Solubilization of Methylene Blue in Reversed Micelles, Effect of Water

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The solubilization of methylene blue in dodecylammonium propionate reversed micelles in cyclohexane depended strongly on the solubilized water content. Methylene blue solubilized in the micelle of which head groups are not completely hydrated by bound water exhibited the new absorption bands at 495 and 270 nm, but these bands did not appear in the micelle containing free water. This dye had the absorption bands at 655 and 290 nm in the latter micelle. The solubilizing process and solubilized state of methylene blue in the reversed micelles were discussed with results obtained.

Introduction

Recently, chemical reactions of the reactants solubilized in reversed micelles are attracting many worker's attention [1, 2] from the interest of biomimic chemistry. The DAP reversed micelle in cyclohexane has a hydrophilic environment inside the micelle, and solubilizes various hydrophilic molecules. The DAP micelle changes its size (aggregation number) depending on the solubilized water content [3]. MB has been most commonly used for studying the photo-oxidation-reduction reaction and photosensitization reaction such as singlet oxygen production [4, 5] and photodynamic action [6, 7]. The solubilized state of reactants is one of the important factors for the chemical reactions in micelle.

The present paper deals with the absorption spectrum of MB solubilized in DAP reversed micellar solutions at various water contents. The solubilizing process and solubilized state of MB were investigated in relation to the solubilized water.

Materials and Methods

MB and DAP purchased from Katayama Chemical Co. were guaranteed reagents. DAP was purified from recrystallization twice from *n*-hexane. Cyclohexane and aqueous solutions (0.1 N) of NaOH and HCl were obtained from Katayama Chemical Co. Laboratory-distilled water was further distilled from alkaline KMnO_4 solution. Bulk pH's (3-12) in

aqueous MB solutions were adjusted with 0.1 N NaOH and 0.1 N HCl, and were measured with a pH-meter (Hitachi-Horiba, type F-7 ss).

Reversed micellar solution was prepared by dissolving DAP in cyclohexane. The DAP cyclohexanic solution was stirred in the dark at 40 °C after adding a definite amount of the concentrated aqueous MB solution (e.g., aqueous solutions of $2.0 \cdot 10^{-3}$, $1.0 \cdot 10^{-3}$ and $4.0 \cdot 10^{-4}$ M MB for the preparation of 0.1, 0.2 and 0.5 M H_2O DAP micellar solutions, respectively). Most of the present experiments were carried out at pH 10.0. The monocationic blue form of MB is stable in the pH range 2-12 [8]. The final concentration of MB was $3.6 \cdot 10^{-6}$ M in all the sample solutions, and that of H_2O was varied from 0.1 to 0.5 M.

The absorption spectrum was measured with a Hitachi spectrophotometer type 124 or 356 at 30 °C under aerobic conditions. An optical vessel (length of optical path, 0.036-0.090 mm) was used for measuring the absorption spectrum of concentrated aqueous MB solutions (10^{-4} - 10^{-3} M).

Results and Discussion

Fig. 1 shows changes in the absorbance of the MB monomer band (655 nm) with the time after mixing of DAP cyclohexanic and aqueous MB solutions. The absorbance unchanged with the time in the DAP micellar solution containing 0.5 M H_2O , but it decreased with the time in the DAP micellar solution containing 0.2 or 0.1 M H_2O . This absorbance decrease was more remarkable in the 0.1 M H_2O DAP micellar solution than in the 0.2 M H_2O DAP micellar one. The absorbance decrease with the time was observed below 0.5 M H_2O . The saturation value

Abbreviations: MB, methylene blue; DAP, dodecylammonium propionate.

