March, 1982] Bull. Chem. Soc. Jpn., 55, 717—720 (1982) 717

Highly Aggregated State of the Dye with the Detergent in the Premicellar Region as Revealed by Resonance Raman Spectra

Masaaki Katsumata, Kazuo Kasatani, Masahiro Kawasaki, and Hiroyasu Sato*
Chemistry Department of Resources, Faculty of Engineering, Mie University, Tsu 514

(Received September 3, 1981)

Resonance Raman spectra of 3,3′-diethylthiacarbocyanine iodide–sodium dodecyl sulfate (SDS) system are characterized by [SDS]-dependent change of band intensities. The change was much larger than that accompanied with the shift of monomer-dimer equilibrium in the aqueous solution. The spectra for $[\mathrm{SDS}] \ll$ critical micelle concentration (cmc) resemble that of the dye in the solid state, indicating the highly-aggregated state of the dye caused by the interaction with the detergent.

The dye-detergent systems are currently attracting considerable interest from several viewpoints, *e.g.*, highly enhanced energy transfer between the dyes in these systems making these as good model membrane systems of chloroplasts,1–6) and the study of photophysical processes of the dye in micelles in relation to the improvement of lasing properties of dyes.7–8)

The presence of peculiar interaction between the dye and detergent carrying the opposite charge has long been known by the color change of the dye which is dependent on the concentration of the detergent (metachromasy).9) Some investigations have been made on this phenomenon by absorption and fluorescence spectra,10–12) and also by conductivity measurements.12) Recently, the fluorescence decay studies provide very efficient means to clarify the nature of the dye-detergent interaction.2,13–14) All of these investigations indicate the formation of some dye-detergent aggregate *below* the critical micelle concentration (cmc), *i.e.*, in the *premicellar* region.

In this paper, the resonance Raman spectra of 3,3′-diethylthiacarbocyanine iodide (DTC), a cationic dye, was studied in the presence of sodium dodecyl sulfate (SDS), an anionic detergent, to elucidate the nature of change dependent on the concentration of SDS (abbreviated as [SDS]) in the aggregation state of DTC, especially in the premicellar region. The interaction of DTC with SDS was discussed in comparison with its interaction with polyethylene glycol mono-[*p*-(1,1,3,3-tetramethylbutyl)phenyl] ether (PGME), a nonionic detergent, with hexadecyltrimethylammonium bromide (HTAB), a cationic detergent, and with potassium poly(vinyl sulfate) (PVSK), an anionic polyelectrolyte.

Experimental

Laser-grade DTC ("Dotite" reagent from Dojindo), protein-research grade SDS (Nakarai Chemicals), liquid-scintillation grade PGME (Nakarai), G. R. grade HTAB (Nakarai), and PVSK (Wako) were used as received. Water was distilled twice. Spectrograde methanol was used without further purification.

Raman spectra were recorded using a JASCO CT-80D double monochromator and a Hamamatsu R-649/C-1050 photomultiplier-photon counting unit. The excitation lines at 457.9 nm, 465.8 nm, 472.7 nm, 476.5 nm, and 488.0 nm were obtained from a Spectra-Physics model 164 Ar^+ laser. Since some color change was observed for DTC–SDS solutions after mixing, the solutions were kept in the dark until the color change became undetectable (more than 30 min). A flow-cell composed of a 2.5 mm i.d. glass tube was used in the measurements to avoid the degradation of the dye by the continued irradiation. Solid samples were measured as spinning KBr discs.

Absorption and fluorescence spectra were recorded on a Shimadzu UV-200 recording spectrophotometer and on a Hitachi 650-10S fluorescence spectrophotometer, respectively.

