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SPECTRAL CONTROL OF METHYL ORANGE AND CYANINE DYES BY SYNTHETIC BILAYER MEMBRANES[1)]

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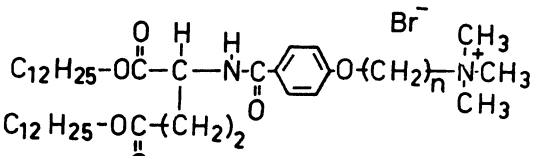
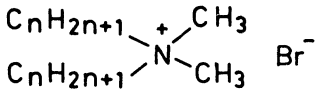
Methyl Orange and cyanine dyes bound to aqueous ammonium bilayer membranes undergo extensive spectral changes which are sensitive to membrane fluidity and small structural changes of the component amphiphile.

Methyl Orange has been used as a solvatochromic probe for the medium polarity, its λ_{max} being located at 464 nm in water and at 420 - 430 nm in organic media.[2)] When it is bound to cationic micelles and cationic polysoaps, a blue shift to 370 - 380 nm can be observed due to formation of the stacked dye molecules.[2-4)] We describe in this paper that further spectral variation is possible by using appropriate ammonium bilayer matrices, and that this technique is readily applicable to cyanine dyes.

Chiral dialkylammonium amphiphiles 1[5)] form stable bilayer membranes, when dispersed in water by sonication (Branson cell disruptor 185, power level 40 W) for 0.5 - 2 min. These membranes, like many other bilayer membranes of synthetic dialkyl amphiphiles,[7,8)] possess physicochemical characteristics common to those of the phospholipid bilayer membrane. Methyl Orange (2.5×10^{-5} M) bound to the aqueous bilayer of L-1 (n = 4), (2.5×10^{-4} M) gives the absorption maximum at 488 nm at 20 - 25°C. This is the largest red shift ever observed for this dye. Interestingly,



1 (n = 2, 3, 4, 5, 6, and 10)



$2C_nN^+2C_1$ (n = 14, 16, 18)

the spectrum is highly sensitive to temperature, as shown in Fig. 1a. A drastic shift of λ_{max} to 424 nm occurs at 28 - 29°C and no further change is observed at 30 - 35°C. The 64-nm shift in λ_{max} by this small temperature change is without precedent. This spectral variation must be produced by the change in membrane fluidity, since the temperature range where the spectral change occurs corresponds almost precisely to the temperature of the gel-to-liquid crystal phase transition (T_c) of the matrix membrane as estimated by differential scanning calorimetry: peak top temperature, 31°C; transition region, 27 - 36°C.[6)]

The spectral variation is highly specific to the chemical structure of the membrane-forming amphiphile. For example, the λ_{max} shift of Methyl Orange embedded

in simple dialkylammonium membrane($2C_nN^+2C_1$, n = 14, 16, 18) was in the reversed direction of that mentioned above; e.g., for $2C_{16}N^+2C_1$ membrane, λ_{max} = 388 nm in the low temperature range(20 - 25°C) and 408 nm in the high temperature range(above 29°C)(Fig. 1b). The spectral shift occurs at 26 - 28°C in accord with T_c of this membrane(28°C). Similar T_c dependence was found for the membrane matrix in which the glutamic acid moiety in 1 is replaced by the L-aspartic acid moiety.

In the case of 1(n = 4), the identical spectral change was observed regardless of whether 1 contains L-, D-, or DL-glutamic acid residue. This indicates that the chiral property of the membrane is not directly responsible for the spectral change. On the other hand, the length of the "spacer" methylene chain, n in 1, is crucial for the spectral shift. The same spectral change as mentioned above for L-1(n = 4) membrane is found in the case of n = 3; however, when n = 2, λ_{max} is located at 367 nm at temperature below T_c(27°C) and shifts to 415 nm upon heating to temperature above T_c. With n = 5 and 10, the T_c dependence is not observed: λ_{max}, 385 to 405 nm. λ_{max} for L-1(n = 6) is at 440 nm at temperature below T_c(42°C) and at 424 nm above T_c. The spectral shift is only 16 nm. These data indicate that the change in the spacer length by one methylene unit causes large differences in the spectral property.

