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Spectroscopic study of cyanine dyes interacting with the biopolymer, DNA

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ABSTRACT

Dyes intercalated into DNA strands or bound to grooves show fluorescence intensity changes and aggregate formation depending on the conditions. In order to establish some empirical rules concerning dye intercalation, spectroscopic studies for the effects of DNA on several series of cyanine dyes with different aromatic rings, conjugated chain length and alkyl substituents were made. Absorption spectra, fluorescence intensity and circular dichroism spectra showed strong dependence on the species of dyes. Combination of preceding studies and these present results indicates that cyanine dyes tend to intercalate into DNA strand if their polymethine bridge was composed of only one carbon. For molecules with the longer chains irregular aggregates were formed by small amounts of DNA, which transformed into complexes composed of multiple dye and DNA strands. These results would serve as a useful guideline for designing of optical functional materials and devices utilizing DNA complex.

1. Introduction

Some organic dyes can form various types of complexes by interacting with the biopolymer DNA, resulting in an interesting modulation of their spectroscopic properties through the influences on their structures and electronic states. A well-established example is ethidium bromide which undergoes enhancement of its fluorescence intensity when intercalated between base pairs consisting of the DNA skeleton [1]. Recently, fluorescence enhancement has attracted considerable attention because it would be applicable to thin film dye lasers with potentially high efficiencies [2–11]. As well as the intercalation, binding of molecules on DNA's groove is also an interesting topic. Many cyanine dyes are known to be bound to the minor grooves of DNA strand, and sometimes the binding shows significant selectivity for DNA sequences, suggesting the recognition and detection of specific base pair sequences [12–16]. Furthermore, because cationic molecules should have electrostatic interaction with DNA due to its poly-anionic property, such electric force has influence on the interaction among dyes and often enhances or suppresses their aggregate formation [17–23]. Utilizing these effects would make it possible to develop novel photonic devices with unique and high performance.

The interaction modes among dyes and DNA are very sensitive to the properties of dyes themselves as molecular size and electronic structures as well as environmental conditions such as concentrations, pH, additives and so on. Therefore, it is worthwhile to make a systematic study on their optical properties. In this study, we employed several kinds of cyanine dyes with different types of aromatic rings, different conjugation length and alkyl substituent length to investigate the effects from DNA by absorption, fluorescence and circular dichroism (CD) spectra in aqueous solutions.

Before getting into the main issue, we will make a brief historical review on this subject. There have been many studies made on the optical properties of cationic cyanine dyes interacting with DNA, and researchers' interests have focused on interaction modes, their structural change, change of optical properties and also their dependence on DNA sequences [13,14,17,18,20–29]. In this paper, we will use the abbreviations $DiXC_n(m)$ for typical cyanine dyes as depicted in Fig. 1 which have been widely used in the preceding researches. We introduce a parameter β for the molar ratio of DNA base pair to dye for convenience, because that would be one of the most important parameters.

Many important studies have been contributed by several groups all over the world. One important work was given by Armitage group who added artificially sequenced oligo-DNA into the solution of $DiSC_2(5)$, finding monomer absorption peak reduction and the increase of dimer peak at shorter wavelength side as long as β was less than 2 [13]. The spectral change accompanied the fluorescence quenching, leading to the conclusion that the dye formed a face-to-face dimer or dimer pair in the minor groove of the DNA strand. They also employed $DiSC_{3+}(5)$, the tricationic

