J Mater Sci (2011) 46:2036–2040
DOI 10.1007/s10853-010-5035-x

Surfactant modified deoxyribonucleic acid films: synthesis, interaction with acridine orange and luminescent properties

Nobuaki Kitazawa · W. Aroonjaeng · M. Aono · Y. Watanabe

Received: 20 July 2010 / Accepted: 26 October 2010 / Published online: 12 November 2010

Abstract Dye-doped deoxyribonucleic acids (DNA)–tetradecyltrimethylammonium (TTA) films have been prepared. Acridine orange, known as a DNA-binding molecule, can be spontaneously doped by immersing the DNA–TTA film in an acetonitrile solution of the dye. Dye-doped samples exhibit two characteristic absorption bands corresponding to the dye monomer and aggregate, respectively. With the elapse of time after immersion, dye molecules undergo an unusual transformation from the aggregate state to the monomer state, and photoluminescence intensity also increases. Dye molecules in the sample exhibit a pronounced enhancement in their photoluminescence intensity than those in PMMA. The photoluminescence intensity of the samples strongly correlates to both of the dye concentration and monomer/(monomer + aggregates) ratio. Not only the hydrophobic interaction but also the electrostatic force between DNA and dyes play important roles in the formation of the dye-doped samples. It is surmised that monomers and aggregates disperse within the hydrophobic TTA sites in the early stage, and then a part of monomers presumably intercalate between adjacent base pairs of DNA with the elapse of time.

Introduction

There has been great interest in past decades to synthesize nanostructured materials based on the use of molecular templates [1–3]. Deoxyribonucleic acid (DNA) is one of the potential molecular templates for functional molecules because of its unique double helix structure. In the biochemistry field, it is generally accepted that many cationic molecules having a planar structure interact with DNA [4–6]. There are three types of the interaction as follows: intercalation, groove binding, and external stacking [7, 8]. These molecules are called as a DNA-binding molecule. It is very interesting to note that some DNA-binding molecules show a pronounced enhancement in their fluorescence intensity by the intercalation [5, 9]. Upon intercalation, one molecule is separated spatially between adjacent base pairs. Furthermore, intercalated molecules are expected to be oriented to one direction because base pairs are stacked each other. From the viewpoint of materials science, the most striking interest is the fluorescent intensity enhancement and stabilization of the molecules induced by the intercalation. Therefore, dye-doped DNA would offers interesting opportunities for optical applications if functional molecules such as luminescent, non-linear optical and photochromic dyes for example, were intercalated.

So far, the use of DNA for optical applications was limited due to its hygroscopic properties. Recently, water insoluble DNA-surfactant complexes have emerged by a simple mixing of DNA with cationic amphiphilic surfactants [10, 11], and optical properties of dye-doped DNA-surfactant films have been widely investigated [12–15]. In our previous paper, the authors found that Coumarin dyes were spontaneously incorporated into the DNA-surfactant film [16]. However, no fluorescent intensity enhancement was observed. In order to clarify the interaction between DNA and dyes, it is preferred to use DNA-binding molecules, which show fluorescence intensity enhancement upon intercalation. From the reasons above, acridine orange (AO) was chosen as a doped dye.

N. Kitazawa (✉) · W. Aroonjaeng · M. Aono · Y. Watanabe
Department of Materials Science and Engineering,
National Defense Academy, 1-10-20 Hashirimizu, Yokosuka,
Kanagawa 239-8686, Japan
e-mail: nkita@nda.ac.jp

AO is one of the DNA-binding molecules known to exhibit different absorption and fluorescence properties, depending on the chemical states [17, 18]. The most important feature is that the characteristic green fluorescence is enhanced when AO is intercalated into double strand DNA. The purpose of this study is to synthesize AO-doped DNA-surfactant films and to investigate the chemical states of AO by optical spectroscopy.

Experimental procedure

The following raw materials were purchased and used without further purification. Salmon sperm DNA sodium salts (average base pairs = 300, 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. *sec*-Butyl alcohol [$C_2H_5CH(OH)CH_3$, 99.5%] was used as a solvent. 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. Acridine orange ($C_{16}H_{20}N_3^+Cl^-$, 99%) was used as a doped dye.

