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Supramolecular aggregates of photoreactive aromatics. Structure, photophysics and photochemistry of stilbene and azobenzene phospholipids

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Abstract

Stilbene and azobenzene-derivatized phospholipids have been synthesized as building blocks to study H-aggregate formation, structure and properties in both bilayer vesicles and LB films. The observed H-aggregates in bilayers display similar spectral characteristics to that in LB films and a strong induced circular dichroism spectrum. Studies of interconversion between H-aggregates and monomers or dimers have established relatively small, integral numbers for aggregate sizes. Based on these results as well as molecular cooling simulation, chiral cyclic pinwheel structures are proposed for these H-aggregates. Single crystal structure of a *trans*-stilbene derivative (**4**) has been obtained, and its remarkable similarity in structure to compressed monolayer obtained from molecular simulation and almost identical spectra to H-aggregate of stilbene support the proposed herringbone lattice for these aggregates. The very large arrays of aggregates may consist of a "mosaic" of small aggregates. The presence of aggregates as either stable or metastable entities has special consequences for microscopic as well as macroscopic properties of the medium in which they are generated. The aggregates of azobenzene in DPPC vesicles promote reagent release much more efficiently than the correspondent monomer and dimer. *Trans–cis* photoisomerization of azobenzene aggregates opens a convenient way to establish photoregulatable membrane and related materials.

Keywords: Supramolecular aggregates; Photoreactive aromatics; Stilbene phospholipids; Azobene phospholipids

1. Introduction

The association of aromatic chromophores and dye molecules in condensed phases is generally easily detectable by shifts in absorption and emission spectra, the "type" of aggregate formed is often classified by the characteristics of these spectral shifts [1–4]. Studies of these molecules in microheterogeneous media such as thin films [5–9], micelles, microemulsions [10] and vesicles [11,12] involve frequent encounters with aggregated species, giving rise to many questions as to why and how aggregates are formed as well as to their size, structure and properties relative to the "isolated" molecule in dilute organic solution. In the present paper, we focus on some recent studies in our laboratory which have involved two photoreactive chromophores *trans*-stilbene and *trans*-azobenzene, which have been incorporated into amphiphilic molecules, fatty acids and phospholipids, which have in turn been studied in Langmuir-Blodgett films and aqueous bilayer vesicles. The results presented focus on the novel structure and properties of the small unit aggregates formed by association of these chromophores that can be fairly described as true supramolecular species.

2. Results and discussion

Our investigations into aggregate formation with *trans*-stilbene derivatives began with the synthesis of some stilbene-containing fatty acids (SFAs) whose structures are given in Fig. 1. While these compounds could be dispersed in micelles or vesicles in very dilute dispersions to give absorption and fluorescence similar to that observed for the same compounds in dilute homogeneous solution, their behavior in either pure or mixed (with saturated fatty acids such as arachidate) supported Langmuir-Blodgett films was quite different. As shown in Fig. 2, both the absorption and fluorescence of the SFAs in the LB films are shifted compared with solution; the direction of the shifts is consistent with a "card pack" or "H" aggregate according to the exciton treatment developed by Kasha and Hochstrasser [1,2], using the point-dipole approximation, and the more elaborate treatment of Kuhn et al. [3,4], using an extended dipole-extended

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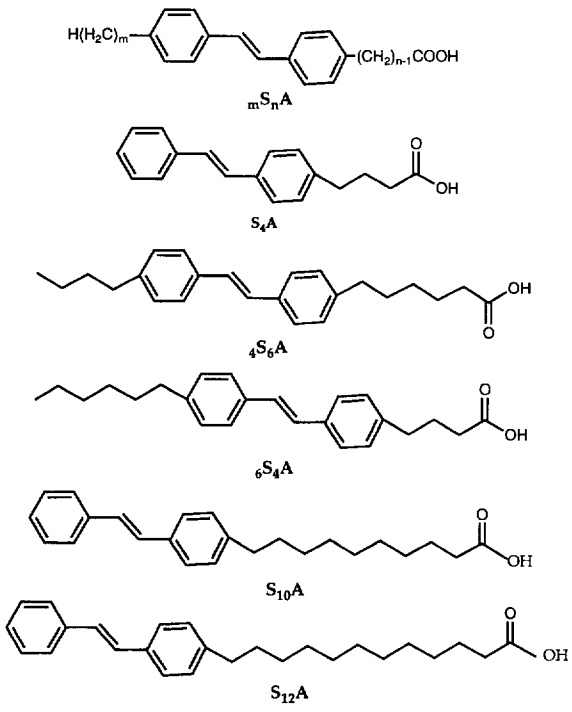

Fig. 1. Strucure of SFAs used in this work.