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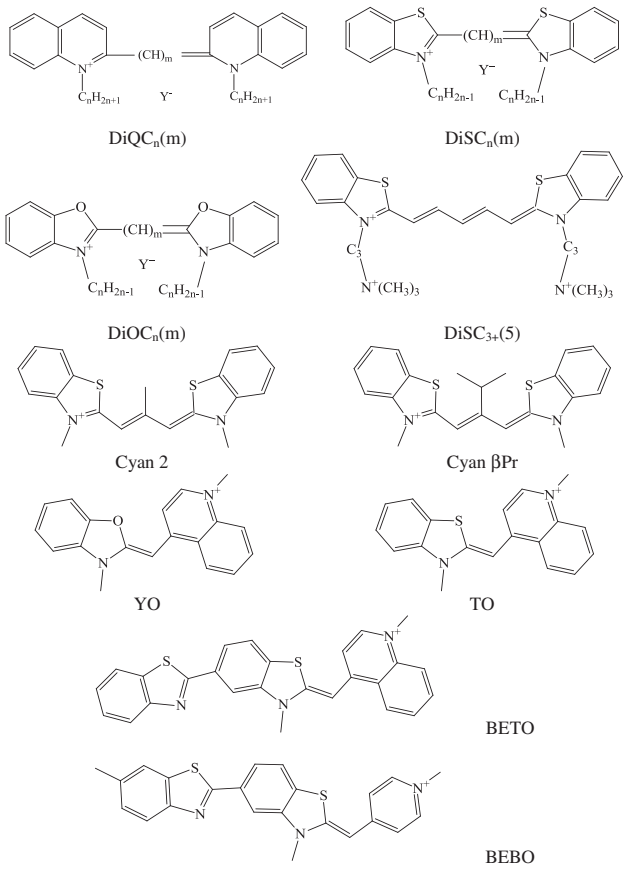

Fig. 1. Molecular structures of cyanine dyes employed for the experiments and referred in discussion, where *Y* means counter anions, and letters '*m*' and '*n*' indicate the conjugation chain length and alkyl chain length, respectively.

version of DiSC$_2$(5) which will weakly interact with DNA [14,17]. Spectral features of the dye were more complex than that of the monocationic counterpart, showing a J-aggregate peak at low temperature when β was about 1.5. The temperature dependence of peak intensities and the spectral shape indicates the structural change of aggregates interacting with DNA. The works of Arimitage's group also showed the strong dependence of spectral properties on sequence of DNA, of which fact suggests the possibility of the DNA sequence recognition. Chen et al. investigated the effects of several types of DNA including hairpin type duplex or quadruplex on DiOC$_2$(5) and found that the fluorescence was quenched by DNA. Combining with other experimental results, they explained it by groove binding of the dye [24]. As examples of the dyes with longer conjugated chain, Davidson et al. examined the IR emissive dye DiSC$_2$(7) and found that the fluorescence intensity was enhanced by adding DNA as $\beta = 100$ [25]. Yarmoluk et al., have made a systematic investigation on cyanine dyes interacting with DNA, showing that Cyan 2 and Cyan βiPr gave fluorescence enhancement at $\beta \sim 50$, and J-aggregate formation when $\beta \sim 1$ [18,26]. Among molecules with shorter conjugation but with an asymmetric structure, investigations of the interaction of thiazole orange or oxazole yellow with DNA have shown by polarization spectroscopy that these dyes are intercalated into DNA [27]. DiQC$_2$(1) which is well known as pseudoisocyanine (PIC) forms J-aggregates in solutions and films under some conditions, and its interaction mode is strongly affected by the existence of DNA [20–23]. Nordén et al. suggested that DiQC$_2$(1) was intercalated into DNA from its linear dichroic spectrum measured for flowing aqueous solution [28]. Vieira Ferreira et al. investigated the DiQC$_2$(1) and DiQC$_2$(3) adsorbed on cellulose crystallites, finding the clear J-band for absorption spectra of DiQC$_2$(1) [29].

From these preceding studies, it seems possible to derive a general rule that cyanine dyes tend to form aggregation when β is comparable or less than unity, but when the β value becomes much larger than 1, fluorescence is usually enhanced and monomer spectral peak shifts to longer wavelength without changing its shape. The spectroscopic properties under $\beta < 1$ depend on the length or size of molecules. We can propose a conjecture that for DiXC$_n$(1) molecules can intercalate into DNA chain even when β is small, but molecules usually form dimer or larger aggregate when *m* (the conjugation chain length) exceeds 1. This study was conducted in order to verify some proposed rules governing DNA-dye interaction. Details of sample preparation will be described in the next section and spectroscopic results will be discussed to clarify the mutual interaction modes between DNA and the dyes.

