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π-Stacked and Unstacked Aggregates Formation of 3,3'-Diethylthiatricarbocyanine iodide, A Near-infrared Dye

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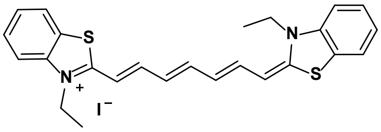
Absorbance and fluorescence spectra of 3,3'-diethylthiatricarbocyanine iodide (DTCI), a near-infrared dye, were measured in methanol and water at different concentrations. DTCI exhibited distinctive monomer and excimer (dimer) fluorescence emission bands on photo excitation in the two solvents. The monomer emission band at around 600-700 nm has been unprecedented, and the dimeric nature of the typical emission band at around 750-850 nm has not been recognized in solution. In the ground state, DTCI is proposed to form π-stacked and unstacked aggregates in methanol and water, respectively, at higher concentrations. The proposed unstacked aggregates in water may be of high molar mass leading to a short relaxation time in NMR analysis which did not show clear signals.

Dyes that emit light in the near infra-red (NIR) region are important in medicinal and physiological fields because they can be applied for diagnosis and imaging of tissues due to the fact that water and haemoglobin do not seriously interfere through scattering and absorbance in the range of around 700-900 nm.[1-6] As NIR dyes need to undergo photo excitation and emission under various environments in practical applications, it is important to obtain information on dependence and variation of their photo physical properties on conditions. This work discloses absorbance, excitation and emission behaviors of 3,3'-diethylthiatricarbocyanine iodide (DTCI) (Figure 1), a representative fluorescent NIR dye,[7-11] in water and methanol which varied sensitively depending on solvent properties and also concentration. Although the nature of the typically observed emission band at around 750-850 nm has not been disclosed, the results of the present work indicate that it arises from excited-state dimeric species. In addition, an emission band at around 600-700 nm was observed for the first time, and it is assigned to monomeric emitting species.

As for studies of π-stacked chromophores, there have been sophisticated reports on DNA assembled alternating dye stacks[12] and amphiphilic pyrene oligomers leading to two-dimensional polymers.[13] In these examples detailed structures of the stacks and their photo electronic properties have been unambiguously identified and presented.

In this work, first, in order to obtain information about ground-state behaviors of DTCI, its absorbance spectra were compared in methanol and in water (Figure 2). It should be noted that DTCI appeared not to be completely soluble in water under the conditions of this work; the aqueous samples are supernatant solutions separated from mixtures of water and DTCI, and the concentrations and in water have been accurately determined by dissolving a small amount of the supernatant solution in a large excess of methanol; absorbance values at 758 nm were used to calculate concentrations on the basis of molar absorptivities in methanol (Figures S1 and S2 in supporting information).



DTCI

Figure 1. Chemical structure of 3,3'-diethylthiatricarbocyanine iodide (DTCI).

While the spectra in methanol indicated rather sharp signals with peaks at around 750 nm, those in water had broad signals in the range of 600-1500 nm. In addition, molar absorptivity in methanol decreased with an increase in concentration. On the other hand, in water, molar absorptivity was higher at a higher concentration, and, in addition, contributions from the longer-wavelength range (800-1500 nm) were more significant at higher concentrations. The spectral characters in methanol may mean "π-stacked aggregate" formation causing hypochromism[14-18] which is facilitated at a higher concentration. On the other hand, the spectral behaviors in water may be interpreted in terms of two different types of aggregation whose contribution varies depending on concentration; aggregation

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x is contributed by π-stacking leading to hypochromicity at lower concentrations while aggregation occurs through more undefined inter-molecular interactions which do not involve π-stacking (“unstacked aggregate”) at higher concentrations.