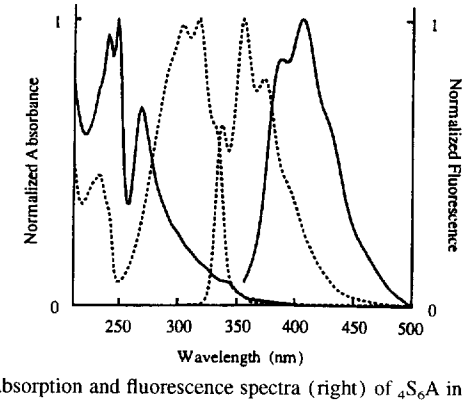

Fig. 2. Absorption and fluorescence spectra (right) of $_4S_6A$ in chloroform (dotted line) and multilayer film (solid line).

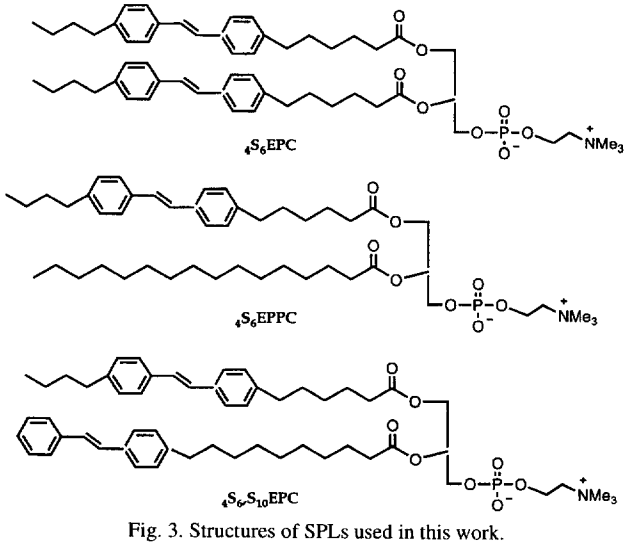

Fig. 3. Structures of SPLs used in this work.

dipole approach. That the shifts observed are due to physical interaction of the chromophores is easily shown by the observation that the LB assemblies can be readily dissolved by organic solvents to "liberate" unreacted SFA quantitatively [13]. Since the SFAs exhibit film-forming properties (isotherms, limiting areas/molecule, etc.) very similar to linear saturated fatty acids such as arachidate, we initially assumed that the formation of a card-pack aggregate was a consequence of forcing the chromophores together in the process of forming the solid LB films at the air–water interface. We expected that dilution of the SFAs with excess arachidate or other saturated fatty acids should lead to the appearance of monomer absorption and fluorescence. Moreover, we expected that mixtures of two or more of the SFAs shown in Fig. 1 should lead to more complex spectra due to the presence of "mixed aggregates" having different relationships between the stilbene chromophores and consequently different exciton splittings. We were surprised to find that aggregate absorption and fluorescence persisted for binary mixtures of SFA (or related diphenylbutadiene or diphenylhexatriene derivatives) with arachidate until rather low dilution, where statistically little aggregate should be expected. We also found that mixtures of two or more SFAs gave LB films having very similar spectra to those of pure SFA and no clear evidence of the occurrence of any "mixed aggregates" [9–14]. Interestingly, a number of studies suggested that mixtures of SFAs and related functionalized amphiphiles gave rise to films consisting of "zones" of like-aggregated SFAs which are far from homogeneous. All of these observations suggested that aggregation of the SFAs in LB films was a more complex process than initially suspected and that the aggregates might be more stable and more complex than a simple sandwich or card pack, as might be anticipated from simple packing considerations. Difficulties in studying the dynamics of aggregate formation in LB films led to our preparation and study of stilbene phospholipids (SPLs) (Fig. 3), which could be used as more complex building blocks for formation of aggregates in bilayer vesicles with hosts such as the naturally occurring phospholipids, such as dimyristoyl

