Influence of hemicyanine dye structures on spectral properties of their supramolecular complexes with amylose

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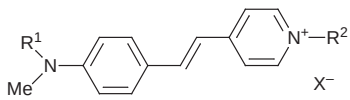
Hemicyanine chromophores bearing long-chain alkyl substitutents on the donor and/or acceptor ends have been prepared, and absorption and emission spectra of their supramolecular complexes with amylose have been studied in order to relate them to their supramolecular structures.

We have developed a novel strategy[1] based on supramolecular complexation of alkyl-substituted hemicyanine dyes (guest) by amylose (host). This not only prevents chromophore aggregation but also provides many added advantages such as improved thermal stability[2] and enhanced hyperpolarizabilty.[3] The unique feature of this system is the development of a supramolecular self-poling[4] in solution-cast thin films.

Since the guest dye fluoresces strongly in the inclusion,[1,5,6] the dye itself can be utilized as a probe to monitor the binding interaction with the host amylose, depending on external conditions such as temperature (thermochromism).[7] Hemicyanine dyes have also been studied as potential voltage-sensitive probes in biomembranes.[8,9] The dyes exhibit a negative solvatochromism,[10,11] in that spectral shifts of absorption and fluorescence, in response to the solvent polarity, occur to the blue and to the red, respectively. However, in lipid membranes, their spectral behavior is reversed, exhibiting a further blue-shift beyond the absorption in water. This is interpreted to be due to the differential solvation of the chromophore oriented in a lipid bilayer.[12] Here we discuss spectral properties of the supramolecular chromophores (which are uniaxially oriented in the host cavity) when they (dyes **1** and **2**) are mono-functionally



1 R^1 = Me, R^2 = $C_{16}H_{33}$, X = Br
2 R^1 = $C_{16}H_{33}$, R^2 = Me, X = I
3 R^1 = R^2 = $C_{16}H_{33}$, X = Br

derived by blocking one of the sensing partners (amino head and pyridinium tail) with a long alkyl chain and retaining (deactivating) it inside the cavity, thereby allowing the other unit to function for polar sensing. In this respect, one of the present hemicyanine–amylose supramolecules, which contains dye **2**, can be regarded as a model of the dye probe imbedded in the lipid bilayer. Spectral results are compared to demonstrate how the chromophore structure is reflected in the sensing activity of their supramolecular complex.

Visible absorption and emission spectra of dyes **1**–**3** in various DMSO–water mixtures (without amylose) reveal significantly different aggregation tendencies. While dye **1** exhibits negligible aggregation even at Φ_{DMSO} = 0.2, spectral data indicate that aggregation (*ca*. 420 nm) of dyes **2** and **3** occurs below Φ_{DMSO} = 0.55 and 0.75, respectively. This suggests that for the fixed alkyl chain length, substitution at the amine end of the chromophore (relative to the pyridinium end) has a greater influence on the overall hydrophobic character of the molecule.

The spectral properties of dyes **1**–**3** in DMSO-rich mixtures ($\Phi_{DMSO} \geqslant 0.75$) containing amylose† are nearly identical to those of comparable mixtures without amylose, since no inclusion (only free dye state) occurs even in the presence of amylose. However, significant differences due to the presence of amylose are observed as the Φ_{DMSO} of the solvent mixture is decreased. In particular, a dramatic enhancement of fluorescent emission (Fig. 1) is observed at $\Phi_{DMSO} \approx 0.6$ for all three dyes, indicative of supramolecular confinement of the chromophore by the helical amylose.[3] The greater fluorescence enhancement due to the inclusion complexation of dyes **2** and **3** relative to **1** presumably reflects stronger binding of more hydrophobic amino residue by amylose. For all three dyes, the full inclusion of amylose is attained at $\Phi_{DMSO} \approx 0.5$, as evidenced by the