Representative temperature dependence of λ_{max} is shown in Fig. 2. Sharp changes in λ_{max} are observed at T_c in the membrane matrix of 1(n = 2 and 4) in the opposite directions, but the change is less drastic for $2C_{16}N^+2C_1$ membrane.

The observed λ_{max} values cover a range of 367 to 488 nm, as against the "normal" λ_{max} value of 420 - 430 nm in organic media. The blue shift beyond the normal λ_{max} range is caused by stacking of the dye molecules in the parallel orientation(H-type aggregation), as already discussed in the micellar and other systems.[2-4] On the other hand, the red shift may be attributed to dye stacking in the head-to-tail orientation(J-like aggregation).[9-10]

The influence of dye aggregation on the λ_{max} shift is clearly shown in Fig. 3. The largest red shift is observed in the rigid membrane matrix of L-1(n = 4) at the molar ratio of 1:10([Methyl Orange]/[L-1(n = 4)]). The red shift decreases with dilution of Methyl Orange and λ_{max} reaches 455 nm at the ratio of 1:500. It is presumed that the aggregated dye is not present at the latter molar ratio, and the dye stacking alone should account for the red shift of 33 nm(488 - 455 nm). The λ_{max} value is independent of the molar ratio in the fluid membrane matrix at temperature above T_c. This λ_{max} value(424 nm) is typical of that in common organic media, indicating that dye aggregation and/or specific interaction with the membrane is not significant in the case of the fluid membrane. The λ_{max} difference of 455 - 424 = 31 nm at the molar ratio of 1/100 to 1/500 is thus produced by the change in the microenvironment of the membrane due to phase transition. Since the λ_{max} value in the fluid matrix is normal, the 31-nm red shift may be ascribed to the specific orientation of unaggregated Methyl Orange at the rigid membrane surface.

In the case of the $2C_{16}N^+2C_1$ membrane matrix, the large blue shift observed is produced by dye aggregation, as also shown in Fig. 3. The λ_{max} shift due to dye aggregation is small at temperatures above T_c, but is much larger at temperatures below T_c.

It is noteworthy that the two types of the rigid membrane matrix promote dye

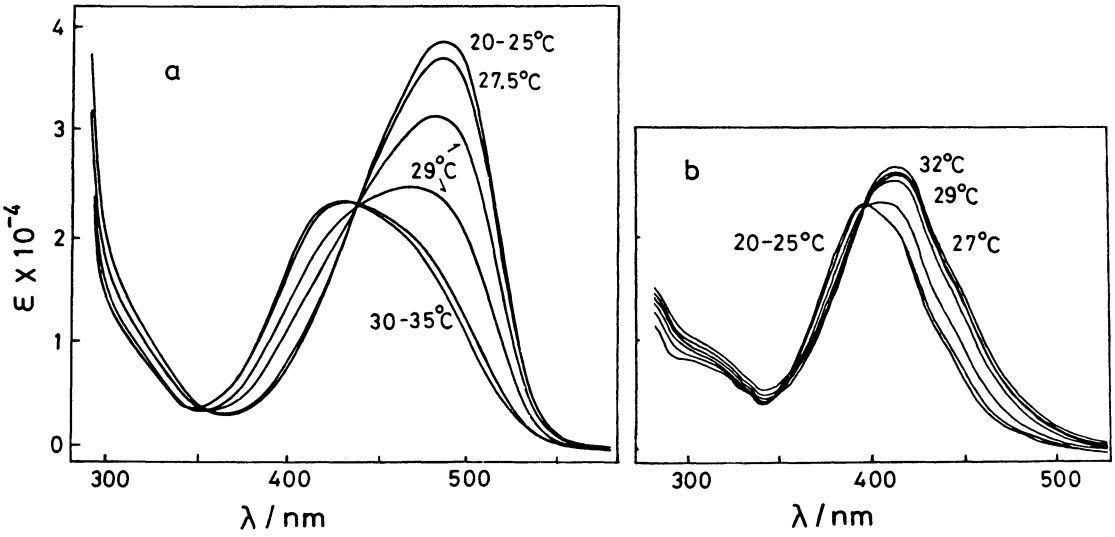


Fig. 1. Temperature dependence of absorption spectrum of Methyl Orange (2.5×10^{-5} M) bound to the bilayer membrane(2.5×10^{-4} M).
a. L-1(n = 4), b. $2C_{16}N^{+}2C_{1}$.