The cmc of SDS was determined conductometrically to be 7.4±0.1 mM (at 25±0.1 °C).[†]

Results

DTC–SDS Solutions. Some of the Raman spectra of DTC–SDS solutions are shown in Fig. 1, together with that of the aqueous solution. The concentration of the dye is 1.0×10^{-5} M. The excitation wavelength is 488.0 nm. The spectra changed with [SDS]. The spectrum at $[\mathrm{SDS}] \ll \mathrm{cmc}^{15)}$ has a large difference from that of the aqueous solution. The difference decreased with [SDS], and the spectrum above cmc was again similar to that of aqueous solution. While the positions (in cm^{-1}) of Raman bands did not show appreciable shift, their relative inten-

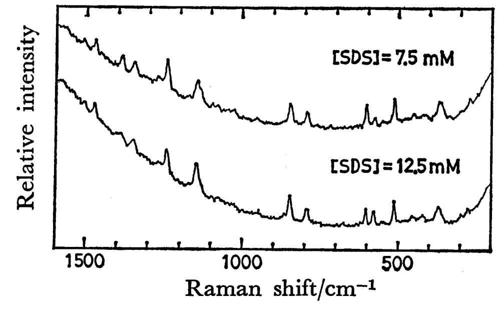

Fig. 1. Raman spectra of aqueous solution of DTC and DTC–SDS, $[\mathrm{DTC}]=1.0\times10^{-5}$ M, exciting wavelength: 488.0 nm.

[†] $1\ \mathrm{M}=1\ \mathrm{mol/dm^3}$, $1\ \mathrm{mM}=1\times10^{-3}\ \mathrm{mol/dm^3}$ in this paper.

sities varied substantially as a function of [SDS], as shown in Fig. 2 for some of the bands. The intensities of each band were corrected for reabsorption in the cell. The intensity of 841 cm^{-1} band was chosen as an internal reference standard.[16] The bands can be classified into three groups by their [SDS]-dependent behavior: (a) the intensity increases for [SDS]$\ll$cmc, (b) the intensity was almost independent of [SDS], and (c) the band disappeared for [SDS]$\ll$cmc. The most peculiar is the band at 569 cm^{-1}, which belonged to the last group.

Aqueous and Methanol Solutions of DTC. The Raman spectra of aqueous solution of DTC were measured in the concentration range 1×10^{-5} M—1×10^{-4} M. The tenfold change in the concentration is accompanied with a large shift in the monomer-dimer equilibrium as shown by the absorption spectra (Fig. 3). The 554 nm and 507 nm peaks can be attributed to monomer and dimer, respectively.[14] However, the intensity change of Raman bands was found to be rather small, as depicted in Fig. 4. The absorption spectra of DTC in methanol show that DTC is present essentially as a monomer, because (1) the shape of the absorption band does not show any appreciable concentration dependence, and (2) it is similar to that of dilute ([DTC]$<2.0\times10^{-5}$ M) aqueous solution, apart from a small red shift. The Raman spectrum of methanol solution of DTC (4.0×10^{-5} M) was quite similar to that of aqueous solution (1.0×10^{-5} M). These results indicate that the shift of monomer-dimer equilibrium in the aqueous solution causes only a small change in the relative intensities of Raman bands.

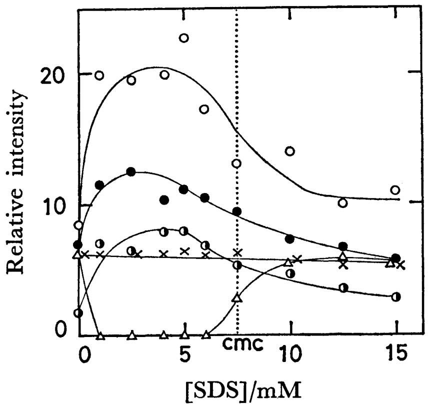

Fig. 2. Dependence of Raman intensities on [SDS]. △ 569 cm^{-1}, ● 601 cm^{-1}, × 791 cm^{-1}, ○ 1238 cm^{-1}, ◑ 1383 cm^{-1} band, [DTC]$=1.0\times10^{-5}$ M, exciting wavelength: 488.0 nm.

