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Spectroscopic study of cyanine dyes interacting with the biopolymer, DNA

Yutaka Kawabe*, Sho Kato

Chitose Institute of Science and Technology, 758-65 Bibi, Chitose 066-8655, Japan

A R T I C L E I N F O

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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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* Corresponding author. Tel./fax: +81 123 27 6067.
E-mail address: y-kawabe@photon.chitose.ac.jp (Y. Kawabe).

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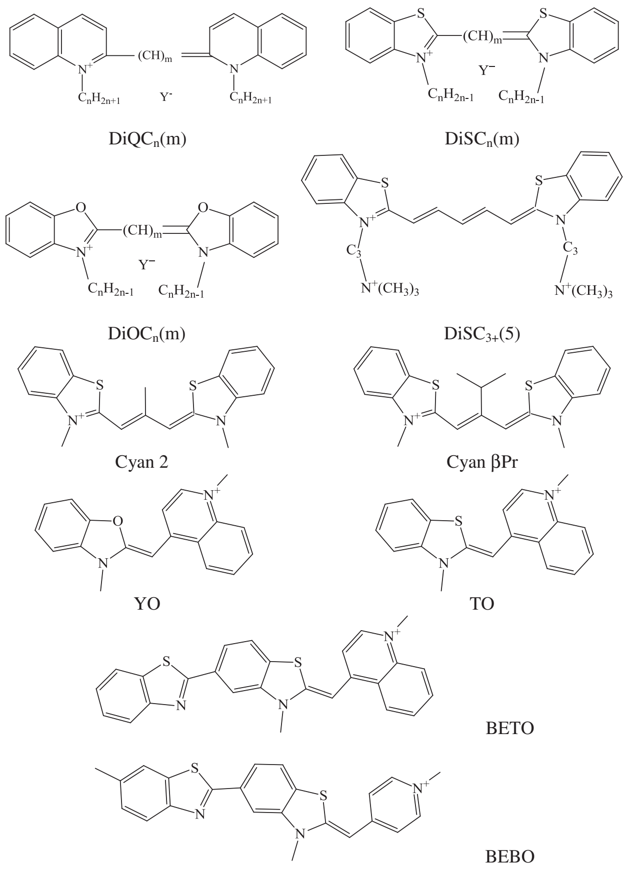

Fig. 1. Molecular structures of cyanine dyes employed for the experiments and referred in discussion, where *Y* means counter anions, and letters '*m*' and '*n*' indicate the conjugation chain length and alkyl chain length, respectively.

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	$DiOC_2(1)$	$DiOC_2(3)$		
		$DiOC_3(3)$	$DiOC_2(5)$	
		$DiOC_6(3)$		