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The Formation of Ground-State Nonfluorescent Complex between Chlorophyll a and Methylviologen in Methanol Solution

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An enhanced quenching of fluorescence of chlorophyll a by methylviologen in methanol solution was found to proceed via both dynamic and static processes. It was shown that the formation of a ground-state "dark" complex between the reactants is responsible for the latter process.

Because of their importance in biological and photochemical processes, chlorophyll a (Chl) and methylviologen (MV^{2+}; 1,1'-dimethyl-4,4'-bipyridinium) cation have attracted much interest.[1] For example, photochemistry of electron donor-acceptor or charge-transfer (CT) complexes of MV^{2+} and fluorescence quenching of excited molecules by MV^{2+} have been the subject of recent intense research.[2,3] It has been pointed out that formation of ground-state CT complexes between MV^{2+} and amine-based sacrificial electron donors such as EDTA and triethanolamine has serious implications for the full understanding of typical water photoreduction systems.[4] In this connection, Harriman et al.[5] and Okura et al.[6] reported photoredox properties of the ground-state complexes between metalloporphyrins and viologens in aqueous solution. Recently we have given a preliminary account of fluorescence quenching of Chl by MV^{2+} in aqueous surfactant solutions.[7] In the present study, a special attention was paid to the anomalous quenching of Chl fluorescence by MV^{2+} in a methanol solution. We wish to report that crucial event in the system is the formation of a Chl-MV^{2+} complex which results in an enhanced quenching of Chl fluorescence.

Chl (Wako, >99.8%),[8] MV^{2+} dichloride (Sigma, 99%), and methanol (Dotite Spectrosol) were used without further purifications. Steady-state corrected fluorescence spectra were measured with an excitation wavelength of 435 nm on a Hitachi 850 spectrofluorometer. Fluorescence decay curve was measured with a Horiba Model NAES-1100 time-resolved spectrofluorometer. The decay curves were described by a single exponential decay, even when MV^{2+} was present. In the quenching experiment, fluorescence intensity and lifetime were measured at 674 nm as a function of MV^{2+} concentration at a constant concentration of Chl (4×10^{-6} mol dm^{-3}). All measurements were made at 25 °C in aerated solutions.

In general, fluorescence intensity (I) and fluorescence lifetime (τ) are related to quenching rate constant (k_q) and concentration of quencher ([Q]) by the Stern-Volmer equation (Eq. 1);

$$I_0/I = \tau_0/\tau = 1 + k_q\tau_0[Q] \quad (1)$$

where I_0 and τ_0 are fluorescence intensity and fluorescence lifetime in the absence of quencher, respectively. Figure 1 shows two types of the Stern-Volmer plots, I_0/I vs. [Q] and τ_0/τ vs. [Q]. The latter plot yielded a straight line (a dashed line in Fig. 1), from the slope of which the k_q value was obtained to be 7.2×10^9 mol^{-1} dm^3 s^{-1} by using τ_0 of 5.7 ns. As can be seen in Fig. 1, however, the plot of I_0/I vs. [Q] is apparently concave upwards.

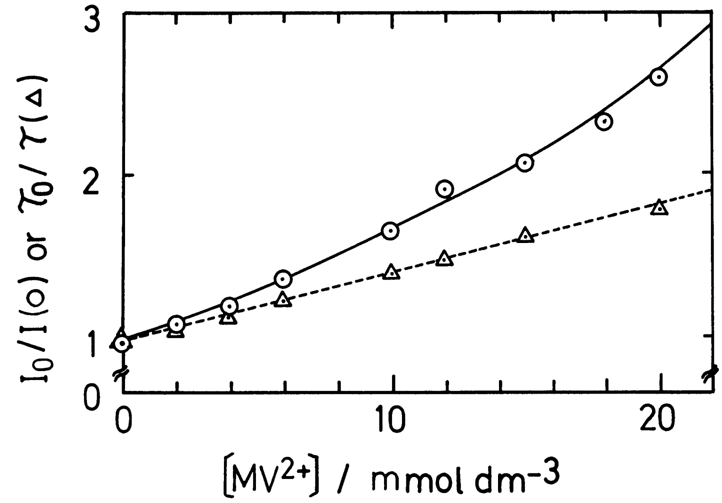

Fig. 1. Stern-Volmer plots for the fluorescence quenching of Chl by MV^{2+}. o: I_0/I vs. $[MV^{2+}]$; Δ: τ_0/τ vs. $[MV^{2+}]$; solid line: calculated from Eq. 2 using parameters given in the text; dashed line: straight-line fit of lifetime data to Eq. 1.

