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Selective Fluorescence Quenching of Chlorophyll a-N-Methylmyristamide System by Methylviologen in Aqueous Sodium Dodecyl Sulfate Solution

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Addition of methylviologen to the chlorophyll a (Chl)-N-methylmyristamide system in aqueous sodium dodecyl sulfate solution led to the selective fluorescence quenching of monomeric and aggregated Chl species in the concentration range of surfactant below and above the critical micelle concentration, respectively.

The elucidation of photophysical and photochemical properties of chlorophyll a (Chl) at various interfaces is of great interest in relation to its central role in the primary process of photosynthesis in vivo. In order to obtain an insight into the specific interaction of Chl with surrounding protein molecules in thylakoid membrane,[1] we have carried out the preliminary studies of the interaction of Chl with an amphiphilic amide in the presence of surfactants;[2] these studies were based on some early work on the particulate model system of photosynthesis.[3] We reported that in solution of surfactant such as sodium dodecyl sulfate (SDS) Chl associates strongly with N-methylmyristamide (NMMA) to give rise to the premicellar aggregates even in the concentration range below the critical micelle concentration (CMC).[2] The present paper deals with the selective fluorescence quenching of the Chl-NMMA system by methylviologen (MV^{2+}) cation in aqueous SDS solution.

Chl, NMMA, MV^{2+} (paraquat; 1,1'-dimethyl-4,4'-bipyridinium) dichloride, and SDS were essentially the same as those employed previously.[2,4,5] Laboratory deionized water was distilled twice. The sample solutions containing SDS were buffered at pH 9.5.[5] The concentrations of Chl, NMMA, and MV^{2+} were fixed,

respectively, at 4 X 10^{-6}, 5 X 10^{-4}, and 1 X 10^{-3} mol dm^{-3} throughout whole experiments. In order to avoid the effect of reabsorption and reemission of fluorescence, absorbance at an excitation wavelength (435 nm) was kept always below 0.05. All measurements were made at 25 °C for aerated solutions.

Figure 1 shows the SDS-concentration ([SDS]) dependences of absorption and fluorescence spectra of the Chl-NMMA-MV^{2+}-SDS system. The CMC of SDS in the present system (pH 9.5) was determined conductometrically to be ca. 5 mmol dm^{-3}. We can see that new absorption bands centered at 675 and 684 nm and a new fluorescence band centered at 687 nm appear dominantly at SDS concentrations below and around CMC. In a previous study of the Chl-NMMA-SDS system, we have revealed that the 684 and 687 nm bands are marker bands characteristic of the formation of solute-rich induced micelle (SRIM), in which Chl associates strongly with NMMA.[2] We have also shown that MV^{2+} forms SRIM with SDS.[5,6] In the present system, therefore, it is also reasonable to assume the formation of SRIM consisting probably of Chl, NMMA, MV^{2+} and surfactant ions. This in turn suggests that the Chl molecules associated with NMMA[2] can interact further with MV^{2+} molecules, since they are considered to locate in close neighborhood of MV^{2+} in SRIM. The formation of SRIM will necessarily lead to a remarkable fluorescence quenching of the Chl species by MV^{2+}.

In Fig. 2 is shown the [SDS] dependence of the difference spectrum between the fluorescence spectra in the presence and absence of MV^{2+}. It can immediately be seen that the 687 and 678 nm fluorescence components are preferentially quenched by MV^{2+} below and above CMC, respectively. The 678 nm component has been attributed to the Chl monomer species solubilized in SDS micelles above CMC.[2,7] Thus, we can conclude safely that MV^{2+} quenches selectively the Chl species associated with NMMA in the concentration range of SDS below CMC, but monomeric species above CMC.

The apparent degree of fluorescence quenching, E_Q, defined by $1 - I/I_0$, was measured at 678 and 687 nm as a function of [SDS], where I_0 and I are the fluorescence intensities of Chl-NMMA system in the absence and presence of MV^{2+} at a given [SDS], respectively. The typical results are shown in Fig. 3.[8] It is evident that efficient quenching occurs even at SDS concentrations below CMC. The observed enhanced quenching at lower SDS concentrations below CMC can reasonably be ascribed to the formation of foregoing SRIM. An another remarkable feature of Fig. 3 is that the [SDS] dependence of E_Q can distinctly be divided into three regions of