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Fig. 1. Changes in the absorbance of MB with the time after mixing of DAP cyclohexanic and aqueous MB solutions. Concentrations of MB and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively; Curves 1, 2 and 3, DAP reversed micellar solutions containing 0.1, 0.2 and 0.5 M H_2O , respectively; $\bullet$ (1) and $\bullet$ (2), absorbances after adding 0.4 M H_2O to Curve 1 solution and 0.3 M H_2O to Curve 2 solution, respectively; bulk pH's of aqueous MB solutions and H_2O added, 10.0; temperature, 30 °C.

at 20 min was 0.002 for the 0.1 M H_2O DAP micellar solution and 0.13 for the 0.2 M H_2O DAP micellar one. A little higher absorbance than that in the 0.5 M H_2O DAP micellar solution was obtained by adding 0.4 M H_2O to the 0.1 M H_2O DAP micellar solution or 0.3 M H_2O to the 0.2 M H_2O DAP micellar one at the saturation state (20 min), as shown by the arrow in Fig. 1. This indicates that the solubilization of MB depends on the procedure of the sample preparation. The final concentration of MB in Curves 1, 2 and 3 was equally $3.6 \cdot 10^{-6}$ M. The initial absorbances in these curves could not be measured, but they may highly depend on the MB concentration in its aqueous solution before mixing it with DAP cyclohexanic solution.

Fig. 2 shows the absorption spectra of MB in aqueous and DAP reversed micellar solutions. Curve 1 is the absorption spectrum of MB at 3 min after mixing of aqueous MB and DAP cyclohexanic solutions. The final concentrations of MB and H_2O were $3.6 \cdot 10^{-6}$ and 0.2 M, respectively. The concentration of MB in the aqueous solution used for the sample preparation was $1.0 \cdot 10^{-3}$ M, and Curve 3 is the spectrum measured with the optical path of 0.036 mm. The absorption bands at 655 and 600 nm are attributed to the monomer and dimer of MB, respectively. The dimer band in Curve 3, was higher than the monomer one, but the latter band in Curve 1 became inversely higher than the former. This indicates that large part of the dimers in aqueous solution were solubilized in DAP micelles after dissociated to monomers in the process of the DAP

Fig. 2. Absorption spectra of MB in bulk aqueous and DAP micellar cyclohexanic solutions. Curves 1 and 2, measured at 3 and 20 min after mixing of aqueous MB and DAP cyclohexanic solutions, respectively; Curve 3, measured with the optical path of 0.036 mm (aqueous solution of $1.0 \cdot 10^{-3}$ M MB); Curve 4, aqueous MB solution; final concentration of MB, $3.6 \cdot 10^{-6}$ M in Curves 1, 2 and 4; concentrations of H_2O in Curves 1 and 2, 0.2 M; concentration of DAP, $8.0 \cdot 10^{-2}$ M; bulk pH of aqueous MB solution, 10.0; temperature, 30 °C. The MB concentration involved in the optical path was same in Curves 1 to 4.

micellar formation. Curve 2 is the spectrum 20 min after the mixing (saturation state in Fig. 1). The spectrum in the aqueous MB solution ($3.6 \cdot 10^{-6}$ M) is shown as Curve 4, where most of MB molecules are present in the monomer form. The monomer bands in Curves 1 and 2 are blue-shifted by 5 nm compared with that in Curve 4. This blue-shift may be caused by the interaction between MB and the micro-field inside the micelle.

The decrease in the monomer band with the time in Fig. 1 accompanied the appearance of a new band at 495 nm. The appearance of the 495 nm band was more pronounced in DAP micellar solution with lesser H_2O content as shown in Fig. 3. The monomer and dimer bands in the visible region almost disappeared in the 0.1 M H_2O DAP micellar solution. Further, the 290 nm band in aqueous MB solution was replaced by a new broad band at 270 nm

Fig. 3. Absorption spectra of MB in DAP reversed micellar solutions at various H_2O concentrations 20 min after mixing of aqueous MB and DAP cyclohexanic solutions. Concentrations of MB and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively; Curves 1, 2 and 3, solubilized H_2O of 0.1, 0.2 and 0.5 M, respectively; Curve 4, after adding 0.4 M H_2O to Curve 1 solution; bulk pH's of aqueous MB solutions and H_2O added, 10.0; temperature, 30°C .