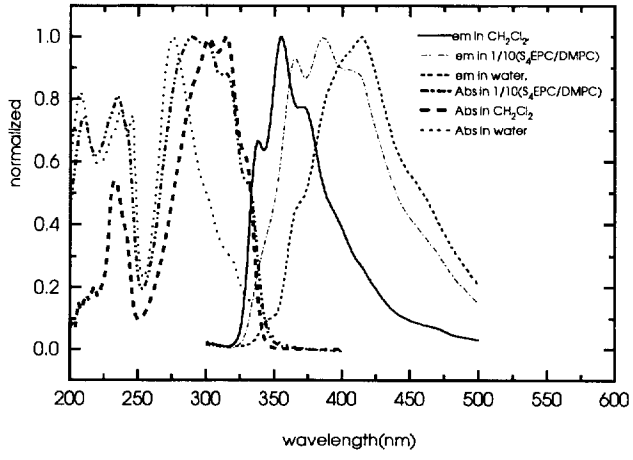

Fig. 4. Absorption (abs) and fluorescence (em) spectra of **1** in chloroform, DPPC and water.

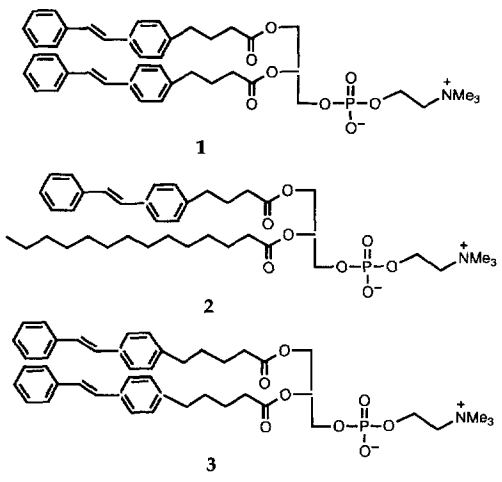

Fig. 5. Structures of the shorter chain SPLs used in this work.

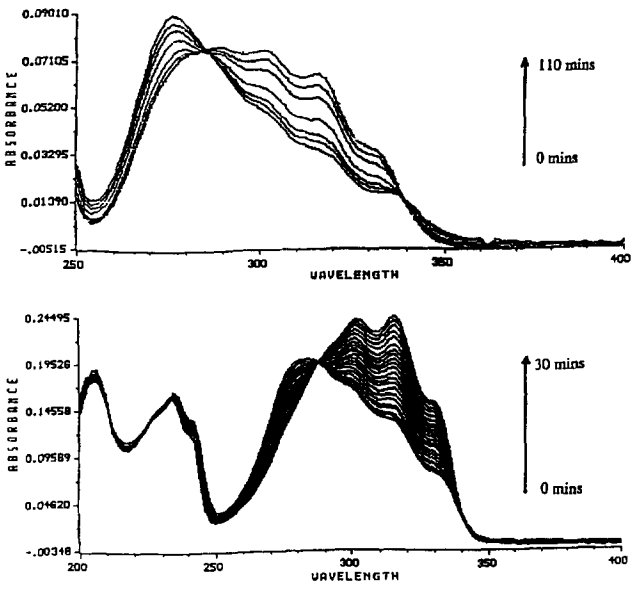

Fig. 6. (a) (upper trace) Dilution of aqueous solution of **1** with DMPC as a function of time. (b) (lower trace) Dilution of aqueous solution of **2** with DMPC as a function of time.