First, DNA–TTA complexes were synthesized by the mixing of an aqueous DNA solution with TTA-chloride. The DNA–TTA precipitates were collected by filtration, dried at room temperature in an atmospheric pressure followed by suction drying (approximately 0.1 Pa) at 60 °C for 24 h. Next, DNA–TTA films were prepared by the casting DNA–TTA solution (100 μL) on SiO_2 glass substrates, and then dried at room temperature for 48 h. The concentration of DNA–TTA was fixed at 5.0 wt%. The dye-doped DNA–TTA films were prepared by the following methods. The DNA–TTA film was dipped in an approximately 3×10^{-4} wt% AO solution for 10 s to 1 h. Acetonitrile (CH_3CN, 99.8%) was used as a solvent. This method is defined as "immersion method" hereafter. After immersion, samples were rinsed with distilled water, dried by air blow and then kept in a plastic container. Note that the expression "as-prepared" means just after drying and "after 1 month" means that the sample has been stored for 1 month, respectively. For comparison, AO-doped DNA–TTA films were also prepared by the casting AO and DNA–TTA co-dissolved *sec*-butyl alcohol solution (DNA–TTA concentration; 5.0 wt%) on SiO_2 glass substrates, and then dried at room temperature for 48 h. This method is defined as "casting method" hereafter. The AO/DNA–TTA weight ratio was varied from 1/10 to 1/1600.

The thickness of the 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). Absorption spectra were fitted using Gaussian function to separate the AO absorption band. Commercially available program (PeakFit, Sea Solve Inc.) was employed for fitting. Photoluminescence (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.

Results and discussion

The solubility of DNA in organic solvents drastically improves in the presence of cationic amphiphilic surfactants. Preliminary experiments showed that DNA–TTA complexes were highly soluble in methyl alcohol, ethyl alcohol, and *sec*-butyl alcohol but insoluble in water, acetonitrile, and tetrahydrofuran. DNA–TTA films prepared by the casting method were homogeneous, highly transparent but amorphous. The film thickness of AO-doped DNA–TTA and PMMA films were approximately 4.4–5.3 and 4.2–4.8 μm, respectively.

Figure 1 shows optical absorption spectra of as-prepared AO-doped DNA–TTA films with different immersion time. As was described in the previous paper [16], DNA–TTA films showed a pronounced absorption band at 255 nm, corresponding to the π–π^* transition of nucleobases [19]. DNA–TTA films before immersion show no absorption and PL peaks over the wavelength range of 350–700 nm. Absorption spectra of the AO-doped DNA–TTA films consist of two absorption bands at 470 and 500 nm. These absorption bands have been attributed to the AO aggregate

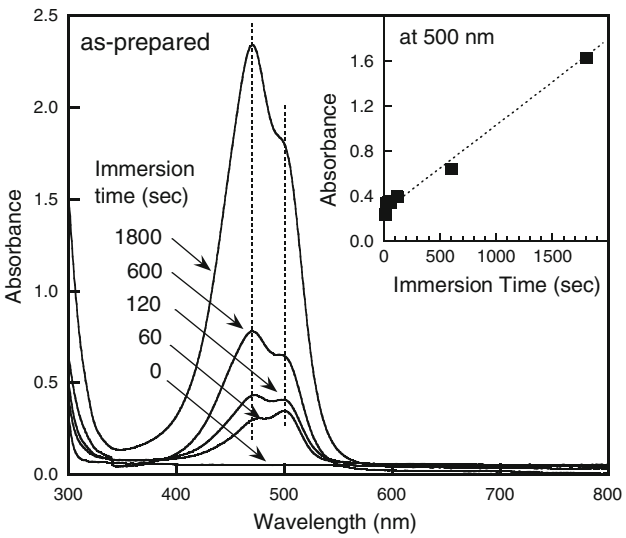

Fig. 1 Optical absorption spectra of as-prepared AO-doped DNA–TTA films with different immersion time. The *inset* shows the change is the absorbance at 500 nm as a function of the immersion time

($\lambda = 470$ nm) and the monomer ($\lambda = 500$ nm), respectively [20, 21]. As seen in the inset, it is clear that these absorption bands increase with increasing immersion time, suggesting that AO molecules are incorporated into DNA–TTA spontaneously.

Optical absorption spectra reveal that AO molecules in the sample undergo an unusual transformation from the aggregate state to the monomer state with elapse of time after immersion. It is interesting to note that the absorbance of the AO monomer further increases with the elapse of time after immersion. On the contrary, the absorbance of the AO aggregates decreases. Changes in the monomer/(monomer + aggregates) ratio as a function of the immersion time are shown in Fig. 2. Hereafter, the ratio is denoted as the M/(M + A) ratio. The M/(M + A) ratio is approximated by the $S_{monomer}/(S_{monomer} + S_{aggregates})$ ratio. $S_{monomer}$ and $S_{aggregates}$ mean the peak area due to the monomer and the aggregates, respectively. The M/(M + A) ratio of as-prepared samples decreases with increasing the immersion time. With the elapse of time after immersion, the M/(M + A) ratio increases and approaches a constant value. When the immersion time was less than 60 s, the M/(M + A) ratio remains constant for 1 day. For the immersion time >120 s, the ratio approaches a constant for 1 month.