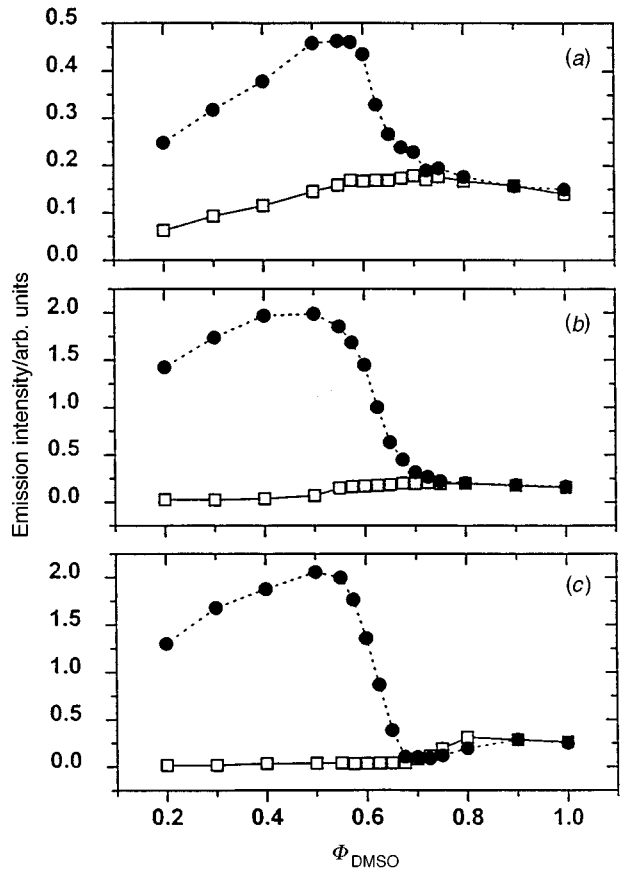


Fig. 1 Integrated fluorescence intensity (480–760 nm) *vs*. Φ_{DMSO} (volume fraction of DMSO) for dyes **1**–**3** in DMSO–H_2O mixtures with (●) and without (□) added amylose: (*a*) dye **1**, (*b*) dye **2** and (*c*) dye **3**. [Dye] = 1.5 × 10^{-5} M in all cases, and [Amylose] = 1.0 × 10^{-3} M for solutions containing amylose. Excitation wavelength = 425 nm.

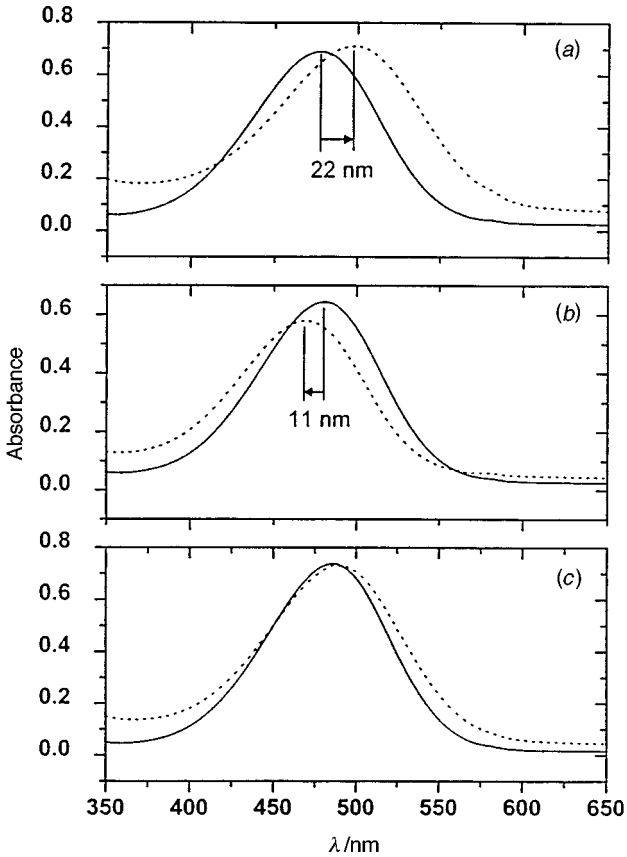


Fig. 2 Visible spectra of dyes **1**–**3** in the free state (—) compared with those of their inclusion complexes (···): (*a*) dye **1**, (*b*) dye **2** and (c) dye **3**. Free dye spectra were recorded at Φ_{DMSO} = 0.90. Spectra of included dyes were recorded at Φ_{DMSO} = 0.50, [amylose] = 1.0×10^{-3} M and [dye] = 1.5×10^{-5} M in all cases.

maximum fluorescence emission‡ of the supramolecular complex. The gradual decrease in emission intensity of the included dye in water-rich mixtures ($\Phi_{DMSO} < 0.50$) is not due to the separation of the dye from the amylose inclusion. It can be therefore attributed, as previously suggested,[3] to conformational changes (*i.e.* swelling) of the amylose helix induced by increasing the solvent polarity of the medium.

