Controlled Organization of Cyanine and Merocyanine Dyes at the Surface of Synthetic Bilayer Membranes

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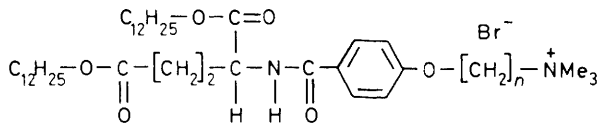
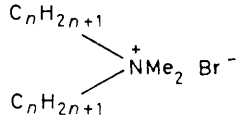
Some water-soluble cyanine and merocyanine dyes bound to a synthetic bilayer membrane show drastic spectral changes which are sensitive to the phase transition and the chemical structure of the membrane.

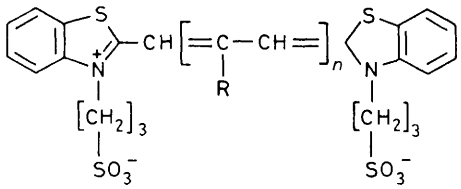
Cyanine and merocyanine dyes play an important role as sensitizers in photographic processes.[1] The spectral properties of these dyes are known to be strongly affected by their aggregation behaviour, and extensive efforts are being made to control the mode of dye aggregation. The peculiar properties of biological pigments are often attributed to the specific organization of the pigment molecule at the surface of the biomembrane. Therefore, we thought it would be interesting to see if the organization of cyanine and merocyanine dyes could be controlled by their binding to a synthetic bilayer membrane which has been developed in these laboratories.[2] We describe herein unprecedented spectral changes of these dyes resulting from specific organization at the surface of some synthetic bilayer membranes. The peculiar spectral



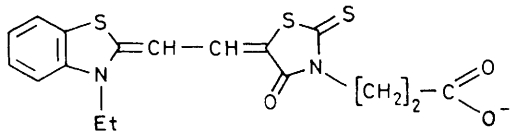
L-(**1**), n = 2, 3, 4, 5, 6, and 10
D-, DL-(**1**), n = 4



(**2**), n = 14, 16, and 18



(**3**) **a**; R = Me, n = 1
b; R = H, n = 2
c; R = H, n = 3



(**4**)

properties observed (unusual red shifts; drastic spectral changes with temperature and membrane structure) are explained in terms of specific organization of the dye and the physical state of the membrane.

The dialkylammonium amphiphiles (**1**) and (**2**) form stable bilayer membranes[3,4] (vesicles and lamellae) when dispersed in water (Branson cell disruptor 185; sonication time 0.5—2 min). These membranes possess physicochemical characteristics similar to those of biolipid bilayer membranes. For instance, the synthetic bilayer undergoes a crystal–liquid crystal phase transition in a manner similar to that of the lecithin bilayer.[3,5]

Some cyanine and merocyanine dyes show unusual spectral behaviour when bound to the synthetic membrane.† For example, the absorption maximum of the dye (**3b**) is located at 653 nm (ϵ_{max} 150 000) in methanol, at 580 and 648 nm (ϵ_{580} 60 800; ϵ_{648} 100 000) in aqueous buffer, and at 673 nm (ϵ_{max} 130 000) in the cetyltrimethylammonium bromide (CTAB) micelle. However, when it is bound to the membrane matrix of (**1**; n = 4) at 20 °C, a remarkable red shift with an enhanced ϵ value is observed: λ_{max} 720 nm (ϵ_{max} 300 000) (Figure 1).

It is also notable that the red shift is highly sensitive to temperature. As shown in Figure 1, λ_{max} changes drastically between 720 and 667 nm in the narrow temperature range of 27—30 °C, with a corresponding marked decrease in ϵ, but remains unchanged outside this range. The 53-nm shift in

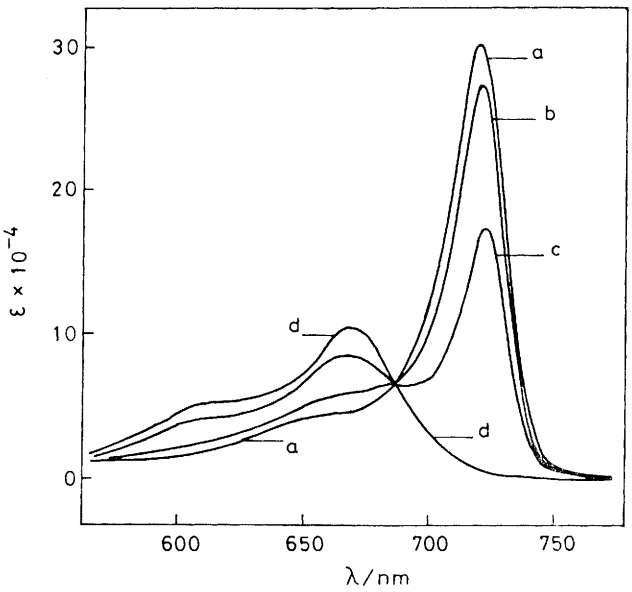


Figure 1. Temperature dependence of the visible absorption spectrum of the cyanine dye (**3b**) bound to the aqueous bilayer membrane L-(**1**; n = 4); [(**3b**)] = 5.0 × 10^{-6} M, [L-(**1**; n = 4)] = 2.5 × 10^{-4} M; a; 20—25 °C, b; 27 °C, c; 29 °C, d; 30—35 °C.