Figure 3 shows PL spectra of as-prepared AO-doped DNA–TTA films with different immersion time. Clear PL bands are observed for the AO-doped DNA–TTA films at 540 nm. The AO monomer exhibits a characteristic green PL at approximately 530 nm [17, 18]. Therefore, the PL band at 540 nm observed for the as-prepared samples corresponds to the AO monomer. As the immersion time increases the PL at 540 nm is significantly quenched. This

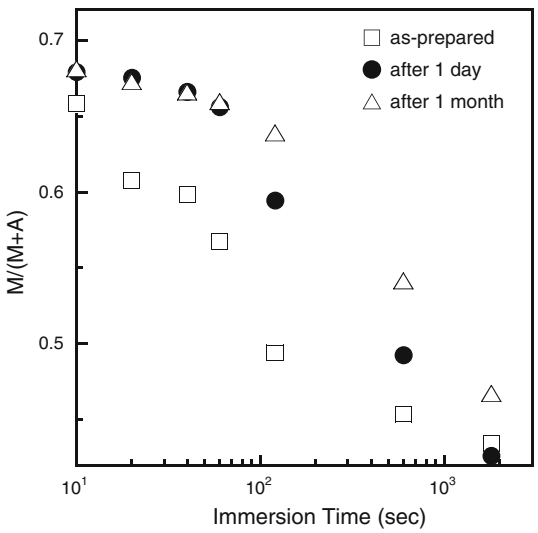

Fig. 2 Changes in the monomer/(monomer + aggregates) ratio of the AO-doped DNA–TTA films as a function of the immersion time

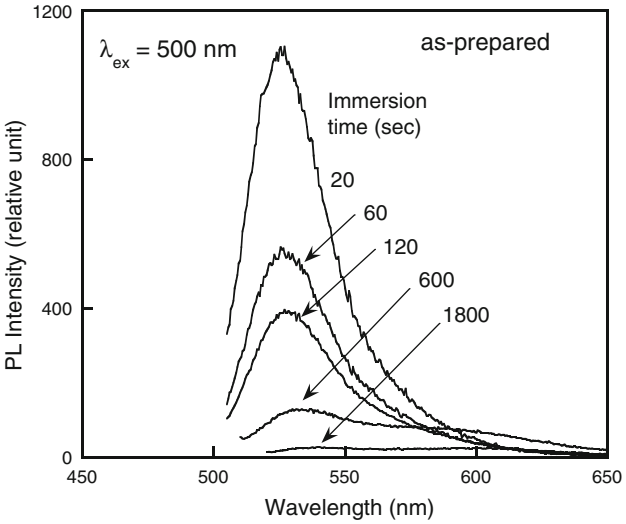

Fig. 3 PL spectra of as-prepared AO-doped DNA–TTA films with different immersion time. The excitation wavelength was 500 nm

can probably be explained by concentration quenching. On the contrary, a weak PL band at 600 nm appears as the immersion time increases longer than 10 min. The PL band at 600 nm can be attributed to the AO aggregate.

Changes in the maximum PL intensity of the AO-doped DNA–TTA films as a function of (a) immersion time (b) absorption coefficient and (c) M/(M + A) ratio are plotted in Fig. 4. For comparison, the AO-doped PMMA films were also prepared by a spin-coating method (2000 rpm, 1 min). Values of the absorption coefficient and the PL intensity were estimated at 500 and 540 nm, corresponding to the AO monomer. As seen in the Fig. 4a, the PL intensity of as-prepared samples decreases with the immersion time. But the PL intensity increases with the elapse of time and finally approaches a certain value. When the immersion time was less than 120 s, the PL intensity increases with the elapse of time. After 1 week, the PL intensity of each sample remains almost constant. The saturated PL intensity shows a maximum at the immersion time of 20 s, and then decreases. For the immersion time >120 s, on the contrary, no increase in the PL intensity is observed. It should be noted that absorption and PL spectra of the AO-doped DNA–TTA films prepared by the casting method remain independent of the elapse of time. Both of the absorption and PL spectra showed no change even after 9 months storage. As is evident from Fig. 4b, the fluorescence intensity enhancement is observed for the AO-doped DNA–TTA film. For example, the maximum PL intensity is 10 times as large as than that of the AO-doped PMMA films when the absorption coefficient of each film is the same ($\alpha = 750$ cm^{-1}). However, the PL intensity is very sensitive to the AO concentration compared with that of the AO-doped PMMA films. In Fig. 4c, there exist strong

