approximately 5–6 μm in thickness.

Figure 2 shows visible–UV optical absorption spectra of a DNA–TTA film on SiO_2 glass substrates. As seen in the figure, no absorption peaks are observed over the

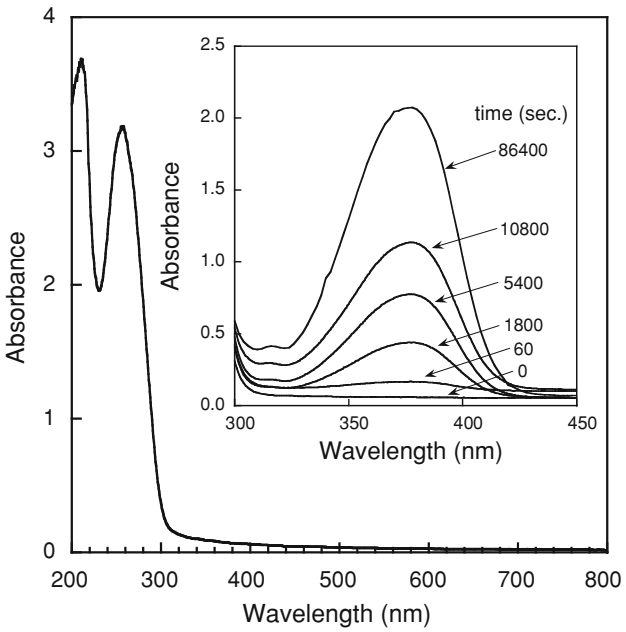

Fig. 2 Visible–UV optical absorption spectra of a DNA–TTA film on SiO_2 glass substrates. The inset shows absorption spectra of C1-doped DNA–TTA films with different immersion time. Note that distilled water was used as a solvent for C1 solution

wavelength range of 300–800 nm. The sample shows a pronounced absorption band at 255 nm in wavelength. There is general consensus that DNA in an aqueous solution has a specific absorption band at about 260 nm in wavelength, which has been attributed to the nucleobases of DNA [11]. These results lead us to an important suggestion that DNA–TTA films can be successfully formed, and DNA maintains its double helix structures. The inset shows absorption spectra of C1-doped DNA–TTA films with different immersion time. Note that distilled water was used as a solvent for C1 solution. C1-doped DNA–TTA films showed a clear absorption peak at 380 nm. The absorption peak has been indexed as an electronic transition of C1. It is clear that absorption due to C1 increases with increasing immersion time.

Changes in the absorbance at 380 nm of the C1-doped DNA–TTA film as a function of immersion time are shown in Fig. 3. The absorbance increases continuously with increasing immersion time, when distilled water was used as a solvent for C1 solution. On the contrary, the absorbance shows constant (almost 0 abs.) when acetonitrile was used. From these results, C1 can be successfully doped into DNA–TTA films by the intercalation method using aqueous C1 solution. In order to clarify the C1 concentration in the samples, C1-doped samples were also prepared by casting using C1 and DNA–TTA co-doped solution. The inset of Fig. 2 shows the relationship between the C1/DNA–TTA weight ratio and absorbance at 380 nm. An approximately linear relationship exists between them.

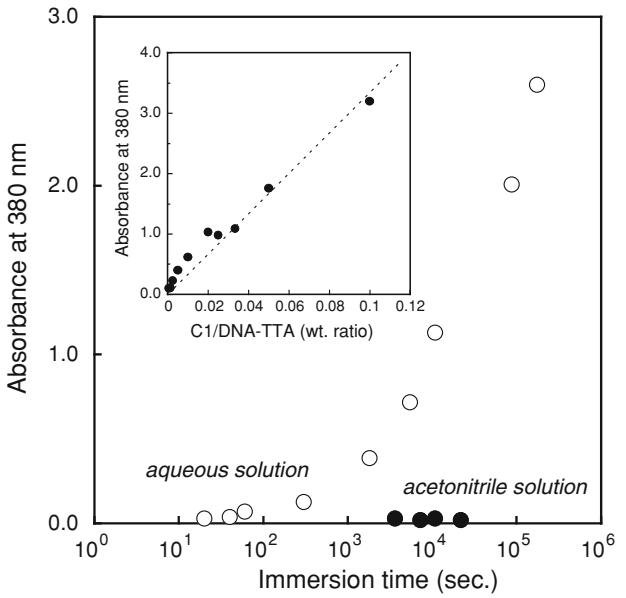

Fig. 3 Changes in the absorbance at 380 nm of the C1-doped DNA–TTA film as a function of immersion time. The inset shows the relationship between the C1/DNA–TTAC weight ratio and absorbance at 380 nm

