Selective Binding of a Cyanine Dye at the Surface of Ammonium Bilayer Membranes

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Selective binding of anionic cyanine dyes at the surface of closely related ammonium bilayers was confirmed by dye-transfer experiments.

We report herein that ammonium bilayer membranes provide surprisingly specific binding sites for cyanine and other dyes. The binding sites, though not defined precisely at present, are molecular spaces created near the surface of the synthetic bilayer, and possess functions comparable to those of host molecules such as crown ethers, cyclodextrins, and cryptands.

We have shown that spectral properties (absorption, fluorescence, circular dichroism) of some anionic dyes are specifically altered upon binding to ammonium bilayer membranes.[1—4] A subsequent survey demonstrated that the combination of dyes and bilayers is remarkably specific in terms of spectral changes,[5] suggesting that selective binding sites are created at the bilayer surface.

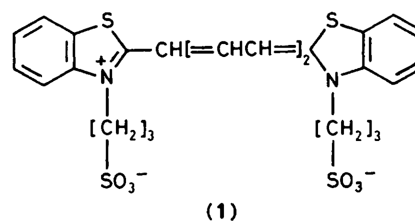
The absorption spectrum of the pentamethine cyanine dye (**1**) changes extensively when the dye is bound to the structurally closely-related double-chain ammonium bilayers (**2**)—(**6**) in water: *e.g.*, $\lambda_{max.}$ 666 nm, bilayers of (**2**),[4] (**5**),[6] and (**6**);† 720 nm, bilayers of (**3**)[4] and (**4**).[4] $\lambda_{max.}$ Values near 666 nm are observed in common organic media or in aqueous micelles; therefore, the bilayers of (**2**), (**5**), and (**6**) conceivably produce non-specific binding sites. In contrast, the red-shifted peaks at 720 nm are attributable to the formation of 'J-like aggregates'.[7] This means that the cyanine dye is bound to the bilayers of (**3**) and (**4**) in specifically aggregated forms. Minor variations in structure such as are present in compounds (**2**)—(**5**) can give rise to very different binding sites.

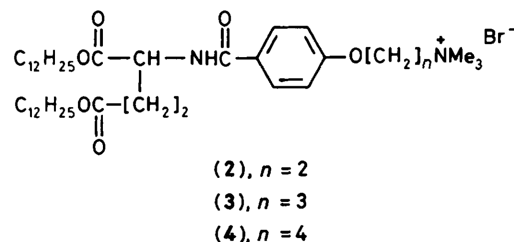
The presence of different binding sites can be examined by various experiments, a typical one involving dye transfer. The cyanine dye is first bound to the fluorocarbon bilayer of (**5**) ($\lambda_{max.}$ 666 nm). Upon addition of only 10 mole % of the related hydrocarbon bilayer of (**4**), the original peak diminishes and a new peak at 720 nm increases with time. The change is completed in *ca.* 1 h. This proves that the dye is transferred from the fluorocarbon bilayer to the hydrocarbon bilayer, since a differential-scanning calorimetry examination indicates that the two bilayer systems remain unmixed under the experimental conditions. Further evidence for selective dye binding is as follows: if the dye is first bound to the bilayer membrane of (**4**) ($\lambda_{max.}$ 720 nm), the spectrum does not change on addition of a 100-fold excess of (**5**). When the dye is added to a mixture of the separate bilayer aggregates of (**4**) and (**5**), it is initially bound to the two bilayers, (*i.e.*, two absorption peaks are seen), but is gradually transferred to (**4**) in *ca.* 1 h. The favoured binding of the dye to (**4**) in preference to (**5**) is schematically illustrated in Figure 1.

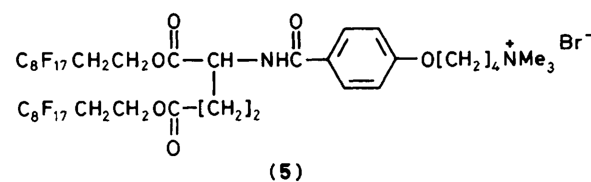
The selective dye binding to the bilayer of (**4**) is also observed when the fluorocarbon bilayer is replaced by the bilayers of (**2**) or (**6**). Preferential binding is observed towards the bilayer of (**3**) in the presence of (**2**). The cyanine dye is preferentially bound to the bilayer upon which 'the J-like aggregates' are formed.