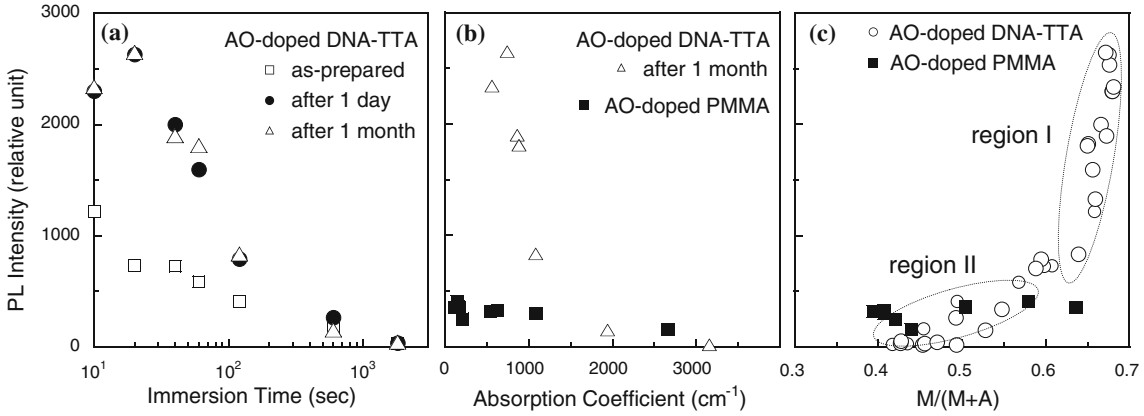

Fig. 4 Changes in the maximum PL intensity of the AO-doped DNA–TTA films as a function of **a** immersion time, **b** absorption coefficient, and **c** M/(M + A) ratio. The PL intensity of the AO-doped PMMA films was also plotted for comparison. Values of the absorption coefficient were estimated at 500 nm, corresponding to the monomer absorption

correlation between the PL intensity and M/(M + A) ratio for the AO-doped DNA–TTA films. For the samples with M/(M + A) < 0.6 (region I), no fluorescence intensity enhancement is observed. For the samples with M/(M + A) > 0.65 (region II), the fluorescent intensity enhancement occurs. On the contrary, the PL intensity of the AO-doped PMMA films is independent of the M/(M + A) ratio. One possible explanation is that the fluorescence efficiency of the AO in PMMA is still low regardless of its concentration and chemical state.

Dye-doped DNA–TTA films have been prepared by the immersion method. The important considerations in this study are the driving force of the intercalation, the chemical state of AO and fluorescence intensity enhancement. DNA is composed of hydrophobic base pairs (inside of double helix) and of hydrophilic phosphate chains (outside of double helix). Fundamentally, AO molecules first interact with phosphate chains by the electrostatic force, and then intercalate between adjacent base pairs [17, 18, 22]. In the case of 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 the DNA double helix (core) is surrounded by the hydrophobic alkyl chain of TTA ions (shell). Taking the core–shell structure of DNA–TTA complexes into account, one can surmise that AO molecules diffuse within the hydrophobic TTA sites by the hydrophobic interaction if DNA–TTA is a more preferable solvent than acetonitrile. In the early stage, it is believed that both of the monomer and aggregate are dispersed within the hydrophobic alkyl chains of TTA ions. Considering the DNA–TTA complex structure, there exists other possible doping sites as follows: the core–shell interface between phosphates and TTA ions, major and minor grooves and between base pairs. Most of DNA-binding molecules, including AO, consist of benzene ring with cationic NH– groups. It has been reported that the NH– group interacts with the π-electrons of the benzene ring by the intermolecular force like a hydrogen bond [23, 24]. It is quite reasonable that the AO molecule should exist in the monomer state between adjacent base pairs owing to the spatial limitation of the DNA–TTA complex. Although there has not been any direct evidence that most AO molecules are intercalated, the increase in the M/(M + A) ratio with the elapse of time can be explained by assuming that intercalation occurs. An additional remark is that the fluorescence intensity enhancement is observed for the AO-doped DNA–TTA films. Extensive studies on the fluorescence intensity enhancement of ethidium bromide, one of the DNA-binding molecules, have been reported. Olmsted and Kearns [25] proposed that the fluorescence intensity enhancement of ethidium bromide upon intercalation was attributed to a reduction in the rate of excited state proton transfer to solvent molecules. Other possible mechanism of which explains the fluorescence intensity enhancement has been proposed as follows: hydrophobic effect [26], solvent quenching through complex formation, hydrogen-bonding, or proton transfer [27, 28] and interchange of electronic states [29]. Upon intercalation, the environment and conformation of the AO monomer should be modified. Either one or both of these changes might induce the fluorescence intensity enhancement of AO. At this stage, however, the experimental evidence observed in this study is insufficient to clarify the satisfactory mechanism for the fluorescence intensity enhancement.

