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FLUORESCENT STILBENE, DIENE AND TRIENE SURFACTANTS AS PROBES OF REACTIVITY IN AMYLOSE INCLUSION COMPLEXES[1]

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Abstract—The surfactant diphenylbutadiene and diphenylhexatriene derivatives, trans,trans-1-[4-(3-carboxypropyl)-phenyl]-4-[4-butylphenyl]-1,3-butadiene (4B4A) and trans,trans,trans-1-[4-(3-carboxypropyl)-phenyl]-6-[4-butylphenyl]-1,3,5-hexatriene (4H4A), respectively, have been found to form highly fluorescent inclusion complexes with amylose in aqueous dimethylsulfoxide. The fluorescence of the complexed guests is comparable in efficiency to that of some intrachain surfactant trans-stilbenes and greater than that of earlier-studied monosubstituted stilbenes. Dissociation constants (K_d*) for the inclusion complexes are shown to be mainly determined by the effective chain length for several comparable surfactants. A preliminary study of quenching of the excited singlet of 4H4A indicates that the complexed probe is less reactive towards a variety of quenchers by roughly one order of magnitude.

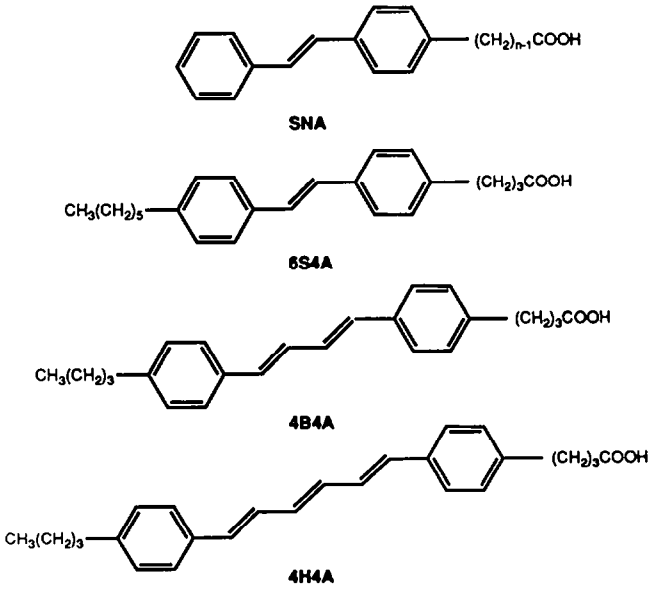
INTRODUCTION

The ability to form inclusion complexes in which a "guest" molecule is incorporated into a cavity in a larger host has been demonstrated for several different sets of reagents with a wide array of substances ranging from zeolites, cyclodextrins, to cryptates and crown ethers serving as sequestering hosts.[2-10] In most cases the host provides a fairly rigid pocket or binding site into which the guest can be incorporated. The driving force for host-guest complex formation can be provided by specific bonding interactions or, in several cases for aqueous solutions, by entropically-driven hydrophobic effects associated with release of structured solvent from both host and guest. Complex formation between the glucose polymer amylose (starch) and various organic reagents has been observed in a number of cases in aqueous or partially aqueous solution.[11-16] In these systems the type of complex formed appears more dependent upon the guest than on a cavity provided by the host since the amylose is a flexible linear polymer which can exist in a variety of conformations. In aqueous or partially aqueous solutions amylose exists in a metastable conformation (interrupted helix) such that it tends to complex linear hydrophobic molecules with the formation of relatively stable complexes. It is generally believed that the guest induces formation of a helical coil which surrounds the substrate and effectively shields it from the solvent and other solutes present in solution.[13,15]