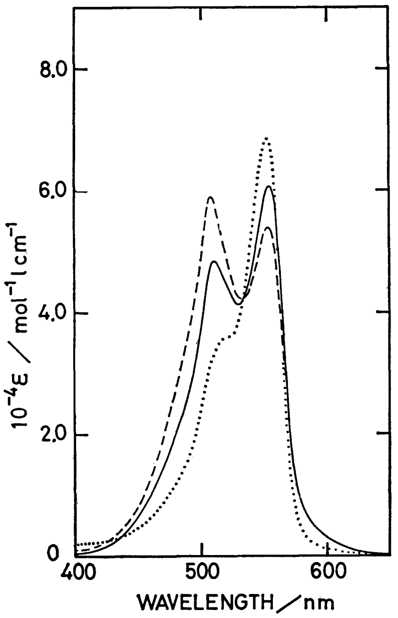

Fig. 3. Absorption spectra of aqueous solution of DTC. [DTC]: --- 1.0×10^{-4} M, —— 5.0×10^{-5} M, ······ 1.0×10^{-5} M.

Solid Spectrum (KBr Disc). The Raman spectrum of DTC in KBr disc is shown in Fig. 5. It is to be noted that relative intensities of the bands in the spectrum of DTC–SDS at [SDS]$\approx$2.5 mM closely resemble those of the solid. The 569 cm^{-1} band disappeared completely in the solid spectrum.

DTC–PGME, -HTAB, and -PVSK. Absorption and Raman spectra of these systems were studied. PGME, a nonionic detergent, has a deaggregation

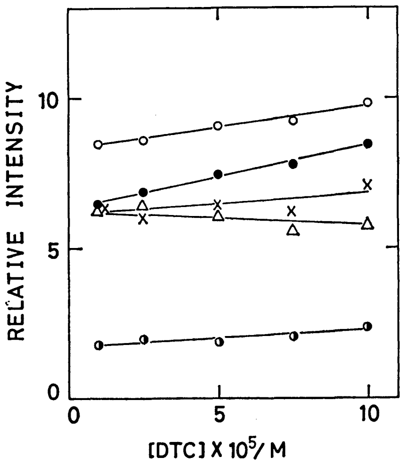

Fig. 4. Dependence of Raman band intensities on [DTC], exciting wavelength: 488.0 nm. The symbols the same as those in Fig. 2.

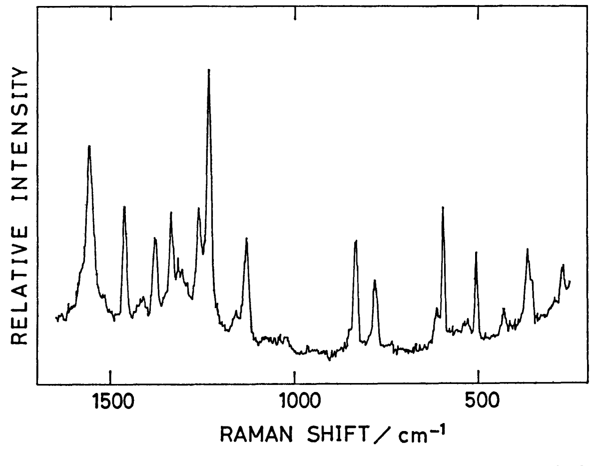

Fig. 5. Raman spectrum of solid DTC (KBr disc), exciting wavelength: 488.0 nm.

effect on DTC as revealed by the decrease of the intensity of dimer band (at 507 nm). However, the corresponding change in the Raman spectra was very little. HTAB, a cationic detergent, did not give any appreciable change in either of absorption and Raman spectra. The positive charge of the HTAB micelle will repel the cationic dye, DTC. PVSK, an anionic polyelectrolyte, (1×10^{-2} g/l—10 g/l)-DTC(1×10^{-4} M) gave the absorption spectra and Raman spectra which are very similar to those of DTC–SDS ([SDS]$\approx$2.5 mM), showing the interaction of $-OSO_3^-$ groups on the polymer chain with DTC.