(Curve 1). In the 0.2 M H_2O DAP micellar solution, the monomer, dimer and 290 nm bands were still present (Curve 2). The 495 nm band did not appear in the 0.5 M H_2O DAP micellar solution, and the content ratio of dimer to monomer was comparatively high compared with that in the 0.2 M H_2O DAP micellar solution (Curve 3).

Next, when 0.4 M H_2O was added to the Curve 1 solution, the monomer (652 nm) and 290 nm bands developed remarkably (Curve 4). But the dimer band was much weaker than that in Curve 3. The shape of Curve 4 quite resembled to that of Curve 4 (aqueous MB solution) in Fig. 2. Namely, Curve 3 was not obtained by adding 0.4 M H_2O to the Curve 1 solution. This indicates that the dimers were bound to 0.5 M H_2O DAP micelles as monomer on the formation of the 0.5 M H_2O DAP micelle from the 0.1 M H_2O DAP micelle (redistribution of MB in DAP micelles). The absorbances at 495 nm (A_{495}) and monomer (A_{655}) bands in DAP micellar solutions at various water contents are shown in Fig. 4. The absorbance was measured 20 min after mixing of aqueous MB and DAP cyclohexanic solutions. A_{495} decreased rapidly with increasing the

water content, but A_{655} inversely showed a rapid increase.

A_{655} and A_{495} are plotted in Fig. 5 at various bulk pH's in 0.2 M H_2O DAP micellar solutions. At lower pH's below 5, A_{655} had a high constant value but A_{495} vanished. At higher pH's above 5, A_{655} showed a slight decrease and A_{495} did a gradual

Fig. 4. Absorbances at 495 (A_{495}) and 655 (A_{655}) nm bands of MB as a function of H_2O concentration. Concentrations of MB and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively; Curves 1 and 2, A_{495} and A_{655} , respectively; bulk pH's of aqueous MB solutions and H_2O added, 10.0; temperature, 30°C .

Fig. 5. Absorbances at 495 (A_{495}) and 655 (A_{655}) nm bands of MB at various bulk pH's. Concentrations of MB, DAP and H_2O , $3.6 \cdot 10^{-6}$, $8.0 \cdot 10^{-2}$ and 0.2 M, respectively; Curves 1 and 2, A_{495} and A_{655} , respectively; temperature, 30 °C.

increase. These changes may be related to the binding state of MB which depends on the dissociation state of the head groups (amine and hydroxyl) in DAP reversed micelles.

The results obtained are summarized as a diagram shown in Fig. 6. On the formation of the reversed micelles containing low concentrations of H_2O such as 0.1 and 0.2 M, MB molecules in aqueous solution are fractionated by DAP micelles. However, large part of dimers present in aqueous solution dissociate to monomers when solubilized in the micelles. Some monomers may be stably bound to the DAP micelles about 20 min after mixing of aqueous MB and DAP cyclohexanolic solutions under the present experimental conditions. The state of MB molecules in the 0.5 M H_2O DAP micelles which was obtained by adding H_2O to 0.1 M H_2O or 0.2 M H_2O DAP micelles was different from that in the 0.5 M H_2O DAP micelles prepared originally as stated above.

Correl *et al.* [3] described that the aggregation number of DAP micelle is strongly dependent on the solubilized water content. Parameters for 0.08 M DAP reversed micelles were obtained at various solubilized H_2O contents from the data of Correl *et al.* These are given in Table I. The average

Fig. 6. A diagram for the solubilization of MB in DAP reversed micelles. States of (1), (1') and (1''), immediately after mixing of aqueous MB and DAP cyclohexanolic solutions, respectively; states of (2), (2') and (2''), after solubilization of MB in DAP micelles; shaded portion, bound water; dotted portion, free water; ■, MB monomer; ■—■, MB dimer.