In previous investigations we have observed that amylose or its water-soluble carboxymethyl derivative can form complexes with photochemically reactive surfactant or hydrophobic molecules; in several cases complex formation can be readily monitored by a coincident change in photophysical properties or photoreactivity of the guest.[17-19] Two cases we have studied in detail include a series of surfactant trans-stilbene derivatives[17,19] and some aromatic ketones.[18] For the latter we observed that formation of carboxymethylamylose inclusion complexes

in water reduces sharply the quantum efficiency of the Norrish Type II photoelimination; the reduction in reactivity is attributed to a reduction in γ-hydrogen atom abstraction rates for the complexed guest.[18] This effect is in accord with the complexed guest having an "extended" configuration in a cavity providing a reasonably hydrophobic and restricted environment. Our studies of the stilbene derivatives with amylose in dimethylsulfoxide (DMSO)-water solutions provide a similar picture for the environment of the complexed guest.[17] In this case our results show enhanced fluorescence and reduced trans → cis isomerization to be a characteristic feature of the complexed trans-stilbene chromophore. The surfactant molecules we have used in these studies have also been investigated in microheterogeneous media such as micelles, vesicles and Langmuir-Blodgett multilayers; comparison of the behavior of these molecules in the presence of different "hosts" provides a contrasting picture of the different microenvironments present on the photochemical time scale.[20,21] As will be developed subsequently, many of the results obtained thus far suggest that the amylose-incorporated surfactant guests experience a microenvironment similar to that encountered when the same probes are solubilized within the ordered hydrocarbon portion of the low temperature phase of saturated phospholipid bilayers.

In the present paper we report on the formation of amylose complexes with the surfactant diphenylbutadiene (4B4A) and diphenylhexatriene (4H4A) vinylogues of the intrachain surfactant stilbenes, 6S4A and 4S6A. Our results show that these molecules with the photoreactive chromophore "inserted" in the hyydrocarbon chain of a fatty acid have comparable compatability for complex formation to unsubstituted fatty acids or the previously-studied terminal-chain trans-stilbenes, S6A and S12A. Attractive features of the hexatriene 4H4A include its absorption and fluorescence at wavelengths conveniently red-shifted compared to the stilbenes and, more importantly, its longer fluorescent lifetime. The latter has allowed us to carry out a comparison of quenching of excited singlet states of bound and free 4H4A with a variety of reagents. The results reported here indicate that the amylose host provides a constrained environment which results in consistently lower rates for bimolecular quenching processes than for uncomplexed triene.



RESULTS AND DISCUSSION

As reported earlier, addition of amylose to 1:1 (v/v) DMSO-water solutions containing the surfactant stilbenes S4A, S6A or S12A results in an increase in fluorescence and a concommitant reduction of the trans → cis isomerization efficiency.[17] Although the quantum efficiency of

fluorescence increases, there is no alteration in either the absorption or emission spectra up to a concentration of 7.21×10^{-5} M (1.0%) amylose. trans-Stilbene itself shows no changes in absorption or fluorescence upon addition of up to 1% amylose to the DMSO-water solutions.[17] The increase in fluorescence can be used to evaluate the extent of complex formation.[17,22] Assuming the amylose contains n binding sites, the concentration of amylose sites is nA, where A is the concentration of amylose and the equilibrium is given in eqn (1), where S and C are the

$$S + nA \underset{1/K_d^*}{\overset{K_a^*}{\rightleftharpoons}} C \tag{1}$$

concentrations of fluorescent probe and host-guest complexes, respectively. In terms of initial concentrations of S and A and the equilibrium concentration of C, the equilibrium is expressed by eqn (2). Under conditions used in this study $nA_o >> C$ and the expression can be simplified and

$$K_d^* = \frac{1}{K_a^*} = \frac{(nA_o - C)(S_o - C)}{C} \tag{2}$$

solved for C (eqn 3). The change in total fluorescence efficiency can also be related to C,

$$C = \frac{nA_o S_o}{K_d^* + nA_o} \tag{3}$$

assuming characteristic fluorescence efficiencies, ϕ_f^c and ϕ_f^s, for complexed and uncomplexed guest, respectively, as indicated in eqn (4). Combination of eqns (3) and (4) leads to

$$\frac{[C]}{[S_o]} = \frac{\Delta\phi_f}{(\phi_f^c - \phi_f^s)} \tag{4}$$

eqn (5), which predicts a linear relationship between $1/\Delta\phi_f$ and $1/A_o$ with a slope/intercept

$$\frac{1}{\Delta\phi_f} = \frac{1}{\phi_f^c - \phi_f^s}\left(1 + \frac{K_d^*}{nA_o}\right) \tag{5}$$

ratio = K_d^*/n. As reported earlier using a similar relationship to eqn (5), such plots are linear for S4A and S6A.[17] The limiting fluorescence quantum yield for these terminal chain stilbenes is $\phi_f = 0.23$ or about 2.5-3 times that in a nonviscous homogeneous solution.