Effect of Excitation Wavelength. When we measure the resonance Raman spectra of DTC–SDS solutions as a function of [SDS] by exciting at a fixed wavelength, the change in the resonance conditions is inevitable because the absorption spectra changes with [SDS], as shown in Fig. 6. The observed results should be attributed to the joint effect of (1) change in the aggregation state of the dye, and (2) change in the resonance condition. The latter effect can be divided into those due to (2-a) the change in absorption spectra by the shift of monomer-aggregate equilibrium and (2-b) the simple wavelength shift of absorption bands due to environmental effect. The effect (2-a) has the common origin as (1), and what is to be investigated in this study. If the observed results are solely due to (2-b), they are only apparent results, nothing to do with the change in the aggregation state of the dye. In case the observed results are solely due to (2-b), the same results will be obtained by the change in the excitation wavelength. The effect of excitation wavelength on the relative Raman band intensities (the intensity of 841 cm^{-1} being taken as a standard) was studied for aqueous solutions ([DTC]$=1.0\times10^{-4}$ M and 1.0×10^{-5} M) and methanol solution ([DTC]$=4.0\times10^{-5}$ M). Although the effect of excitation wavelength did exist as shown in Fig. 7 taking [DTC]$=1.0\times10^{-5}$ M as an example, there was no direct relationship to the classification of (a), (b), and (c) of [SDS]-dependent intensity change mentioned above. Therefore, the observed [SDS]-dependent change cannot be attributed to the change of resonance condition caused by the shift in the absorption bands (*i.e.* effect 2-b).

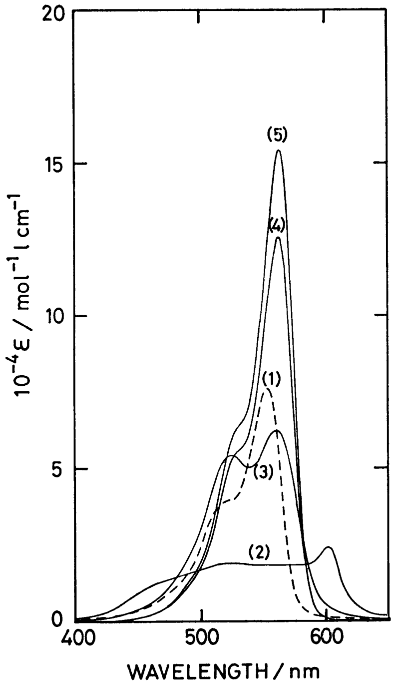

Fig. 6. Absorption spectra of aqueous solution of DTC–SDS.
[DTC]$=1.0\times10^{-5}$ M, [SDS]: (1) 0 mM, (2) 2.5 mM, (3) 5 mM, (4) 7.5 mM, (5) 15 mM.

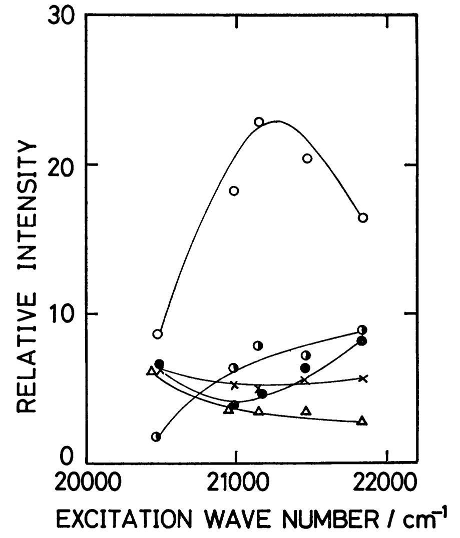

Fig. 7. Dependence of Raman band intensities on excitation wave number, △ 569 cm^{-1}, ● 601 cm^{-1}, × 791 cm^{-1}, ○ 1238 cm^{-1}, ◑ 1383 cm^{-1} band. [DTC]$=1.0\times10^{-5}$ M.