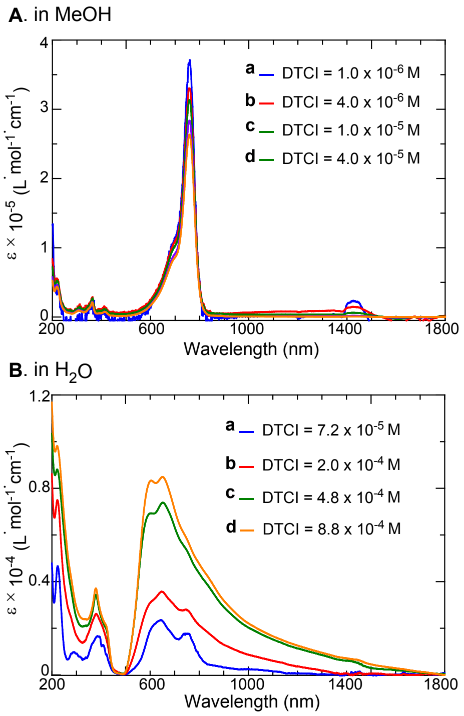

Figure 2. Absorbance spectra of DTCI in methanol (A) and in water (B): [DTCI] = 1.0 x 10^{-6} M (a), 4.0 x 10^{-6} M (b), 1.0 x 10^{-5} M (c), and 4.0 x 10^{-5} M (d) in A and 7.2 x 10^{-5} M (a), 2.0 x 10^{-4} M (b), 4.8 x 10^{-4} M (c), and 8.8 x 10^{-4} M (d) in B. [room temperature, 1-mm cell]].

The proposed difference in aggregate formation in methanol and water may also have connection to the difference in spectral shape in the two solvents. The facts that the spectra in water are more significantly contributed by the longer-wavelength signals (800-1500 nm) at higher concentrations and that the main peak at around 750 nm in methanol is red-shifted with respect to the main signals in water (600-700 nm) may mean that aggregation in methanol may be mainly contributed rather by J-aggregates and π-stacking in water rather by H-aggregates. Aggregates are characterized by absorbance peak shifts with respect to the band due to isolated molecule; J- and H-aggregates indicate red- and blue-shifted absorbance, respectively.[19] Although it is not clear where exactly isolated DTCI indicates a signal in absorbance spectra in methanol and water in Figure 2, it is proposed to be excited at around 650 nm through the discussions about emission and excitation spectra (Figure 4).

Temperature effects on absorbance spectra of DTCI were studied at 0-60°C at 1.0 x 10^{-6} M and 4.0 x 10^{-5} M in MeOH and at 20-80°C at 7.2 x 10^{-5} M and 8.8 x 10^{-4} M in H_2O (ESI Figures S4 and S5). In MeOH, spectral intensity decreased with no clear change in spectral shape on elevating solution temperature, suggesting that π-stacked aggregates may be stabilized or reinforced at a higher temperature. On the other hand, in H_2O, spectral shapes largely changed on elevating solution temperature where the peak at around 750 nm which seems to be common between in MeOH and in H_2O became rather dominant. These results may mean that π-stacked aggregates are favored at a higher temperature both in MeOH and H_2O.

Additional information was obtained about the different aggregation behaviors in methanol and water through NMR studies (Figure 3). Figure 2 shows the ^{1}H NMR spectra of DTCI measured in methanol-d$_4$ and D_2O. Clear signals relevant to the chemical structure of DTCI (Figure 1) were observed in methanol-d$_4$ whereas no signals were detected in D_2O even at a concentration twice as high as that in methanol-d$_4$. These results suggest that spin relaxation time in D_2O is much shorter than that in methanol-d$_4$. It is assumed that the proposed unstacked aggregates in water may have a much greater size and may be more rigid in structure than the π-stacked aggregates in methanol. The broad absorbance band in the longer-wavelength range (800-1500 nm) may be based on polymeric aggregates in DTCI in water (Figure 2B).

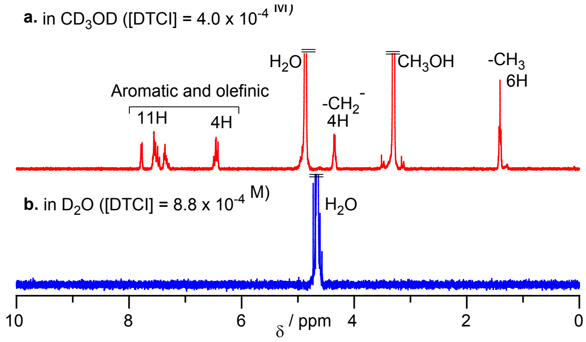

Figure 3. ^{1}H-NMR spectra of DTCI in methanol-d4 at 4.0 x 10^{-4} M (a) and in D_2O at 3.6 x 10^{-4} M (b) [400 MHz, ambient temperature].