The intrachain stilbenes 4S6A and 6S4A also exhibit an increase in fluorescence when amylose is added to dilute solutions of them in 1:1 DMSO-water; for these compounds the limiting quantum yields for fluorescence (ϕ_f^c) approach unity.[19] Table 1 compares the fluorescence efficiencies measured for several stilbenes and related compounds in the presence and absence of amylose. The fluorescence lifetime (also given for several probes in Table 1) for the intrachain stilbene 6S4A

Table 1. Effect of amylose on fluorescence of stilbenes, dienes and trienes in aqueous DMSO

Guest	ϕ_f^{solv} [a]	ϕ_f^{Am} [b]	τ_{solv}, [c] ns	τ_{Am}, [d] ns
S	0.034	0.037		
6S4A	0.20	1.0	0.5	1.75
DPB	0.03	0.06		
4B4A	0.14	0.35[e]	0.3	1.0
DPH	0.08	0.21		
4H4A	0.15	0.80	1.5	3.4

[a] fluorescence in 1:1 DMSO-water [b] fluorescence in the presence of 1% amylose [c] fluorescence lifetime of probe in the absence of amylose; a species having the same lifetime is also observed upon amylose addition [d] "long-lived" fluorescence component observed when amylose is added [e] 0.5% amylose. All samples were undegassed.

shows two components in the presence of amylose; the predominant component in 1% amylose has a lifetime of 1.75 ns which is very close to the 1.7 ns limiting radiative lifetime for trans-stilbene.

In contrast to nonsurfactant trans-stilbene, its vinylogues trans,trans-1,4 diphenyl-1,3-butadiene (DPB) and trans,trans,trans-1,6-diphenyl-1,3,5-hexatriene (DPH) show some increase in fluorescence when amylose is added to solutions of 1:1 DMSO-water containing either probe (Table 1). The increases are modest compared to those observed for their surfactant counterparts 4B4A and 4H4A; for the latter the pronounced changes that occur are consistent with formation of complexes having properties similar to those of the complexed intrachain stilbenes 6S4A and 4S6A. Dissociation constants calculated for the surfactant stilbenes, 4B4A and 4H4A are listed in Table 2.[23] The values for n, the number of binding sites per amylose segments, estimated on the basis of the degree of polymerization (857) and number average molecular weight (1.39×10^5) assuming a 6-helix cavity (see below) and assuming the length of the extended surfactant from molecular models, are also listed in Table 2. For the stilbenes the fluorescence lifetimes are too low to reliably estimate the relative amounts of free and complexed probes; however for both 4B4A and 4H4A the fluorescence lifetimes are significantly longer such that reliably reproduceable weights can be obtained and the expression given by eqn (6) used to calculate K_d^*, where f_f and f_c refer

$$K_d^* = \frac{f_f}{f_c} \cdot nA_o \tag{6}$$

to the fraction or weight of fluorescence from free and complexed probe as deconvoluted from fluorescence decay. For both 4B4A and 4H4A values of K_d^* estimated from several different amylose concentrations gave values in good agreement with one another and with those determined by plots according to eqn (5).

Table 2. Values of the amylose-"ene" complex dissociation constants, K_d^*

Compound	n	K_d^*, M[b]	Method
S4A	74	0.02[a]	plot of eqn 5
S6A	64	0.0097[a]	" "
S12A	47	0.00065[a]	" "
4S6A	53	0.0008	" "
6S4A	53	0.0009	" "
4B4A	53	0.003	" "
4B4A	53	0.003	fluorescence lifetimes, eqn 6
4H4A	47	0.0004	" " "

[a]data from ref. 17. [b]concentration of 1% amylose (A_o) = 7.21×10^{-5} M as determined by viscosimetry.