2. Sample preparations and experiments

We employed more than ten types of water soluble organic dyes and observed the influence of DNA on their optical properties. The compounds studied are addressed in Table 1 by abbreviation DiXC$_n$(*m*) and their structures are shown in Fig. 1. All the samples were purchased from Sigma–Aldrich or Hayashibara, and were used without further purification. In this study, three types of aromatic rings were chosen, that is, DiQC including quinoline rings, DiSC with benzothiazole and DiOC with benzoxazole. These compounds are commercially available for $m = 1$, 3, 5 and 7 (*m* means the length of the conjugated polymethine chain). The length of alkyl chain attached to the nitrogen of the carbazole substituent is given by '*n*', and it was usually 2, except that for DiOC$_n$(1) other materials ($n = 3$, 6) are also available. Counter ions were iodine. Among them, DiQC$_2$(1) have been well known as pseudoisocyanine (PIC) and the characteristics of their J-aggregates have been widely investigated [30].

We have already investigated the absorption and fluorescence spectra of several cyanine dyes, that is, DiSC$_2$(3), DiSC$_2$(5), DiOC$_3$(3) and DiOC$_6$(3), showing that the spectral peaks broadened and their fluorescence was quenched by adding a little amount of DNA [21,22]. In this paper, we also tested the CD spectra of a wider variety of cyanine dyes as well as their absorption and fluorescence properties. Absorption and fluorescence spectra were measured by UV-2400PC (Shimadzu) and RF-5300PC (Shimadzu), respectively. For CD spectra, we used a CD spectrometer J-820 (JASCO).

For the compounds listed in Table 1, we prepared the dye solutions of water/methanol mixture (the ratio is 4/1) co-dissolved with polyvinylalcohol (PVA) and DNA for the spectral measurements varying the amounts of the additive DNA. PVA was used in order to suppress the precipitation of the dyes, and it was also possible to fabricate thin films with high optical quality by spin-coating from the solution as done for J-aggregate study [20,23]. The concentration of PVA and dyes were 48 g/l and 6.3×10^{-4} M, respectively. The molar ratio β was adjusted to be about 0, 0.19, 0.49

Table 1
List of compounds.

Structure	Conjugation chain length (*m*)			
	1	3	5	7
DiQC	DiQC$_2$(1) or PIC	DiQC$_2$(3)	DiQC$_2$(5)	DiQC$_2$(7)
DiSC	DiSC$_2$(1)	DiSC$_2$(3)	DiSC$_2$(5)	DiSC$_2$(7)
DiOC	DiOC$_2$(1)	DiOC$_2$(3)		
		DiOC$_3$(3)	DiOC$_2$(5)	
		DiOC$_6$(3)		

and 0.88. Note that these β values were much less than that usually employed for fluorescence enhancement studies [18,25,27].

3. Experimental results

At first, we show the results for DiQC series in Figs. 2 and 3, which show absorption, fluorescence and CD spectra for $DiQC_2(1)$ iodide, and $DiQC_2(3)$. J-aggregate properties of $DiQC_2(1)$ (PIC) has been widely investigated. Absorption spectra in Fig. 2a) showed that the addition of small amounts of DNA hardly affected monomeric absorption except for a slight red shift as well known for dyes interacting with DNA [26,31]. An additional sharp peak in longer wavelength side indicated the emergence of J-aggregates, but it was not so significant because the dye was less concentrated than the value for efficient aggregate formation [20]. We found that the fluorescence intensity increased by the DNA addition as shown in Fig. 2b). The fluorescence enhancement and clear CD signals in Fig. 2c) indicated that strong interaction between the dye molecules and DNA strand, and supported the Nordén's conjecture that $DiQC_2(1)$ molecules were intercalated between DNA base pairs [28]. The behaviour under the existence of DNA for the molecule with longer polymethine chain as $DiQC_2(3)$ was much different from that of $DiQC_2(1)$. The absorption peak became diffuse and fluorescence was quenched by DNA addition when $\beta < 1$ as shown in Fig. 3(a and b). These effects were apparently caused by DNA because the CD spectra showed the signal from the dye induced by the chiral strands. For longer chain compounds such as $DiQC_2(5)$ and $DiQC_2(7)$, we could not completely eliminate the influence from precipitation of the dye due to comparatively poor solubility. But the similar spectral broadening due to DNA was observed for both materials.