Next, fluorescence emission and excitation spectra of DTCI were measured in methanol and in water at various concentrations (Figure 4). DTCI in most cases exhibited two emission peaks in the 750-850 nm and 600-700 nm ranges on photo excitation, which are suggestive of two distinctive types of excited-state species. The relative intensity of the longer-wavelength emission band increased with an increase in concentration in methanol (Figure 4A) while it decreased with an increase in concentration in water (Figure 4B).

In emission spectra of aromatic compounds, in general, monomeric species emit in a shorter-wavelength range and dimeric excited-state aggregates such as excimer or exciplex emit in a longer-wavelength range.[14-18,20] The longer-wavelength and shorter-wavelength bands in the emission spectra of DTCI may therefore be ascribed to monomer and excimer (dimer) emissions, respectively. This conclusion is in line with the proposal that π-stacked aggregates tend to form at a higher concentration in methanol while π-stacking is more significant at a lower concentration in water. Monomer and excimer emissions of DTCI have been previously proposed for a system in the presence of a cyclodextrin[9] though wavelengths in the literature do not match those in this work; the additive may alter excited-state properties.

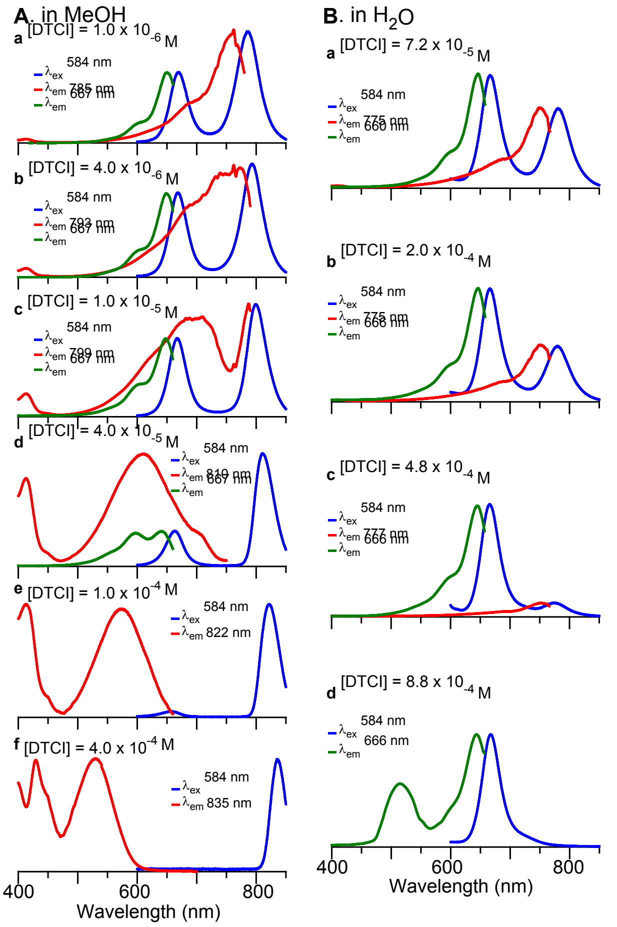

Figure 4. Emission (blue) and excitation (red and green) spectra of DTCI in methanol (A) and in water (B): [DTCI] = 1.0 x 10^{-6} M (a), 4.0 x 10^{-6} M (b), 1.0 x 10^{-5} (c), 4.0 x 10^{-5} M (d), 1.0 x 10^{-4} (e), and 4.0 x 10^{-4} (f) in A and 7.2 x 10^{-5} M (a), 2.0 x 10^{-4} M (b), 4.8 x 10^{-4} M (c), and 8.8 x 10^{-4} M (d) in B. Excitation wavelengths for fluorescence emission spectra (λ_{ex}) and monitoring wavelengths for excitation spectra (λ_{em}) are indicated above relevant spectra. [room temperature, 10-mm cell]

Further, excitation spectral shape varied depending on concentration. In methanol, the peak position of major signal in excitation spectra corresponding to the excimer emission band shifted from ca. 750 nm to ca. 550 nm with an increase in concentration, indicating that higher-energy excitation is more responsible for excimer formation at a higher concentration (Figure 4A, red spectra). This may mean that, although the absorbance spectra of DTCI taken at different concentrations have similar shapes (Figure 3A), the proposed π-stacked aggregates are not uniform in structure but consist of species corresponding to different photo excitation energies including the proposed H- and J-aggregates. Also, π-stacked aggregates composed of different numbers of DTCI molecules or those with different *trans*- and *cis*-isomerism[21,22] compositions may also lead to different excitation energies though details are yet to be studied.