The values of K_d^* tabulated in Table 2 for the series of surfactant stilbenes, 4B4A and 4H4A, follow trends previously encountered with other surfactant molecules. Thus we find that 4S6A and 6S4A have values for K_d^* slightly higher than that of S12A but substantially lower than that of S6A, reflecting the slightly shorter overall chain length of the "extended" molecules in terms of effective numbers of $-CH_2-$ groups. 4H4A, which would be expected to be "longer" than S12A, has a slightly lower value of K_d^* but, surprisingly, 4B4A has an anomalously high value. It is probably reasonable to assume that all of the surfactants used in this study form amylose complexes having similar structures. Force-area studies of the water-insoluble surfactants S12A, 4S6A, 6S4A, 4B4A and 4H4A in films at the air-water interface all indicate that these reagents can be compressed into extended configurations giving solid-like films with cross-sectional

areas/molecule in the range 20-25 $Å^2$; their behavior is quite similar to that of saturated fatty acids under the same conditions.[21,24] Amylose has its glucopyranose units in a C1 chair conformation[25] and several studies indicate that included molecules in linear amylose should occupy helical cavities.[11-16] These helical cavities (6-helix and 7-helix, so-named in terms of the number of glucose units per turn in the helix) have cross-sectional areas of 16 and 38 $Å^2$,[26,27] respectively, for pure amylose. The cross-sectional areas indicated above for the stilbene, diene and triene suggest that in the inclusion complex the helical cavity of the host must be intermediate between a 6-helix and a 7-helix. The resulting structure of the host-guest complex is thus probably not ideal from the point of the host and it is reasonable that complex formation should generally increase with the length of the guest.

The ability of amylose to form host-guest complexes with hydrophobic or surfactant molecules is clearly a function of the solvent used and in turn on its effect on the conformation and stability of the amylose. For amylose solutions in aqueous DMSO Hui and coworkers and others have shown that increasing the DMSO mole fraction results in a tighter and more regular helical structure for the amylose polymer.[28] Thus in pure DMSO amylose exists as a "stiff" or regular helix which resists complete inclusion of a hydrophobic guest; addition of water results in a disordering of the structure such that first there coexist regions of loosely wound helix and random coil. Finally when the solvent composition approaches pure water the conversion to a random coil structure is nearly complete. For substrate-guests in which hydrophobic-lipophilic interactions (which should be dominant when a linear hydrocarbon chain is to be incorporated within the helical cavities described above) govern complex formation K_d^* values are expected to be strongly solvent-sensitive and increase as the fraction of DMSO in the binary mixtures increases; such an effect has been previously observed with the hydrophobic probe N-ethyl-4-hexadecyloxy-carbonyl pyridinium iodide.[28,29] For the surfactant stilbene 6S4A, as the % DMSO (v/v) in binary solvent mixtures with water increases from 50 to 60%, we find that the ratio of fluorescence in the presence and absence of 1% amylose decreases from 6.0 to 1.0.[29] The lack of fluorescence increase upon amylose addition to the solution containing a higher fraction of DMSO suggests either that no complex is formed in this solvent mixture or, perhaps less likely, that any association between host and guest leads to little perturbation of the photophysics of the stilbene-chromophore.[29] These results emphasize then that the ability of amylose to form complexes is a consequence of the polymer being solubilized in a poor solvent and indicate some limitations on the scope of inclusion complex formation of this type.

To return to the question of the precise nature of the constrained environment experienced by an amylose-incorporated guest it should be noted that there is no direct correlation between the extent of fluorescence enhancement and the magnitude of K_d^* Thus the intrachain stilbenes 4S6A and 6S4A have <u>larger</u> K_d^* values than S12A but much greater fluorescence enhancement. This is a reasonable consequence of the location of the chromophore <u>within</u> the linear molecule which is in turn within an elongated helical section of the polymer. For the stilbenes studied the limiting fluorescence quantum yields and lifetimes of the amylose-incorporated molecules are similar to those measured for the same compounds incorporated into saturated phospholipids such as dipalmitoyl phosphatidylcholine (DPPC) below the phase transition temperature, T_c. Our studies of the temperature dependence of fluorescence efficiency at temperatures below T_c for the vesicle-incorporated stilbenes suggest that the enhanced fluorescence is due to an order-limited environment and not simply due to a high medium microviscosity.[20] Such a description probably should also apply to the helical cavity of an amylose-stilbene complex. Interestingly, preliminary studies show sharp differences between the surfactant stilbenes and 4H4A in phospholipid vesicles below T_c with the latter (and to a lesser extent with 4B4A) showing a decrease in ϕ_f in the low temperature phase.[30] The fact that 4H4A and the corresponding stilbenes 6S4A and 4S6A show similar values for K_d^* as well as a comparable enhancement of fluorescence underlines,, as outlined above, that the amylose is a "flexible" complexing reagent in 1:1 DMSO-water which can accept a fairly diverse array of guests.