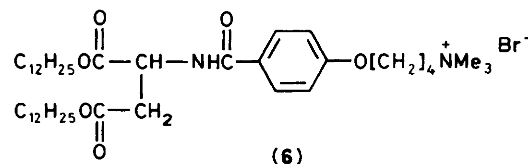
The molecular structure of (**4**) differs from that of (**2**) by two CH_2 units in the spacer portion [(**3**) and (**2**) differ by only one CH_2 unit], from that of (**6**) by one CH_2 unit in the connector portion (glutamate *vs.* aspartate), and from that of (**5**) in the molecular cross sections of the tail (4.8 Å for CH_2 and 6 Å for CF_2). We believe that the cyanine dye is bound to the molecular space created in the spacer portion without disturbing the packing of the double chains, as illustrated in Figure 1, since the dye binding can raise (not lower) the phase transition temperature (T_c).‡ The dimensions of the binding site are, clearly, precisely defined.

It must be emphasized that the binding experiments are conducted at temperatures below the phase transition of the respective membranes. At higher temperatures, the specificities in the absorption spectrum and the binding behaviour are not detectable.









† The amphiphile (**6**) was prepared by a procedure similar to that for (**2**). The products were identified by n.m.r. and i.r. spectroscopy, and elemental analyses.

‡ Examples of the influence of dye binding on T_c are as follows: bilayer (**2**), T_c 27 °C; bilayer (**2**) (10 mM) + dye (**1**) (2 mM), T_c 27 °C; bilayer (**4**), T_c 31 °C; bilayer (**4**) (10 mM) + dye (**1**) (2 mM), T_c 37 °C. The T_c change is related to binding specificity in these cases.

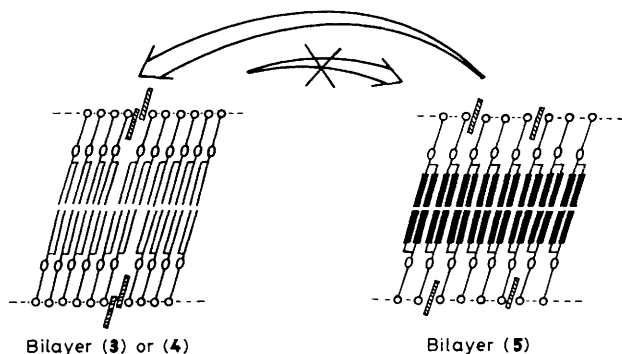


Figure 1. Schematic illustrations of selective dye binding. The dye is bound to the hydrocarbon bilayer preferentially and in a specific manner.

In conclusion, highly specific binding sites (cavities) may be created near the bilayer surface from appropriate amphiphilic components. Selective dye transfer was similarly observed for other cyanine dyes, merocyanine dyes, and azo dyes such as Methyl Orange. Thus, the present findings should be broadly applicable to many dye–bilayer systems. It is noteworthy that the site is produced by self assembly and, therefore, is destroyed or regenerated by controlling the physical state of the bilayer: *e.g.* phase transition and phase separation. Precise definition of the binding site would require single-crystal *X*-ray diffraction studies of the bilayers.

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References

1 N. Nakashima, H. Fukushima, and T. Kunitake, *Chem. Lett.*, 1981, 1207.
2 N. Nakashima, H. Fukushima, and T. Kunitake, *Chem. Lett.*, 1981, 1555.
3 N. Nakashima and T. Kunitake, *J. Am. Chem. Soc.*, 1982, **104**, 4261.
4 N. Nakashima, R. Ando, H. Fukushima, and T. Kunitake, *J. Chem. Soc., Chem. Commun.*, 1982, 707.
5 N. Nakashima, *et al.*, in preparation.
6 T. Kunitake, S. Tawaki, and N. Nakashima, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3235.
7 A. H. Helz, *Adv. Colloid Interface Sci.*, 1977, **8**, 237.