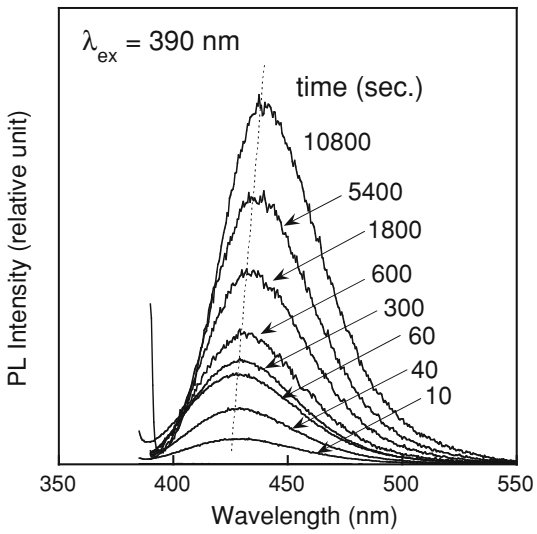

Fig. 4 PL spectra of the samples prepared with different immersion time

From the relationship, about 6 wt% C1 can be doped in the sample when the immersion time was 86400 s.

Figure 4 shows PL spectra of the samples. As seen in the figure, DNA–TTA itself shows no PL. C1-doped samples show broad PL over the wavelength range of 400–500 nm, and its intensity increases with the immersion time. Moreover, the peak position at maximum intensity shifts to a slightly longer wavelength with immersion time.

Changes in the (a) PL intensity and (b) peak position at maximum intensity of C1-doped DNA–TTA and PMMA films as a function of C1 concentration are plotted in Fig. 5. As seen in Fig. 5a, the PL intensity of the C1-doped DNA–TTA film increases with C1 concentration, shows maximum at about 4–5 wt% and then gradually decreases. The PL intensity of the C1-doped PMMA film shows similar tendency. No significant enhancements in the PL intensity are observed for the C1-doped DNA–TTA film compared with C1-doped PMMA films. This can probably be explained that C1 itself has a high quantum efficiency of fluorescence. As shown in Fig. 5b, in contrast, marked differences are observed for the peak position at maximum PL intensity of the films. In the C1-doped PMMA film, the peak position shifts to a longer wavelength with increasing C1 concentration. The amount of peak shift is 35 nm. On the contrary, the peak position of the C1-doped DNA–TTA film shows almost constant at 435 ± 5 nm. The result observed for the C1-doped DNA–TTA film is unusual.

Dye-doped DNA–TTA films have been prepared by the intercalation method in this study. The strong absorption and PL bands corresponding to C1 were observed by intercalation. As shown in Fig. 3, aqueous C1 solution gave strong absorption bands compared with acetonitrile C1 solution. On the other hand, C1 cannot be doped in the

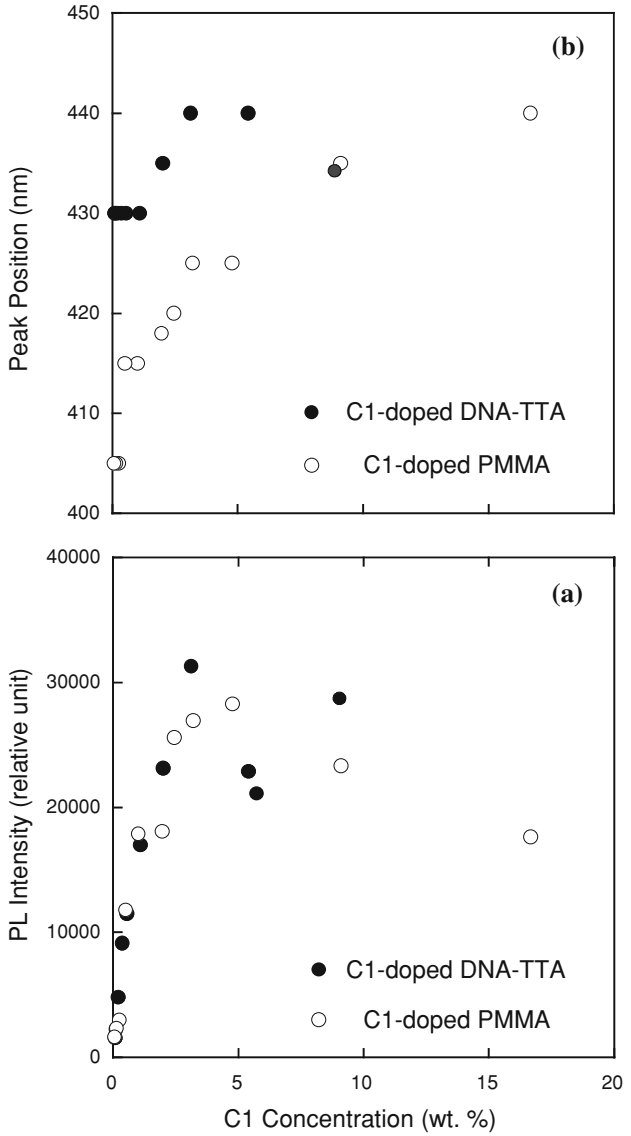

Fig. 5 Changes in the **a** PL intensity and **b** peak position at maximum intensity of C1-doped DNA–TTA and PMMA films as a function of C1 concentration