Our experiments show that AO molecules can be successfully incorporated into DNA–TTA films by the immersion method. AO-doped DNA–TTA films show the fluorescent intensity enhancement. As described, a number of functional dyes can be incorporated in DNA-surfactant films by this technique. Therefore, a suitable choice of dyes could allow the preparation of dye-doped DNA-surfactant films for optical applications.

Conclusion

In this work, AO-doped DNA–TTA films have been prepared and their optical properties have been investigated. Optical absorption spectra reveal that dye molecules in the sample undergo an unusual transformation from the aggregate state to the monomer state with the elapse of time. Dye molecules in DNA–TTA exhibit a pronounced enhancement in their PL intensity than those in PMMA. The PL intensity of the AO-doped DNA–TTA films strongly correlates to both of the dye concentration and M/(M + A) ratio. It is surmised that monomers and aggregates diffuse within the hydrophobic TTA sites, and then monomers presumably intercalate between adjacent base pairs of DNA. Not only the hydrophobic interaction but also the electrostatic force between DNA and dyes play important roles in the formation of dye-doped samples.

References

1. Brinker CJ, Lu Y, Sellinger A, Fan H (1999) Adv Mater 11:579
2. Mitzi DB (1999) Prog Inorg Chem 48:1
3. Antony MJ, Jayakannan M (2010) J Phys Chem B 114:1314
4. Nafisi S, Saboury AA, Keramat N, Neault J-F, Tajmir-Riahi H-A (2007) J Mol Struct 827:35
5. Hilal H, Taylor JA (2007) Dyes Pigments 75:483
6. Hendry LB, Mahesh VB, Bransome ED Jr, Ewing DE (2007) Mutat Res 623:53
7. Saenger W (1984) Principal of nucleic acid structure (in Japanese). Springer-Verlag, New York
8. Bloomfield VA, Crothers DM, Tinoco I Jr (1996) Nucleic acids. University Science Books, Sausalito, CA
9. Yu F, Ding Y, Gao Y, Zhang S, Chen F (2008) Anal Chim Acta 625:195
10. Okahata Y, Tanaka K (1996) Thin Solid Films 284/285:6–8
11. Tanaka K, Okahata Y (1996) J Am Chem Soc 118:10679
12. Kawabe Y, Wang L, Horinouchi S, Ogata N (2000) Adv Mater 12:1281
13. Kawabe Y, Wang L, Nakamura T, Ogata N (2002) Appl Phys Lett 81:1372
14. Yu Z, Li W, Hagen JA, Zhou Y, Klotzkin D, Grote JG, Steckl AJ (2007) Appl Opt 46:1507
15. Krupka O, El-ghayoury A, Rau I, Sahraoui B, JGrote JG, Kajzar F (2008) Thin Solid Films 516:8932
16. Kitazawa N, Miyagawa S, Date K, Aroojaeng W, Aono M, Watanabe Y (2009) J Mater Sci 44:4999. doi:10.1007/s10853-009-3764-5
17. El-Naggar AK, Batskis JG, Teague K, Gamsey L, Bariogie B (1991) Cytometry 12:330
18. Brun AM, Harriman A (1992) J Am Chem Soc 114:3656
19. Wang L, Yoshida J, Ogata N (2001) Chem Mater 13:1273
20. Luchowski R, Krawczyk S (2003) Chem Phys 293:155
21. Lyles MB, Cameron IL (2002) Biophys Chem 96:53
22. Hou X, Xu M, Wu L, Shen J (2005) Colloids Surf B 41:181
23. Munoz MA, Sama O, Galan M, Guardado P, Carmona C, Balon M (2001) Spectrochim Acta A 57:1049
24. Nakanaga T, Buchhold K, Ito F (2002) Chem Phys 277:171
25. Olmsted J III, Kearns DR (1977) Biochemistry 16:3647
26. LePecq JB, Paoletti C (1967) J Mol Biol 27:87
27. Forster TH (1972) Chem Phys Lett 17:309
28. Stryer L (1966) J Am Chem Soc 88:5708
29. Burns VWF (1969) Arch Biochem Biophys 133:420