The triene 4H4A has a considerably longer fluorescent lifetime than the corresponding surfactant stilbenes for both the complexed and uncomplexed species. Thus it is possible to measure dynamic quenching of the respective excited states with reagents soluble in the complexing medium. Table 3 lists rate constants obtained for quenching of complexed and "free" 4H4A in DMSO-water solutions with amylose. While these preliminary results show that the magnitude of the effect is variable and evidently quencher-dependent, it is clear that bimolecular quenching is generally and significantly slower for the amylose-complexed 4H4A compared with the uncomplexed probe. That the effect is generally less than an order of magnitude suggests, in accord with the discussion above, that the helical array of sugar molecules surrounding the guest does not completely seal it off from solutes in the solution. It should be noted that quenching constants obtained with para-benzoquinone in solutions of 4H4A containing no amylose are comparable to those for the free 4H4A in amylose solutions.

Table 3. Quenching of fluorescence of free and complexed triene (4H4A) by different quenchers[a]

Quencher	Concentration	k_q^c[b] ($M^{-1}s^{-1}$)	k_q^f[c] ($M^{-1}s^{-1}$)	k_q^f/k_q^c
I^-	1.0 M	3.3×10^7	2.4×10^8	7.3
1,1'-dimethyl-4,4'-bipyridinium	0.02 M	3.2×10^9	1.8×10^{10}	5.5
Cu^{2+}	0.002 M	1.4×10^{10}	4.7×10^{11}	35
p-benzoquinone	0.002 M	1.1×10^{10}	9.0×10^{10}	8.4

[a]1:1 (v/v) dimethylsulfoxide-water solutions, 0.5% amylose, non-degassed. The quenching was measured by comparing fluorescent lifetimes for both components in the presence and absence of quencher. [b]quenching of the long-lived (τ_o=3.4 ns) component. [c]quenching of the short-lived (τ_o=1.8 ns) component.

Interestingly there appears to be little, if any, oxygen quenching of the relatively long-lived excited singlet of 4H4A whether complexed or free in aqueous DMSO. Thus we find lifetimes for both species are identical within experimental error for deaerated, air-saturated and oxygen-saturated solutions.

In summary, our results indicate that formation of amylose complexes in which motion on the ns timescale is considerably restricted is a fairly general property for linear hydrocarbon or surfactant molecules in aqueous DMSO or other mostly aqueous solutions. Complex formation is governed by hydrophobic interactions and is favored in solutions where the amylose is poorly dissolved and in a metastable conformation. The "constrained environment" of the incorporated guest appears much less restrictive than those provided by more rigid hosts such as cyclodextrins or zeolites, although a move detailed investigation of both ground and excited state reactivity of various incorporated molecules such as 4H4A is certainly needed before any detailed conclusions can be drawn regarding reactivity or selectivity. The sensitivity of K_d^* (or K_c^*) to solvent composition suggests a number of intriguing possibilities for carrier/delivery properties of amylose or modified amylose derivatives which should be fairly conveniently studied with strongly fluorescent reagents such as 4H4A.