number of H_2O molecules per one DAP micelle increases with increasing the solubilized H_2O concentration. Correl *et al.* assumed that one head group binds two H_2O molecules at maximum as bound water and H_2O molecules more than two are bound as free water inside the micelle. According to this assumption, the free water appears in DAP micelles containing H_2O more than about 0.3 M as seen in Table I. Further, about 70 and 40% of the head groups are not hydrated with bound water in 0.1 and 0.2 M H_2O DAP micellar solutions, respectively. Accordingly, the appearance of the 495 nm band depending on the solubilized H_2O concentration (Fig. 4) is explained as follows. After mixing of aqueous MB and DAP cyclohexanolic solutions, DAP

Table I. Parameters for 0.08 M DAP reversed micelles at various water contents. N, average aggregation number of DAP micelle from the data obtained by Correl *et al.* [3]; n, number of head groups (amine and hydroxyl) in one DAP micelle; C_M , micellar concentration; $[H_2O]/C_M \cdot n$, average number of H_2O molecules bound per head group.

$[H_2O]$ (M)	0.1	0.2	0.3	0.4	0.5	0.6
N	6	$1.1 \cdot 10$	$2.0 \cdot 10$	$3.5 \cdot 10$	$5.4 \cdot 10$	$7.7 \cdot 10$
n	$1.2 \cdot 10$	$2.2 \cdot 10$	$4.0 \cdot 10$	$7.0 \cdot 10$	$1.1 \cdot 10^2$	$1.5 \cdot 10^2$
C_M (M)	$1.3 \cdot 10^{-2}$	$7.3 \cdot 10^{-3}$	$4.0 \cdot 10^{-3}$	$2.3 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$
$[H_2O]/C_M$	7.7	$2.7 \cdot 10$	$7.5 \cdot 10$	$1.7 \cdot 10^2$	$3.3 \cdot 10^2$	$6.0 \cdot 10^2$
$[H_2O]/(C_M \cdot n)$	0.6	1.2	1.8	2.5	3.0	3.9

molecules create micelles solubilizing aqueous MB solution. MB dimers are solubilized in DAP micelles directly or after being dissociated to the monomers. The water molecules solvating MB molecule may escape to hydrate the head groups. The naked MB molecule may be bound to the head group unhydrated by bound water. This binding might be caused by the Coulomb and hydrogen bonding interactions as shown in Fig. 7. The binding state created by the proton transfer from amino head

Fig. 7. A model for the binding state of MB inside DAP micelle having the 495 nm band. Arrow, proton displacement.

group to MB molecule as shown by the arrow might be responsible for the 495 nm band. This binding interaction may be lost in the free water range above 0.4 M due to the hydrations of head group and MB molecule.

In conclusion, the solubilization of MB in DAP reversed micelles depended strongly on the concentration of solubilized H₂O. The time after mixing of aqueous MB and DAP cyclohexanic solutions and the procedure of the sample preparation were also important factors, since the solubilization of MB and the micellar formation of DAP are entangled each other. The detailed binding state of MB molecule to DAP micelle is still open to be made clear up in future investigation.

- [1] J. H. Fendler, *Acc. Chem. Res.* **9**, 153 (1976).
- [2] H. Kondo, K. Fujiki, and J. Sunamoto, *J. Org. Chem.* **43**, 3584 (1978).
- [3] G. D. Correl, R. N. Cheser, III, F. Nome, and J. H. Fendler, *J. Am. Chem. Soc.* **100**, 1254 (1978).
- [4] P. B. Merkel and D. W. Kearns, *J. Am. Chem. Soc.* **90**, 6233 (1968).
- [5] A. A. Gorman, G. Lovering, and M. A. Rogers, *Photochem. Photobiol.* **23**, 399 (1976).
- [6] H. Singh and D. Ewing, *Photochem. Photobiol.* **28**, 547 (1978).
- [7] L. S. Jahnke and A. W. Frenkel, *Photochem. Photobiol.* **28**, 517 (1978).
- [8] E. Rabinowitch and L. F. Epstein, *Ind. Eng. Chem.* **63**, 69 (1941).