The experiments for $DiOC_2(1)$ series also showed the similar tendencies as depicted in Fig. 4–6 for $DiOC_2(1)$, $DiOC_2(3)$ and $DiOC_2(5)$. Addition of DNA did not induce changes for absorption spectral shape for $DiOC_2(1)$, but the fluorescence intensity was strongly enhanced by DNA chains as also supported by CD spectra. On the other hand, for $DiOC_2(3)$ and $DiOC_2(5)$ the absorption peaks greatly broadened with DNA and fluorescence intensity was quenched, while CD signals also increased with the increase of DNA. We observed almost the same results for $DiSC_2(1)$, $DiSC_2(3)$ and $DiSC_2(5)$, except that the peak positions were shifted bathochromically by 50–80 nm than those corresponding analogues of $DiOC_2(m)$. Some of these results have been already published elsewhere [22].

In order to investigate the effects from the length of alkyl chain attached to the nitrogen atoms, we conducted the experiments for

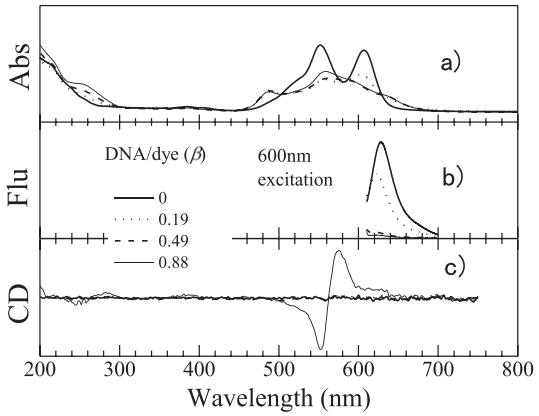

Fig. 3. Absorption, fluorescence and CD spectra for $DiQC_2(3)$.

the cyanine dyes $DiOC_3(3)$ and $DiOC_6(3)$ as well as $DiOC_2(3)$. Figs. 5, 7 and 8 depict the spectra for these three dye species. We can see that there are no significant difference in absorbance and emission among three $DiOC_n(3)$ dyes, while the features of CD spectra were much different from one another and quite little signals were obtained for $DiOC_6(3)$. Because the intensity of CD signal would indicate the interaction strength or the distance of the dye from the DNA chain, the aggregated dye with longer alkyl chain may be considered to be separated from DNA.

4. Discussions

From the results given above, we can derive some empirical rules regarding the optical characteristics of the cyanine dyes interacting with DNA. For short cyanine dyes presented by $m = 1$, no specific change in absorption spectra was observed but fluorescence intensity was enhanced by DNA addition, regardless of the types of cyanine. Dependences of fluorescence intensity on DNA concentration for molecules with $m = 1$ and $m = 3$ are summarized in Fig. 9. The results show the clear distinction due to polymethine chain length. Fluorescence enhancement by DNA seems to be common for small sized molecules, considering the similar effects observed in other asymmetric dyes [27]. In our earlier study, fluorescence was found to be enhanced for a hemicyanine dye, although DNA-surfactant complex and organic solvent were used because of hydrophobicity of the dye [3,32]. From these results, the most probable interaction mode for the short cyanine dyes would be intercalation into the DNA strand. Many preceding studies

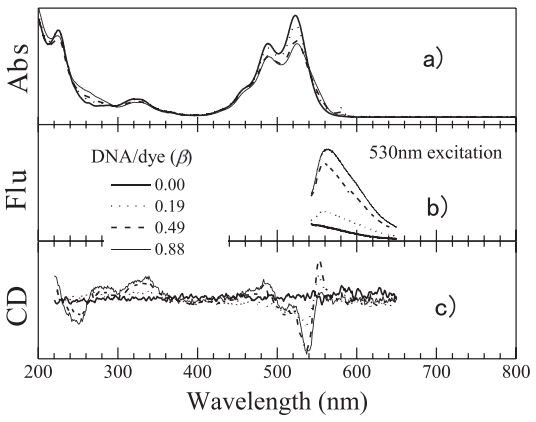

Fig. 2. Absorption, fluorescence and CD spectra for $DiQC_2(1)$ (PIC).