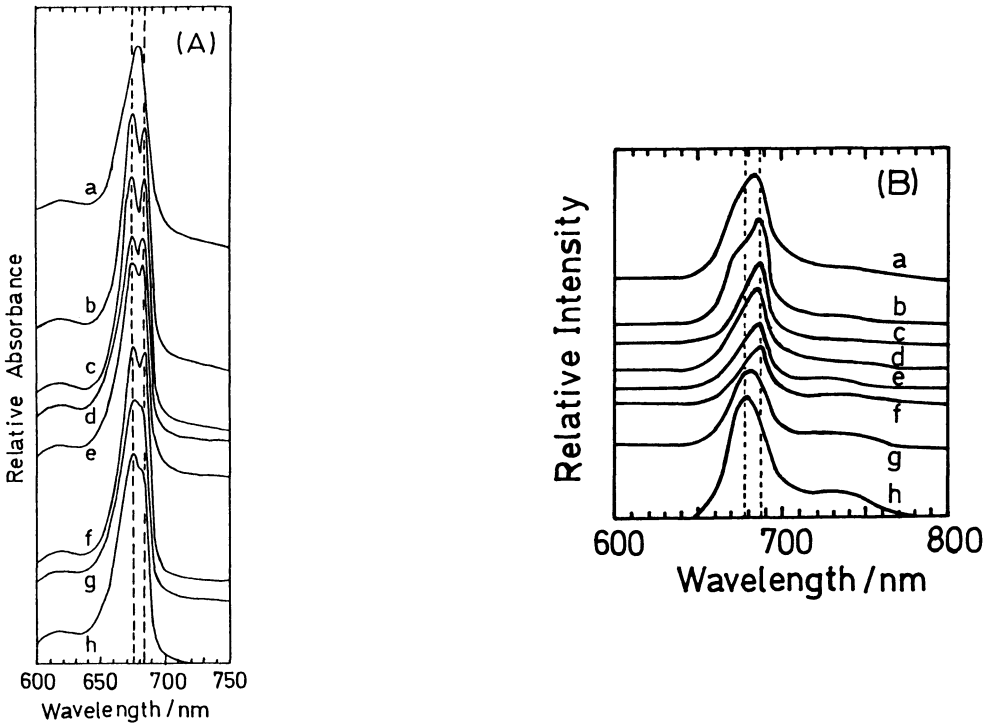

Fig. 1. Red absorption bands (A) and fluorescence spectra (B) of the Chl-NMMA-MV^{2+}-SDS system in aqueous solutions (pH 9.5). (a) [SDS]=0, (b) 1, (c) 2, (d) 3, (e) 5, (f) 8, (g) 15, (h) 30 mmol dm^{-3}.

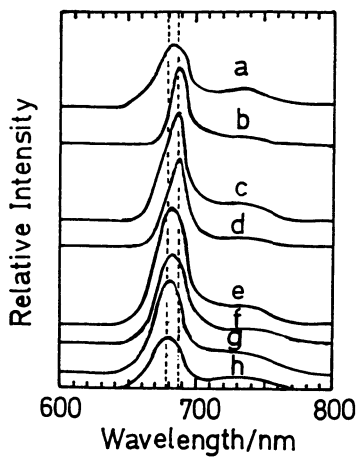

Fig. 2. Fluorescence difference spectra of the Chl-NMMA-MV^{2+}-SDS system. See the figure caption to Fig. 1 for the notation, (a) to (h).

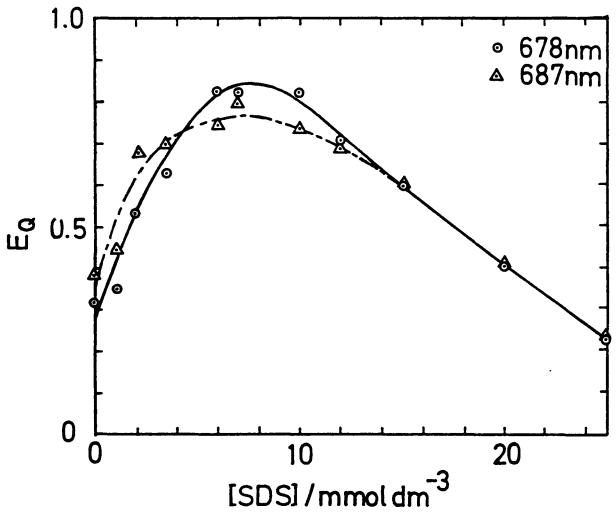

Fig. 3. Variation in the quenching efficiency (E_Q) with [SDS].

[SDS], i.e., (i) belw CMC, (ii) above CMC but below ca. 15 mmol dm^{-3}, and (iii) above ca. 15 mmol dm^{-3}. In regions (i) and (ii), the associated (at 687 nm) and monomeric Chl species (at 678 nm) are preferentially quenched by MV^{2+}, respectively. On the other hand, the change in the 684 nm absorption and 687 nm fluorescence bands observed far above CMC (see Fig. 1), and the decrease in E_Q in region (iii) will be ascribed to two factors: one is the increase in the concentration of the normal micelle which solubilizes Chl as the monomeric species, and the other is the decrease in MV^{2+} concentration in micellar phase with further increase in SDS concentration.

The relative fluorescence yield of the 687 nm aggregated component was low at least by a factor of 5 compared with that of the 678 nm monomeric component; the precise estimation of the fluorescence yields is now in progress. This fact is probably one of the reasons why the selective-quenching phenomenon takes place in the present system. Several factors such as fluorescence yield, formation and deaggregation of SRIM, and interactions among the components of the system, must be responsible for the phenomenon but the results are still wholly unexpected.

Preliminary results on the resonance Raman spectra of the associated Chl species in the absence of MV^{2+} suggest that both the carbonyl group at the 9-position (C_9=O) and the central Mg atom of Chl interact with the amide group of NMMA in micellar SDS solutions.[9] Further Raman spectral studies of the present system are now in progress.

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7) The Chl molecules are considered to be in colloidal states in addition to be solubilized in SRIM, at SDS concentrations below CMC.[2]
8) Since the fluorescence bands of monomeric and aggregated Chl species have some overlap, the fluorescence intensity measured at 678 nm (or 687 nm) contains some contribution from that of aggregated (or monomeric) species. However, this contribution is considered not to be a serious factor.
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