The excitation spectra corresponding to the monomer emission bands in methanol did not vary largely in position with the major peak at around 650 nm at 1.0 x 10^{-6} M~1.0 x 10^{-5} M (Figure 4A a-c). Probably, isolated DTCI molecule may be excited under these conditions at around 650 nm and leads to excited-state monomeric species.

On the other hand, in water, the shapes of excitation spectra with a major peak at around 750 nm corresponding to excimer emission are virtually unchanged at the three lower concentrations, 7.2 x 10^{-5} M, 2.0 x 10^{-4} M, and 4.8 x 10^{-4} M (Figure 4B a-c, red spectra). A very similar tendency is confirmed for the excitation spectra corresponding to the monomer emission at the these three concentrations in water; the three excitation spectra have almost the same pattern with a peak at around 650 nm (Figure 4B a-c, green spectra). These results suggest that, within the lower concentration range from 7.2 x 10^{-5} M to 4.8 x 10^{-4} M in water, π-stacked aggregates formed in the ground-state have rather uniform structure and are excited at around 750 nm leading to excimer emission and that isolated DTCI molecule is excited at around 650 nm leading to monomer emission. The proposed excitation and emission mechanism involving isolated DTCI is common between the systems in methanol and in water.

Only at the highest concentration (8.8 x 10^{-4} M) in water, monomer emission is significantly contributed by excitation at around 500 nm as well as at around 650 nm (Figure 4B d). At this concentration, probably most of DTCI molecules are involved in the rather undefined, unstacked aggregates proposed based on the absorbance spectral features, and DTCI molecules in such aggregates may have higher excitation energies than the isolated ones.

In order to rule out the possibility that reabsorption distorts excitation spectral shapes in Figure 4, the same measurements were conducted through front-face illumination[21] which resulted in the spectral shapes which are virtually the same as those in Figure 4 (Figure S3 in supporting information). The excitation spectra in Figure 4 thus reflect molecular and inter-molecular characters of DTCI but not reabsorption effects.

In conclusion, DTCI shows distinctive monomer and excimer (dimer) fluorescence emission bands in the wavelength ranges of 600-700 nm and 750-850 nm, respectively, and their relative intensity varies depending on solvent and concentration. The monomer emission band has been unprecedented, and the dimeric nature of the typical emission band has not been recognized in solution.

DTCI is proposed to form π-stacked aggregates in methanol regardless of concentration within the range of 1.0 x 10^{-6}~4.0 x 10^{-6} M and also in water at a lower concentration while it appears to form unstacked aggregates in water at a higher concentration which may have a polymeric structure with a large size. In both MeOH and H_2O, π-stacked aggregates seemed to be favored at a higher temperature.

In methanol, π-stacked aggregates having various structures with different excitation energies are responsible for excimer emission whereas, in water at a lower concentration, π-stacked aggregates with more uniform structure than those in methanol lead to excimer emission. In both solvents, monomer emission may arise mainly from isolated DTCI which is excited at ca. 650 nm. It is notable that, in water at the highest concentration, unstacked aggregates as well as isolated DTCI may contribute to monomer emission.

The proposed differences in major aggregate structures in methanol and water may arise from the ionic nature of DTCI; the charged aromatic group of DTCI may attract each other and forms a stack through ionic interactions in methanol while it is well solvated by water molecules preventing the formation of ionic stacking and leading to less ordered, unstacked aggregates in water. However, detailed structures of the proposed aggregates including the number of molecules involved and their relative orientations are not yet known. In addition, photo physical property details such as relative exciton energies and corresponding electronic transition dipole moments are not yet clear. These aspects require further investigations.

It has been reported that π-stacking of ionic dyes can be effectively controlled through binding to macrocyclic host molecule in water.[23-27] The aggregation behaviors of DTCI found in this work may be extended to an alternative method of π-stacking control based simply on solvent properties.

Majority of studies on DTCI emission have been so far conducted in organic solvents, and work about aqueous systems is rare in spite of the fact that medicinal and physiological applications require dye performances in water. The information disclosed in this report would thus be valuable in interpreting data obtained in aqueous systems. Especially, the finding of the two distinctive emission bands based on monomer and excimer may open a way to utilize wavelength distribution (color) information from tissues in bio imaging which may disclose conditions undetectable through single-wavelength detection and analysis based merely on brightness and darkness.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.
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