The characteristic free dye absorption (at *ca.* 475 nm) of dyes **1**–**3** undergoes a spectral shift (Fig. 2) upon complexation with amylose. This is associated with the difference in intramolecular charge-transfer (ICT) transitions between the free dye state and the inclusion state. The ICT band-shifts due to the complexation of the dyes (**1**–**3**), which are determined by comparing visible spectra of each dye in the fully-included and free (unincluded) states, differ in both magnitude and direction, depending on the dye structure. In view of the fluorescence results (*vide supra*), a fully-included dye can be represented by the spectra recorded at Φ_{DMSO} = 0.5 in the presence of amylose. However, it is difficult for dyes **2** and **3** to represent the free dye state in the spectra recorded under identical solvent condition (Φ_{DMSO} = 0.5), due to aggregation of the dyes. Alternatively, the free state of each dye can be best represented by its spectrum recorded at Φ_{DMSO} = 0.90, where no aggregation occurs even in the presence of amylose (mentioned above).

Thus, as shown in Fig. 2, complexation of dye **1** induces a 22 nm red-shift, but a 11 nm blue-shift is observed upon the complexation of dye **2** under comparable conditions. In contrast, the visible λ_{max} for the inclusion state of dye **3** is red-shifted by only 3 nm relative to the free dye. For dye **1**, the position of the alkyl substituent ensures that the charged pyridinium group resides completely within the nonpolar host cavity in the inclusion state. Exclusion of the pyridinium moiety from solvent contact results in the red-shift in visible absorption of the included dye relative to the free dye in this case. In contrast, inclusion of dye **2** confines the alkyl-substituted amino donor in the host cavity, while the pyridinium cation end resides near the edge of the host, being in contact with the bulk solvent polarity. This situation leads to a blue shifting of the absorption band. The common feature in both cases is that the local environment of the sensing units (amino donor and pyridinium acceptor) is differentiated by inclusion formation. Unlike the case of dyes **1** and **2**, both the donor and acceptor moieties of dye **3** are confined in the same environment in the inclusion. This situation seems to have little influence on the ICT in the excited state, leading to a negligible spectral shift.

In solutions of non-inclusion free dye, both the donor-head and acceptor-tail of dye molecules are exposed to a homogeneous environment. The fact that hemicyanine dyes exhibit negative solvatochromism (blue-shift of λ_{max} with increasing solvent polarity)[10,11,13] and that charge-shift occurs in the chromophore by excitation, suggests that the ground state is more stable than the excited state, and that the pyridinium cation in the ground state has a greater influence on the spectral shift relative to the neutral amino donor in the excited state. The opposite spectral shifts of the supramolecules can therefore be interpreted as being due to the fact that, when the pyridinium cation is exposed to a polar environment (dye **2** case), the ground state has a stronger influence on ICT, whereas when it is disposed in a nonpolar environment (dye **1** case), the excited state has a stronger influence on ICT. Accordingly, the spectral behavior of the present amylose–hemicyanine supramolecular complex bearing dye **2** shows a close resemblance of the membrane-embedded case, *i.e.* blue shifts in both absorption and fluorescence spectra relative to the free molecular state.

In summary, the direction of absorption band-shift upon inclusion of amphiphilic hemicyanine dyes by amylose has been related to the dye structure. This is interpreted in terms of the difference in local environments surrounding the sensing units of the chromophore in the inclusion. The charged pyridinium (acceptor) group, being more strongly influenced by the environmental polarity, plays a dominant role in determining the ICT band shift.

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Notes and references

† A low molecular weight (4500 D) amylose (Aldrich) was used for a 1:1 complexation (ref. 1) with the dyes, except for dye **3** with which a 2:1 complexation seems to be more favorable.

‡ The emission band shift due to the inclusion is significant and the direction of the shift depends on the dye structure; the shift of dye **1** is to the red and that of dye **2** to the blue. A minor band shift occurs between different Φ_{DMSO} regions.

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