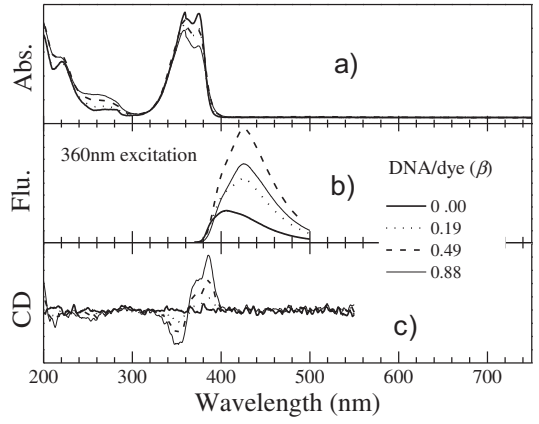

Fig. 4. Absorption, fluorescence and CD spectra for $DiOC_2(1)$.

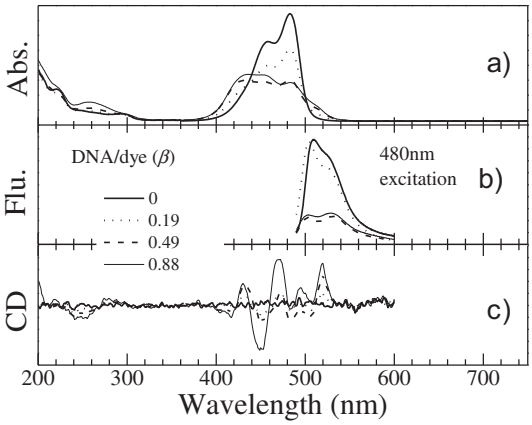

Fig. 5. Absorption, fluorescence and CD spectra for $DiOC_2(3)$.

support this conjecture, for example, the results for oxazole yellow (YO) or thiazole organge (TO) and their longer versions BETO or BEBO showed clear distinction of intercalation and groove binding by molecular length [27].

The behaviour under DNA addition for longer cyanines were quite different. We have already shown the results of fluorescence quenching and spectral broadening in $DiSC_2(3)$ with small amounts of DNA [21]. In this study, we confirmed that the similar broadening was observed both for DiQC and DiOC series. According to molecular exciton theory, interaction between two equivalent molecules results in Davidov splitting consisting of higher and lower energy levels. The higher level is dipole allowed when face-to-face alignment is dominant (H-type) and the lower one is when head-to-tail interaction is dominant (J-type), and the J-aggregate peak is an extreme situation for the latter case [17,33]. Because the observed broadened spectra included both longer and shorter wavelength components, dyes presumably form irregular aggregates not determinable as J or H types. Seifert et al. assigned the spectral change of $DiSC_2(5)$ to dimer formation in minor grooves and they found strong selectivity on the DNA sequence [13]. Although it is difficult to determine whether the aggregates are formed in minor grooves or outside of the strand for our cases, CD spectra show that the aggregates locate at the vicinity of DNA. But it is most reasonable that the aggregates are attached to the outside of DNA by electrostatic force, because there is no trace of dimer absorption when the amount of DNA is much larger than that of the dye as shown before [6,21].

In this study, we did not show the results for the region of $\beta > 1$. However, our former results and studies by other groups showed the enhancement of fluorescence and the recovery of their spectral

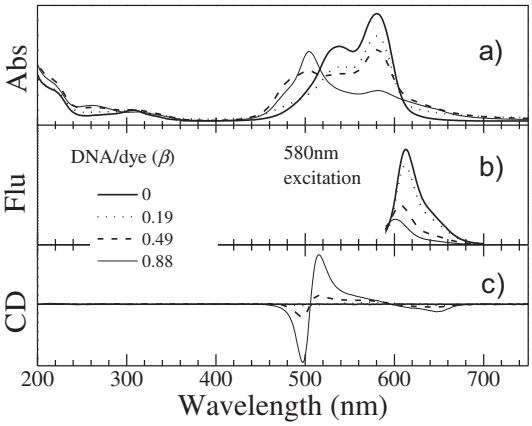

Fig. 6. Absorption, fluorescence and CD spectra for $DiOC_2(5)$.