phosphatidyl choline (DMPC) or dipalmitoyl phosphatidyl choline (DPPC) [15,16]. As shown in Fig. 4, dispersion of a "pure" SPL such as **1** in water led to a clear solution having absorption and fluorescence spectra very similar to those of the SFAs in LB films. In contrast, methylene chloride solutions of the same SPL showed absorption and fluorescence spectra indistinguishable from those of substituted *trans*-stilbene monomers. Dispersions of the SPL with an excess of saturated phospholipid such as DPPC gave absorption and fluorescence spectra somewhat altered from the monomer but quite different from those obtained from pure aqueous dispersions. That the latter spectra could be attributed to a stilbene dimer was shown by the finding that γ-cyclodextrin solutions of either SFAs or $_4S_6$EPC gave nearly identical absorption [17]. This indicated that the aggregate formed in the LB films and phospholipid bilayers was larger than a dimer. Further insights into the size and structure of the aggregates were provided by the study of the shorter chain SPLs **1–3** whose structures are shown in Fig. 5. SPLs **1** and **2** have relatively low T_c values and should be in a more fluid phase at room temperature than the longer chain compounds shown in Fig. 3. Consequently, we anticipated that aqueous dispersions of these compounds should undergo ready interchange with aqueous dispersions of saturated phospholipids such as DMPC, upon mixing at room temperature or above. As shown in Fig. 6, mixing of excess DMPC with aqueous solutions of **1** or **2** leads to a clean spectral evolution from aggregate to dimer or monomer, respectively. The isosbestic points observed in the dilution process suggest that there is a clean conversion from aggregate to monomer or dimer, with the intervention of a range of aggregates of decreasing size. From the very clean equilibration process in dilution of SPLs such as **1–3** with DMPC, it is possible to determine equilibrium constants and binding energies for the aggregation, and more importantly, aggregation numbers. These were found to be 3, 4 and 7 for **1–3**, respectively, corresponding to 6, 4 and 14 stilbene units per aggregate. An additional clue to the aggregate structure was found by examining induced circular dichroism (ICD) spectra for the different SPLs in the various solutions and dispersions. Since the SPLs were always prepared using chiral glyceryl phosphatidyl choline and the "host" phospholipids, DPPC and DMPC are enantiomerically pure, the possibility exists that the achiral stilbene chromophore exists in a chiral environment in the phospholipid bilayers. However no ICD signals (or very weak ones) are detected when the SPLs are present as monomer or dimer. In contrast, the dimer of SFAs or SPLs entrapped in chiral γ-cyclodextrin shows a moderate ICD, characteristic of a chiral excitonic state [18,19]. For the aqueous phospholipid dispersions where the stilbene chromophore is aggregated, there is a very strong ICD spectrum characteristic of an excitonic state which persists during dilution without change in dispersion, until the aggregate disappears. The intensity of the ICD, and the fact [20,21] that it only is observed in the phospholipid dispersions when aggregate is present, suggests that it is not due to a "chiral environment" for an achiral chromophore such is observed with the stilbene monomer or dimer entrapped in a cyclodextrin, but rather due to a chiral structure for the aggregate itself.

Strong support for the idea of a chiral structure for the aggregate also emerges from simulations for the structures of monolayers of the SFAs. A Monte-Carlo cooling simulation [22,23] was applied to examine the lowest energy structures of monolayer clusters of SFAs such as $_4S_6$A and S_4A, whose LB films display similar spectral behaviors to those aggregates in phospholipid vesicles. In each case it was found that several of the lowest energy structures were glide or herringbone arrangements, such as shown schematically in Fig. 7; from these structures the values for several measurable properties, such as the limiting area/molecule and the predicted exciton splittings, can be predicted. As shown in Table 1, the lowest energy glide or herringbone arrangements give remarkable agreement between these measured and predicted

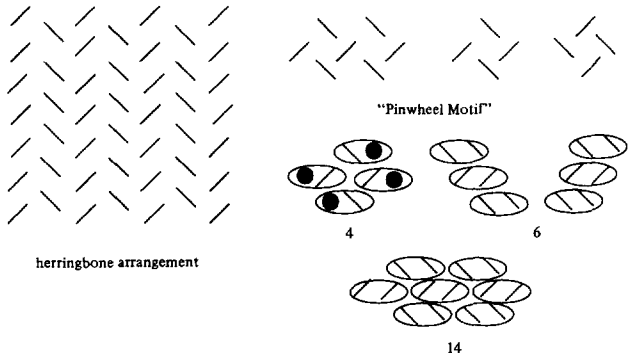

Fig. 7. (left) Schematic glide arrangement of SFA monolayer. (right) Schematic representations of (top) possible cyclic chiral unit structures within a glide layer arrangement (overhead view) and (bottom) possible arrangements of four, three and seven SFA phospholipid units in glide aggregates.

quantities. While the extended glide or herringbone lattice is not chiral, smaller pieces of it are, most notably the "pinwheel" tetramers or other structures as diagrammed in Fig. 7. Taking together the likelihood of a glide structure as the arrangement of an extended aggregate together with the measured aggregation numbers for **1–3**, we come up with the "pinwheel" tetramer as the most likely structure for the smallest or "unit" aggregates. This structure is chiral and accounts nicely for the observed ICD spectra associated with the aggregates. The structure is also attractive, in that it maximizes the number of "T" interactions which have been shown to be stabilizing for benzene and related aromatics (the favored structure for the benzene dimer from both theory and experiment is a "T" arrangement) [24–26].