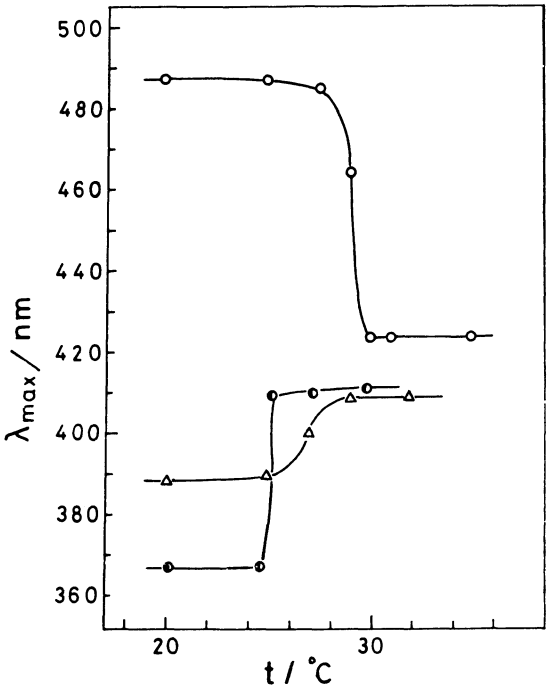


Fig. 2. Temperature dependence of λ_{max} of Methyl Orange(2.5×10^{-5} M) bound to the bilayer membrane(2.5×10^{-4} M). o: L-1(n = 4), ◐: L-1(n = 2), △: $2C_{16}N^{+}2C_{1}$.

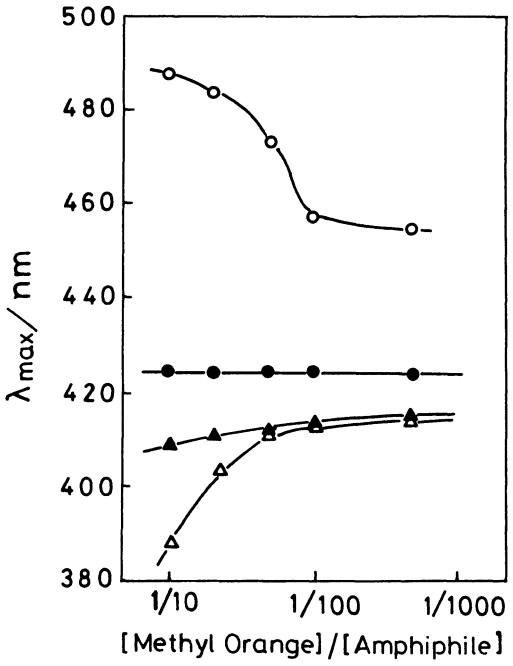
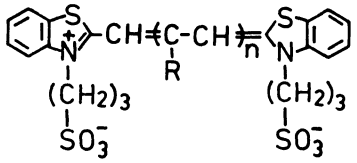


Fig. 3. Plots of λ_{max} of absorption spectra vs. [Methyl Orange]/[amphiphile], Methyl Orange, 1.0×10^{-5} M(constant)
o: L-1(n = 4) (20°C)
●: " (35°C)
△: $2C_{16}N^{+}2C_{1}$ (20°C)
▲: " (35°C)

aggregation in different orientations.

Spectral control by the bilayer membrane can be extended to a variety of cyanine and merocyanine dyes. For example, a considerable red shift was found for 2a bound to the membrane matrix of 1(n = 4): λ_{max} = 721 and 666 nm at temperature below and above T_c, respectively. More interestingly, drastic fluoresence enhancement(quantum yield, 0.64) has been achieved by the interaction of 2b with the bilayer membrane of 1(n = 4; counterion, Cl^-). This value is 30 times larger than that observed in the CTAC micellar system and 250 times larger than those in methanol or in H_2O.[11]



2a(n = 2, R = H)

2b(n = 1, R = CH_3)

In conclusion, the present study establishes that extensive and specific spectral control of Methyl Orange is possible. The λ_{max} shift is presumably produced by the specific orientation and aggregation of the dye molecule at the membrane surface. This conclusion should pave a way to spectral control of dye molecules in general, as briefly described for cyanine dyes. These results should be useful not only in the model study[14-16] of biological chromophores such as membrane-bound chlorophyll but also from the practical point of view.

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