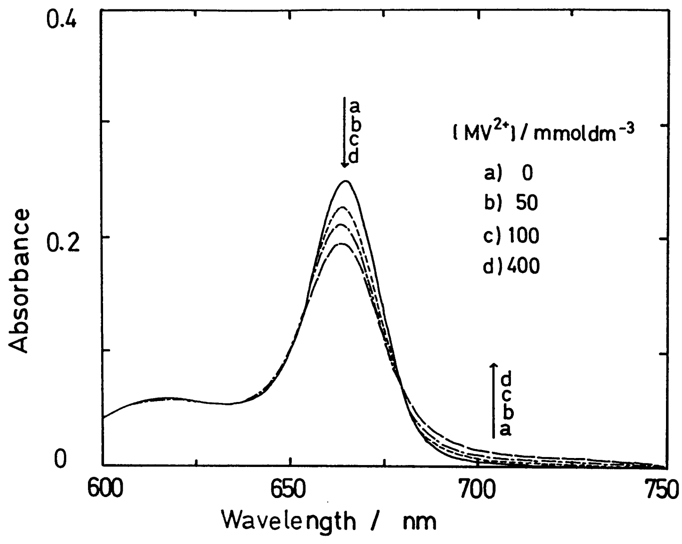

Fig. 2. Absorption spectra of Chl-MV^{2+} system in methanol. Path length: 1 cm.

Such a deviation from the expected linearity can be related to the fact that the fluorescence quenching of Chl by MV^{2+} occurs along with remarkable absorption spectral changes as is shown in Fig. 2, where at least two isosbestic points can be observed at 653 and 678 nm. Although the absorption spectra in the Soret band region (spectra not shown) were somewhat disturbed by additional absorption due to MV^{2+} interacting with Chl, a blue shift (about 2 nm) and two isosbestic points (at about 437 and 452 nm) appeared on an addition of MV^{2+}. These findings suggest the formation of a 1:1 complex between Chl and MV^{2+} under our experimental conditions.

While change in absorption spectrum of Chl was definitely observed (Fig. 2), the shape and position of its fluorescence spectrum were scarcely altered by the addition of various amounts of MV^{2+}. The excitation spectra of Chl monitored at two fluorescence maxima (674 and 731 nm) in the presence of MV^{2+} corresponded fairly well to the Soret absorption spectrum of Chl solution in methanol (data not shown). Furthermore, no emission of the complex was observed upon excitation at wavelengths longer than 700 nm where the absorption band arises predominantly from the complex. These findings suggest that a ground-state nonfluorescent ("dark") complex between Chl and MV^{2+} is formed in methanol solution.

Taking these into account, the anomalous quenching of Chl fluorescence by MV^{2+} can be elucidated by the following modified form of the Stern-Volmer equation:[9,10)]

$$I_0/I = (1 + k_q\tau_0[Q])/(1 - K[Q]) \qquad (2)$$

where $K = \gamma(\varepsilon_c/\varepsilon_0)K_c$ and $K_c = [FQ]/[F][Q]$. Here, γ represents the efficiency of the dynamic quenching reaction, ε_c and ε_0, molar extinction coefficients of the 1:1 dark complex, FQ, and fluorophore (Chl), F, respectively at the excitation wavelength, and K_c, association constant of the complex, FQ. One can rewrite Eq. 2 as:[10)]

$$(1 - (I/I_0))/[Q] = k_q\tau_0(I/I_0) + K \qquad (3)$$

Application of Eq. 3 to the fluorescence-intensity data gave in fact a linear relation (data not shown). Thus, the values of k_q and K can be estimated to be 7.2×10^9 mol^{-1} dm^3 s^{-1} and 15 mol^{-1} dm^3, respectively. The fact that the value of k_q thus obtained is the same as that derived from the plot of τ_0/τ vs. [Q] strongly supports the validity of the dark complex model[9,11)] proposed in this study. The I_0/I values calculated from Eq. 2 with the k_q and K described above are also included in Fig. 1 (a solid line). Agreement between the calculated and the observed I_0/I values is quite excellent.

In another experiments, we determined the association constant, K_c, to be 12 mol^{-1} dm^3 from a Benesi-Hildebrand plot[12)] using absorbance data at 700 nm shown in Fig. 2. Since $K = \gamma(\varepsilon_c/\varepsilon_0)K_c$, we now can estimate $\varepsilon_c/\varepsilon_0$ to be 1.3 at 435 nm using the collisional quenching efficiency γ of 1.0 which is often an assumed value for efficient quenchers.[10)]

In view of the fact that the spectral data reveal the existence of the dark complex between Chl and MV^{2+}, and Eqs. 2 and 3 fit the experimental data satisfactorily, it is reasonable to ascribe the observed anomalous quenching to the result of static quenching. The nature of the dark complex is now under investigation in connection with the contribution of a CT character because Chl and MV^{2+} are well known to be electron donor and acceptor molecules, respectively. It should be noted that to our knowledge, this is the first report on the formation of ground state complex between a biomolecule (Chl) and MV^{2+}. This in turn suggests that many previous studies dealing with Chl and MV^{2+} must be reconsidered, as the case may be, in the light of the present study.

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