Evidence that the pinwheel or unit aggregate may be a key intermediate in the formation of crystals or extended aggregates comes from an X-ray diffraction study of the weakly amphiphilic *trans*-stilbene derivative **4**. This compound forms stable crystals that exhibit fluorescence spectra very similar to those of the aggregates observed for the SFAs and SPLs in films and vesicles, respectively. Moreover, LB films of **4** at the air–water interface and in transferred layers show absorption and fluorescence typical of the "H" aggregated stilbenes. **4** forms crystals suitable for X-ray diffraction studies and we find that the crystal structure of **4** consists of sheets of the amphiphile that are remarkably similar in structure to compressed monolayers; alternate layers have either hydrophilic–hydrophilic (–COOH–HOOC–) or hydrophobic–hydrophobic (–OEt–EtO–) contact such as would be encountered in a "Y" structured multilayer assembly formed by successive transfer of monolayer films onto a support, alternately raised and lowered through the film. The arrangement of individual molecules of **4** within a sheet is nearly identical to the lowest energy glide or herringbone arrangement that emerges from simulations for S_4A. The remarkable agreement between the actual crystal structure measured for **4** and the structures obtained from the simulation and deduced from the above-described experiments, reinforces the idea that the pinwheel structure may be a very stable one for elongated aromatics maximizing the number of stabilizing non-bonded interactions and suggesting that this may be a relatively stable van der Waals molecule. Additional support for the pinwheel tetramer as a true supramolecular species comes from studies of squaraine dye aggregates in solution and microheterogeneous media [27,28]. Several of these dyes have been shown to form aggregates having comparable stability (similar ΔH° and ΔS°) which are also chiral (by ICD) and tetrameric, even though the squaraine chromophore has a structure considerably different from that of *trans*-stilbene. Simulations by the same method described above suggest that the squaraine aggregates also pack into glide or herringbone lattices and predict a similar unit structure to that for the *trans*-stilbene derivatives.

The SFAs and SPLs in LB films and aqueous bilayers, respectively, show very low photochemical reactivity. Irradiation with ultraviolet light leads to a relatively slow bleaching of the chromophore to give products whose identity is currently under investigation [29,30]. From studies carried out thus far, it appears that there is little or no photoisomerization to produce the corresponding *cis*-stilbene derivatives. In order to obtain compounds which might exhibit reversible photoisomerization in both monomeric and aggregated situations in films and vesicles, we have recently synthesized and

Table 1
Predicted unit cell, surface area/molecule and spectral shifts for $_4S_6A$ and S_4A Monolayer

	Layer type	a^a	b^a	γ^a	Surface area	λ_1^c	λ_2^c	Ratio[d]	Energy[e]
$_4S_6A$	glide	6.34	6.81	90.00	21.59	269	329	0.13	
	glide	4.48	11.31	90.00	25.32	284	320	0.35	1.96
S_4A	glide	6.29	6.52	90.00	20.50	264	330	0.00	
	glide	6.74	6.47	90.00	21.79	268	330	0.00	1.18
Exptl					22[f]	270[g]	333[g]	0.16[g]	

[a] Unit cell dimensions in angstrons; angle in degree.
[b] Surface area/molecule in A^2.
[c] Exciton spectra peaks in nanometers. λ_1 and λ_2 for the glide layer as a result of the Davydov splitting.
[d] Computed ratio of the oscillator strength $f(\lambda_1)/f(\lambda_2)$ for the Davydov splitting.
[e] Energy above the apparent global minimum in kilocalories.
[f] Data for $_4S_6A$ monolayers.
[g] Data for S_6EPC vesicles.