Discussion

The dye-detergent interaction was studied with Raman spectra by several authors on several occasions. Verma and Wallach[17)] reported the intensities of 1530 cm^{-1} (–C=C– stretching) and 1165 cm^{-1} (=C–C= stretching) of *β*-carotene were reduced by the addition of SDS. Kim *et al.*[18)] found the change of relative intensities of Raman bands at 1420 cm^{-1} and 1155 cm^{-1} of Methyl Orange (MO, an anionic dye) on addition of HTAB below and above cmc. Takenaka and Nakagawa[19)] studied the monolayer of MO–HTAB adsorbed at the interface between carbon tetrachloride and aqueous solution. They found a shift of band frequencies of MO in the monolayer from those in bulk aqueous solution toward those of solid MO, indicating that the MO molecules in the monolayer were in an environment similar to a crystal field. Koyama and Matsuo[20)] found that 594 cm^{-1} band in the azobenzene–behenic acid system was present in the molten state, but absent in the solid state. They stated that this band can be used as a probe of the aggregation state of the system.

The [SDS]-dependent change of Raman spectra presently studied is not due merely to the change of resonance condition caused by the shift in the absorption bands, and is due to the change of aggregation state of the dye. For [SDS]≪cmc (*e.g.*, 2.5 mM), the dye is in the aggregation state like in the solid. As the dye is considered to be in a highly aggregated state in the solid, the state of the dye for [SDS]≪cmc can also be characterized as a highly aggregated state. The comparison with aqueous solution shows that the aggregation state of the dye in SDS solution with [SDS]≪cmc and in solid state is different from that of the dimer in a concentrated aqueous solution. The data in Fig. 2 show that the intensity maxima of bands characteristic to the highly aggregated dye lie around [SDS]=2.5—3.0 mM. When [SDS] approaches cmc, the spectra show the intermediate feature between [SDS]=2.5—3.0 mM and [SDS]>cmc.

The study of absorption and fluorescence spectra, fluorescence intensity and decay behavior as a function of [SDS][14] revealed that there are two regions below cmc. The region [SDS]≪cmc is characterized with a very broad absorption band and the anomolous long-wavelength fluorescence (655 nm). The dye-detergent salt (with nearly 1:1 stoichiometry) as proposed by Mukerjee and Mysels[12] for pinacyanol-SDS seems to be formed. "The highly aggregated dye" must be found in such a dye-detergent aggregate. The resemblance of absorption and Raman spectra to those of DTC–PVSK supports this assumption. For some dye-detergent systems, the dye-detergent aggregate forms a large particle and precipitates from the solution in the low [SDS] region. In the DTC–SDS system presently studied, very minute solid particles seem to be formed in the solution. The formation of such particles can be monitored by the appearance of naturally emitting lines of Ar^+ laser in the observed Raman spectra. These lines are found around [SDS]=1.0 mM. Since the maximum intensities of the bands characteristic to the highly aggregated dye were observed around [SDS]=2.5—3.0 mM, we can safely say that the observed bands are due to dye-detergent aggregate, but not due to solid particles.

For 5.0×10^{-3} M≤[SDS]<cmc, the absorption bands of monomer and dimer dye were observed along with the monomer fluorescence.[14] (Dimers are non-fluorescent.) The energy transfer efficiency between dyes are highly enhanced in this [SDS] region. The presence of dye-rich induced micelles as proposed by Mukerjee and Mysels[12] is plausible in this region. As far as the Raman spectrum is concerned, this region is not characterized by any peculiar feature, but by the intermediate one between [SDS]≪cmc and [SDS]>cmc. In other words, the gradual change of dye-detergent aggregate to micellized monomer dye did not show any peculiarity in the Raman spectra.