λ_{max} and the large ϵ_{max} change within this small temperature range is without precedent. This spectral variation must be due to a change in membrane fluidity, since the temperature range where the spectral change occurs corresponds almost precisely to the temperature of the crystal–liquid crystal phase transition (T_c) of the matrix membrane as estimated by differential scanning calorimetry (peak temperature, 31 °C; transition region, 27—36 °C).[3,4] These spectral changes are reversible, although ageing is required before the low-temperature spectrum is fully restored. In the case of the membrane (**1**; n = 4), identical spectral shifts were observed regardless of whether (**1**) contains an L-, D-, or DL-glutamic acid residue. This indicates that the chiral property of the membrane is not directly responsible for the spectral change.

Similar red shifts and enhanced ϵ_{max} values are found for the combination of the dyes (**3a**) and (**3c**) and many other anionic and zwitterionic cyanine dyes with the membrane (**1**; n = 3, 4, 5, 6, or 10). With the membrane (**1**; n = 2), the spectral shift occurs for the dye (**3a**), but not for (**3b**) or (**3c**).

This spectral control can be extended to a variety of merocyanine dyes. For example, λ_{max} for the dye (**4**)‡ bound to the membrane (**2**; n = 16) is 495 nm in the range 20—25 °C and shifts to 515 nm above 29 °C (Figure 2). This spectral change at 26—28 °C is in accord with T_c for this membrane.[5] A spectral shift at T_c in the opposite direction is found for the dye (**4**) bound to the membrane (**1**; n = 2, 3, 4, 5, 6, or 10). *E.g.*, λ_{max} for the dye (**4**) is at 549 nm with the membrane (**1**; n = 4) at temperatures below T_c and at 536 nm above T_c. It is noteworthy that two types of spectral control (both blue and red shift) could be achieved by the synthetic membrane. The blue shift may apparently be ascribed to parallel stacking (H-like aggregation)[1] of the dye molecules, and the red shift probably results from head-to-tail orientation (J-like aggregation).[1,6]

The influence of dye aggregation on the shift in λ_{max} values was clarified by dilution experiments. λ_{max} for the dye (**3b**)

† Solutions of dyes (purchased from Nippon Kanko-Shikiso Co.) were added to the aqueous membranes at pH 8.0, 0.01 M-Tris buffer.

‡ λ_{max} 523 nm in methanol and 540 nm in aqueous buffers.

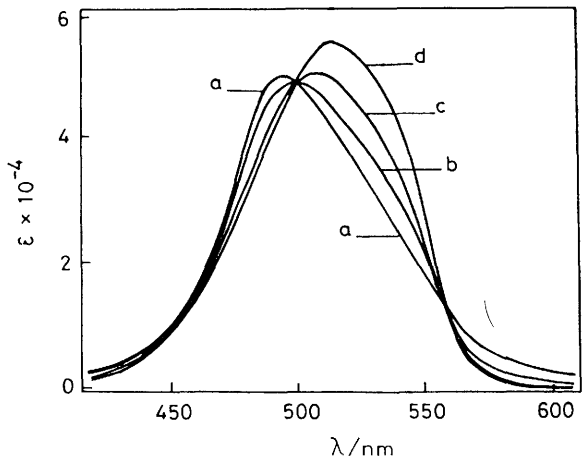


Figure 2. Temperature dependence of the visible absorption spectrum of the merocyanine dye (**4**) bound to the aqueous bilayer membrane (**2**; $n = 16$); [(**4**)] = 2.0×10^{-5} M, [(**2**; $n = 16$)] = 5×10^{-4} M; a; 20—25 °C, b; 27 °C, c; 28 °C, d; 30—35 °C.

bound to the rigid (below T_c) membrane of (**1**; $n = 4$) is at 720 nm for molar ratios of 1 : 10 to 1 : 500 {[(**3b**)] : [(**1**; $n = 4$)]}, whereas it is at 693 nm for a ratio of 1 : 5000. However, the λ_{max} value (667 nm) in the fluid membrane matrix at temperatures above T_c is independent of the molar ratio. This λ_{max} value is almost identical with that in methanol, indicating that dye aggregation and/or specific interaction is not significant in the fluid membrane. Since dye aggregation presumably does not occur at a ratio of 1 : 5000, the 26-nm red shift (693—667 nm) must be caused by orientational fixation of the unaggregated dye at the rigid membrane surface.

In conclusion, the present study establishes that controlled organization (specific orientation and aggregation) of cyanine and merocyanine dyes can be achieved by taking advantage of the specific surface structure of the rigid synthetic bilayer. As a result, extensive spectral control is possible. Although the spectral property of cyanine dyes bound to lecithin liposomes and biomembranes has been investigated,[7] spectral variations as large as described here have not been reported. Thus, the present results should be useful in model studies[8] of membrane-bound biological pigments, apart from their interest from the practical point of view.

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