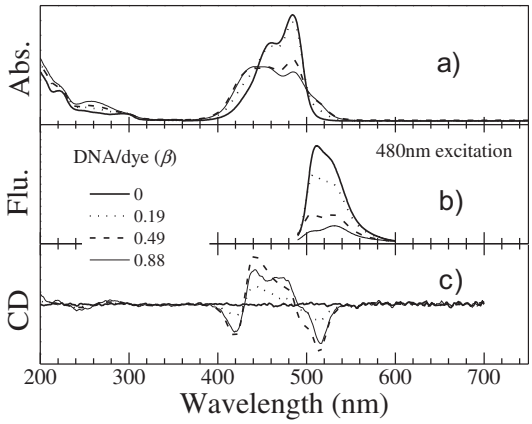

Fig. 7. Absorption, fluorescence and CD spectra for $DiOC_3(3)$.

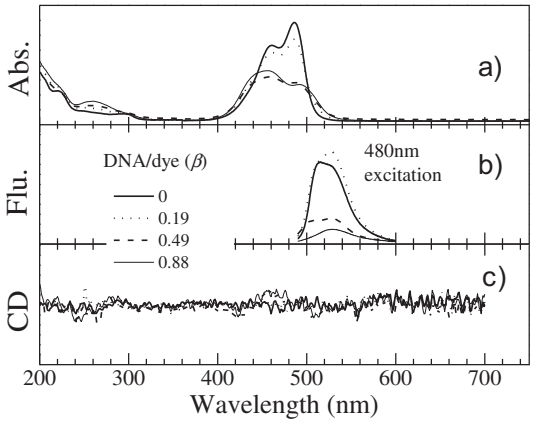

Fig. 8. Absorption, fluorescence and CD spectra for $DiOC_6(3)$.

shapes with a slight bathochromic shift [6,21,31,34]. In this region, apparently dye molecules exist as monomers and the red shift can be assigned to the formation of complexes made from DNA strand and a large number of monomeric molecules.

Elongation of the alkyl chain will give a structural modulation to molecules without the significant effects on electronic wave functions. The effects from the chain for $DiOC_n(3)$ series seemed to be limited because no significant changes were observed in their absorption and fluorescence spectra. But there were very complex

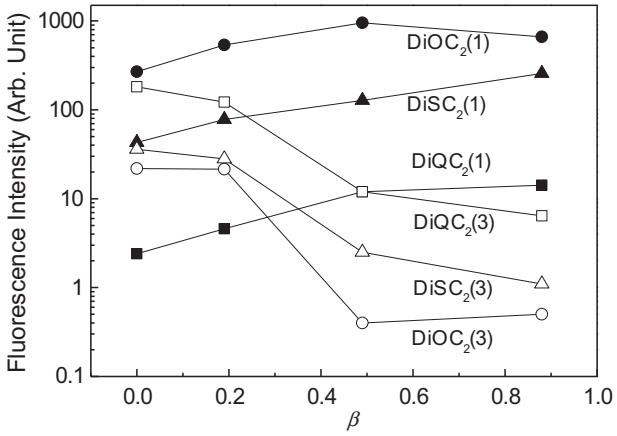

Fig. 9. Dependence of fluorescence peak intensity on the DNA/dye molar ratio for three types of cyanine dyes with $m = 1$ and 3.

structures in the CD spectra, which must be a reflection from the interaction between DNA and each dye molecules, although we did not yet make an explanation for the CD shapes. In order to investigate the effects for intercalation, we should make a similar series of compounds with shorter conjugation length [35]. Since the CD spectral shapes were known to be very sensitive to the interaction among molecules, proper analysis would give useful information about complex structure [36].

5. Conclusions

We systematically studied the spectroscopic properties of several types of cyanine dyes interacting with DNA in aqueous methanol, PVA containing solutions. Through the experiments conducted under different DNA/dye ratio, we found that molecular size is an important factor for their interaction modes. For molecules with a short polymethine chain (that is, $m = 1$), dye intercalates into DNA strand even when DNA concentration is small. In this case, fluorescence increases monotonically with DNA concentration until it saturates and no significant changes in spectral shape were observed. When the molecular chain is much longer, monomeric molecules form complex aggregates with small amounts of DNA accompanied by fluorescence quenching. However, an excess amount of DNA recovers and even enhances the fluorescence efficiency and recovers spectral shape indicating that dye molecules and DNA strand form polyionic complex. These results will be useful for the development of optical functional devices utilizing dye-DNA complex.

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