For [SDS]>cmc, the absorption spectra draw nearer to the monomer dye. The Raman spectra were similar to those of aqueous and methanol solutions. The deaggregation effect of SDS on the dye did not give any significant change in the Raman spectra.

In summary, resonance Raman spectra proved to be a very useful tool in elucidating the aggregation state of the dye in the dye-detergent systems. The 569 cm^{-1} band works as an useful monitor of the aggregation state of DTC.

The present authors would like to thank Dr. Katsunosuke Machida, Kyoto University, for measurement of solid spectra and for helpful discussions. They are grateful to Miss Yuko Hanyu for her assistance. This study is partly supported by a Grant-in-Aid for Scientific Research No. 447006 from the Ministry of Education, Science and Culture.

References

1) G. S. Singhal, E. Rabinowitch, J. Hevesi, and V. Srinivasan, *Photochem. Photobiol.*, **11**, 531 (1970).

2) E. Lohoczki and J. Hevesi, *Dokl. Akad. Nauk SSSR*, **206**, 1158 (1972).

3) Y. Kusumoto and H. Sato, *Chem. Phys. Lett.*, **68**, 13 (1979).

4) Y. Usui and A. Gotou, *Photochem. Photobiol.*, **29**, 165 (1979).

5) H. Sato, Y. Kusumoto, N. Nakashima, and K. Yoshihara, *Chem. Phys. Lett.*, **71**, 326 (1980).

6) T. Matsuo, Y. Aso, and K. Kano, *Ber. Bunsenges. Phys. Chem.*, **84**, 146 (1980).

7) G. A. Kenney-Wallace, J. H. Flint, and S. C. Wallace, *Chem. Phys. Lett.*, **32**, 71 (1975).

8) H. E. Lessing and A. Von Jena, *Chem. Phys.*, **41**, 395 (1979).

9) The color change was used in the "spectral change method" of determining cmc. *Cf.* M. L. Corrin and W. D. Harkins, *J. Am. Chem. Soc.*, **69**, 683 (1947).

10) N. Mataga and M. Koizumi, *Nippon Kagaku Zasshi*, **75**, 35 (1954).

11) N. Mataga and M. Koizumi, *Bull. Chem. Soc. Jpn.*, **27**, 197 (1954).

12) P. Mukerjee and K. J. Mysels, *J. Am. Chem. Soc.*, **77**, 2937 (1955).

13) J. Knof, F. J. Theiss, and J. Weber, *Z. Naturforsch. Teil A*, **33**, 98 (1978).

14) H. Sato, M. Kawasaki, K. Kasatani, Y. Kusumoto, N. Nakashima, and K. Yoshihara, *Chem. Lett.*, **1980**, 1529.

15) Although cmc shows a small shift towards smaller [SDS] on addition of the dye, the shift is rather small. The shift of 0.5—0.6 mM was reported in the literature (Refs. 3 and 12).

16) The addition of external standard, like MnO_4^- or SO_4^{2-} was tried. It was not successful, however, because the line was too weak, or because the added substance gave some effect on the aggregate formation.

17) S. P. Verma and D. F. H. Wallach, *Biochem. Biophys. Acta.*, **401**, 168 (1975).

18) B. K. Kim, A. Kagayama, Y. Saito, K. Machida, and T. Uno, *Bull. Chem. Soc. Jpn.*, **48**, 1394 (1975).

19) T. Takenaka and T. Nakagawa, *J. Phys. Chem.*, **80**, 475 (1976).

20) Y. Koyama and T. Matsuo, "Laser Raman Bunkogaku To Sono Oyo," ed by T. Shimanouchi, M. Tasumi, and I. Harada, Nankodo, Tokyo (1977), Chap. 11.