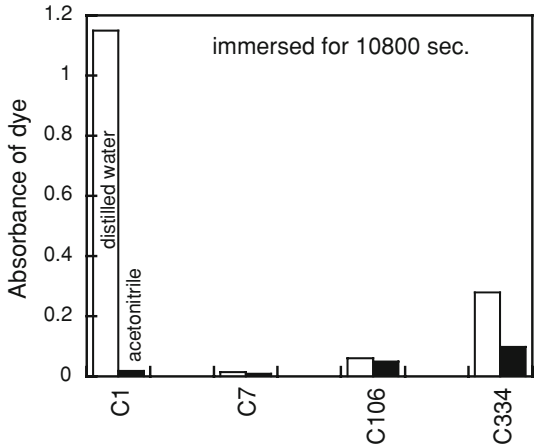

Fig. 6 Absorption intensities of dye-doped DNA–TTA films. Note that the immersion time of each sample was 10800 s

samples when acetonitrile was used as a solvent. This can be explained by compatibility between a solvent and a solute. It is well recognized that DNA is composed of hydrophobic base pairs and of hydrophilic phosphate chains. In the DNA–TTA film, the hydrophilic phosphate chain has been modified with $(CH_3)_3N^+(CH_2)_{13}CH_3$ ions by replacing Na^+ ions, and thereby DNA–TTA posses hydrophobic properties. In addition, C1 can be regarded as a hydrophobic dye. Therefore, one can surmise that C1 in aqueous solution diffuses into hydrophobic sites of DNA–TTA films by hydrophobic–hydrophobic interactions because DNA–TTA is a more suitable solvent for C1 than distilled water. However, the hydrophobic–hydrophobic interaction alone is insufficient to establish the doping mechanism. To clarify the doping mechanism, other Coumarin dyes have been investigated. Absorption intensities of dye-doped DNA–TTA films are shown in Fig. 6. Note that the immersion time of each sample was 10800 sec. Even if C7 and C106 are hydrophobic dyes, these dyes are scarcely incorporated in the DNA–TTA films even when distilled water was used as a solvent. These results lead us to an important conclusion that not only hydrophobic–hydrophobic interactions but also molecular structures of dyes play prominent roles in the formation of dye-doped DNA–TTA films. At this point, however, there has not been any evidence that C1 is completely intercalated into the base pairs of DNA double helix. It is surmised that C1 can be stayed inside the double helix of DNA and/or between alkyl chains of TTA as monomers. In many laser dyes, it is generally accepted that the fluorescence peak shifts towards longer wavelengths with increasing dye concentration. Actually, the PL peak position shifted towards longer wavelengths with C1 concentration in the case of PMMA matrices. But the PL peak position of the C1-doped DNA–TTA film appeared to be almost constant, suggesting that C1 could be incorporated independently as a monomer.

To our knowledge, the preparation of neutral dye-doped DNA–surfactant films is first described in this study. C1 can be successfully incorporated independently as a monomer into DNA–TTA films by immersing the film in aqueous dye solution. As described, a number of cationic laser dyes can be incorporated in DNA–surfactant films by this technique. Our preliminary experiments show that the doped-dyes are mainly incorporated as monomers compared with those in PMMA matrices. Moreover, it has been reported that nonlinear optical [10] and photochromic dyes [12] can be incorporated in DNA–surfactant films. Therefore, a suitable choice of dyes could allow the preparation

of dye-doped DNA–surfactant films for thin film laser, nonlinear optical and photochromic applications.

Conclusion

In this study, dye-doped DNA–surfactant films have been prepared by an intercalation method and their optical properties have been investigated. C1, known as a neutral laser dye, can be successfully incorporated into DNA–TTA films by immersing the film in aqueous dye solution. About 6 wt% C1 can be doped in the sample when the immersion time is 86400 s. Dye-doped samples show pronounced optical absorption and PL bands due to C1. An enhancement of fluorescence intensity cannot be achieved, but C1 can be incorporated independently as a monomer. Not only hydrophobic–hydrophobic interactions but also molecular structures of dyes play prominent roles in the formation of neutral dye-doped DNA–TTA films. It is our belief that the doped dyes stay inside the double helix of deoxyribonucleic acid and/or between the alkyl groups of surfactants.

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