EXPERIMENTAL

Syntheses

1. 4H4A: 5-(p-butylphenyl)-penta-2,4-dienal was prepared by a procedure analogous to that reported by Marshall and Whiting for the unsubstituted analogue.[31] Thus a solution of 19.4 g (0.133 mol) p-(n-butyl)-benzaldehyde (Kodak) in dried tetrahydrofuran (THF) was added dropwise with stirring to a cooled solution of the magnesium bromide salt of 4-methoxybut-3-ene-1-yne in THF (from 14.2 g (0.17 mol) 4-methoxybut-3-ene-1-yne and 0.16 mol of ethylmagnesium bromide) to yield 12.8 g (50%) of a crude dark red oil.

A Wittig reaction was carried out by dissolving 4.3 g (0.00785 mol) of the phosphonium salt of methyl 4-(p-bromomethylphenyl)-4-oxo-butanoate, BrTK4E, (prepared by the procedure reported by Mooney et al.[21]) in a mixture of 25 ml CH_2Cl_2 and 80 ml THF (distilled from $LiAlH_4$). To this solution 5.0 g (0.0234 mol) of the crude dark oil of 5-(p-butylphenyl)-penta-2,4-dienal dissolved in 20 ml THF, 5.0 g potassium carbonate and 25 mg of 18-crown-6-ether was added. The mixture was refluxed under nitrogen and the product was checked by thin layer chromatography (TLC) using CH_2Cl_2 as eluent. A brightly fluorescent yellow spot was detected at an R_f=0.4. The liquid was poured off, leaving potassium carbonate behind. The solvent was evaporated and the remaining solid was separated on a silica column, again using CH_2Cl_2 as eluent, leaving the triphenylphosphine oxide impurity on the column. The fractions collected contained a brightly fluorescent product with R_f=0.4. The solvent was evaporated and the product recrystallized from hexane yielding 1.5 g (47.5%) of a yellow solid (mp=145-147°C).

The keto-ester, 4HK4E, was reduced by a Clemmensen reduction. Zinc metal (Mallinckrodt, 0.9 g, 20 mesh) was washed with 4 ml of 10% hydrochloric acid for 1 min. followed by two washings in distilled water. To the zinc 4 ml of amalgamating solution (5 ml concentrated hydrochloric acid, 50 ml water, and 5 g mercuric chloride) were added and the mixture was agitated for 1 min. The zinc was washed four times with distilled water and placed in a flask containing 4 ml of 25% hydrochloric acid, followed by the addition of 0.214 g (0.0053 mol) of 4HK4E and 8 ml of toluene. The mixture was refluxed with vigorous stirring. The TLC, eluted with CH_2Cl_2, showed a brightly fluorescent spot at an R_f=0.8 which indicated complete disappearance of the starting material. This reduction was repeated six more times, and the combined toluene layers were dried with anhydrous sodium sulfate. The solvent was evaporated and the product passed through a silica column using CH_2Cl_2 again as eluent. The solvent was evaporated from the fractions containing the condensation product which was recrystallized once from hexane and once from acetone-water, yielding 0.735 g (49%) yellow crystals (mp=128-130°C).

Hydrolysis of the ester was achieved by refluxing a mixture of 0.335 g (0.00086 mol) of the ester with 4 pellets of potassium hydroxide, 10 ml acetone and 5 ml water for two hours with stirring. The mixture was concentrated under an aspirator vacuum to a volume of 10 ml. The pH was adjusted to 2-3 by the addition of 25% hydrochloric acid. The precipitate was filtered and the fiiltrate was extracted with ether (3 x 20 ml). The precipitate and the ether layer were combined, evaporated and recrystallized from acetone-water, yielding 0.15 g (44.8%) of the yellow solid 4H4A (mp=158-160°C). ^{1}H NMR ($CDCl_3$) 0.9 (3H, t, CH_3), 1.3-1.6-1.95 (6H, 3m, CH_2), 2.4 (2H, t, CH_2COOH), 2.56 (2H, t, $ArCH_2$), 6.48-6.56 (4H, m, Vinyl), 6.8-6.86 (2H, m, VinylAr), 7.1 (4H, d, m-ArH), 7.32 (4H, d, o-ArH); UV (methylcyclohexane) λ_{max}=358 nm.

2. 4B4A: A solution of 3.24 g (0.02 mol) p-butylbenzaldehyde and 6.0 g (0.02 mol) formylmethylenetriphenylphosphorane (prepared by a procedure reported by Trippett and Walker[32]) in 150 ml benzene was refluxed under oxygen-free nitrogen for 30 hours. The solvent was removed under reduced pressure and 2.9 g (90.6%) crude p-butylcinnamaldehyde, a viscous liquid, was separated on a silica column, using CH_2Cl_2 as eluent. This was dissolved in a mixture of 25 ml CH_2Cl_2 and 80 ml THF (distilled from $LiAlH_4$). To this solution 3.8 g (0.007 mol) of the phosphonium salt of BrTK4E, 5.0 g potassium carbonate and 25 mg of 18-crown-6-ether were added. The work up was analogous to that used for 4TK4E. TLC indicated a spot at R_f=0.6 (CH_2Cl_2). After recrystallization from ethanol, a yield of 1.3 g (50%) of yellow crystals of the keto-ester, 4BK4E, (mp=146-148°C) was obtained. The Clemmensen reduction of 1.3 g 4BK4E by the procedure described above for 4HK4E furnished 0.65 g (52%) of a yellow solid (mp=90-92°C and R_f=0.85, CH_2Cl_2) after recrystallization from ethanol. This ester, 0.35 g (0.00097 mol), was hydrolyzed as described above giving 0.128 g (38%) of 4B4A as a yellow solid (mp=138-140°C) after recrystallization from acetone-water. ^{1}H NMR ($CDCl_3$) 0.92 (3H, t, CH_3), 1.34-1.59-1.96 (6H, 3m, CH_2), 2.37 (2H, t, CH_2COOH), 2.6 (4H, m, $ArCH_2$), 7.2 (4H, m, Vinyl), 7.15 (4H, d, m-ArH), 7.4 (4H, d,o-ArH); UV (cyclohexane) λ_{max}=318 nm.

Materials and Methods

Amylose (Aldrich, mol. wt. ~1.5 x 10^5) was used as received.[23] Millipore filtered water (0.22 μm) was used in all studies and glass distilled dimethylsulfoxide (DMSO) was from OmniSolv. Potassium iodide and copper sulfate were vacuum dried overnight at 37°C. Methyl viologen dichloride was used as received from Aldrich and benzoquinone was vacuum sublimed.

A solution of 2% amylose/DMSO was prepared by dissolving 2.0 g amylose in 100 ml DMSO. To 25 ml of this solution, 25 ml water were added dropwise with stirring to yield a 1% amylose/H_2O:DMSO (1:1) solution. The appropriate amount of a probe stock solution in chloroform (Baker, spect. grade) was evaporated under an N_2 stream. To this the appropriate volumes of 1% amylose and aqueous DMSO (1:1) were added to obtain the desired concentrations of amylose in aqueous DMSO. Typical probe concentrations were ~5 x 10^{-6}M. The concentration of 1% amylose has previously been determined by viscosimetry to be 7.21 x 10^{-5}M.[23] The resulting solutions were bath sonicated for 20 minutes. For the quenching studies the appropriate amount of quencher was dissolved in aqueous DMSO (1:1) and added with an equal volume of 1% amylose to a flask coated with 4H4A to yield solutions of 4H4A/0.5% amylose + quencher in which ~80% of the probe was complexed with the amylose (as determined by the integration of fluorescence lifetimes). Again, solutions were bath sonicated for 20 minutes prior to use.

Fluorescence Quantum Yields

A reference solution with a known quantum yield was prepared with the same optical density as the probe for each solution studied. References included tr-S/MCH, DPB/CH and DPH/CH at 25°C with quantum yields of 0.05, 0.44 and 0.80, respectively. The integrated intensity of the emission band was compared for the probe-containing solution to that of the corresponding reference over the same wavelength region with constant excitation wavelength. All experiments were done at 25°C which was controlled by a Haake A81 temperature bath.

Instrumentation

Ultraviolet spectra were recorded on an IBM 9430 UV-Visable spectro-photometer. Fluorescence spectra were recorded on a SPEX 111CM spectrofluorimeter. Fluorescence lifetime measurements were obtained by a single photon counting method using a PRA Fluorescence Lifetime Instrument, interfaced to a PDP 11/23 microcomputer.

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