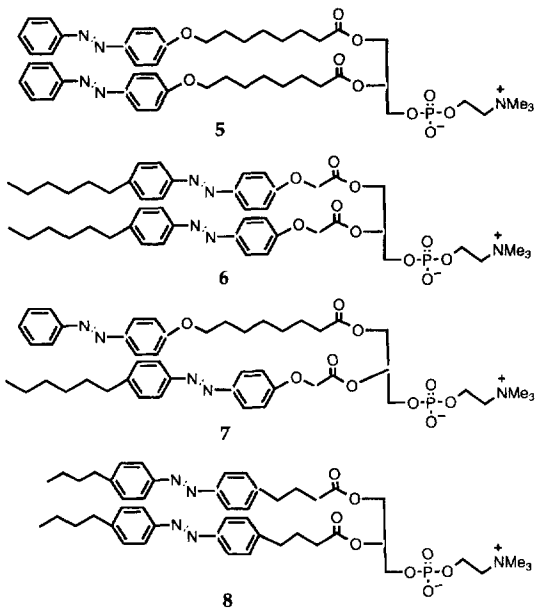

Fig. 8. Structures some APLs **5-8**.

studied some azobenzene phospholipids with structures very similar to those of the stilbene derivatives described above. Fig. 8 gives the structures of azobenzene phospholipids (APLs) **5–8** which have been the focus of some recent investigations. Analogous to their stilbene counterparts, **5–8** show blue-shifted absorption spectra when dispersed in water, compared to their spectra when dissolved in organic solvents such as methylene chloride. When dispersed in water with excess of DPPC or DMPC, a slightly blue-shifted but intermediate spectrum is obtained. None of the APLs show fluorescence; however the ICD spectra in different media parallel those for the SPLs suggesting that the isolated achiral chromophore is too far from the chiral center in the glyceryl head group to impart a strong chirality when the chromophore is isolated or dimeric. The strong ICD that is observed for dispersions of the APLs in water is consistent with the idea that the azobenzene aggregate is chiral and the presence of a chiral center in the phospholipid head group leads to a diastereomeric interaction favoring one form of the chiral aggregate.

In contrast to the SPLs, the APLs undergo clean photoisomerization upon irradiation with ultraviolet light in all of the media mentioned above. Especially interesting is the isomerization when dispersions of **5–8** in pure water are irradiated. The aggregated *trans*-azobenzene is observed to undergo photoisomerization as followed by monitoring the UV-VIS spectrum; however the product *cis*-azobenzene that is produced has an absorption spectrum similar to that of the *cis*-azobenzene generated from irradiation of dilute organic solutions of the *trans*-azobenzenes. This suggests that the photoproduct from irradiation of *trans*-azobenzene aggregates may be a non-aggregated *cis*-azobenzene. This is reinforced by following the photoisomerization by ICD (Fig. 9); as the *trans* is converted to *cis*, the ICD signal decreases to near zero, then reappears as the *cis* is reconverted to the *trans* photochemically. The conversion of *cis* to *trans* for the APLs

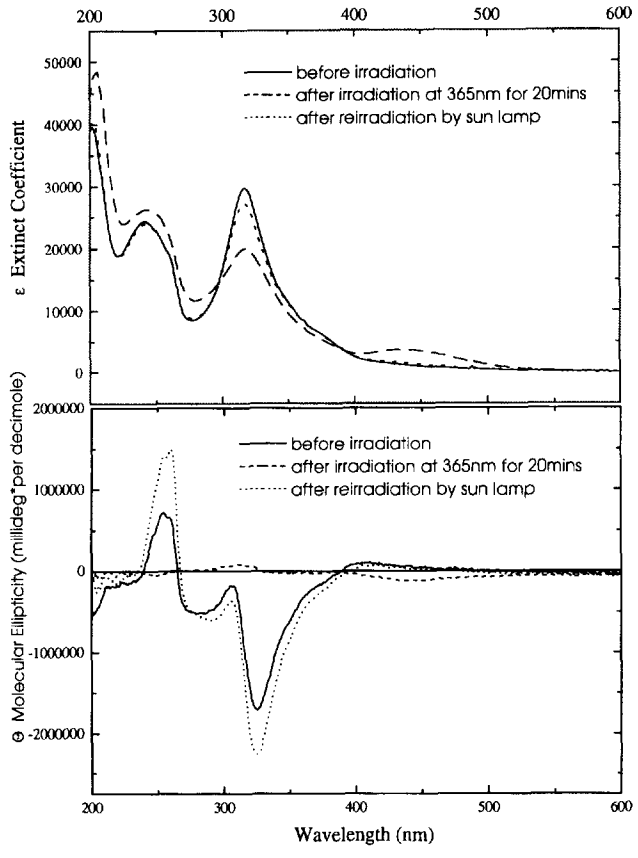

Fig. 9. Absorption (upper) and ICD (bottom) of **5** vesicles before and after irradiation.

occurs either thermally (hours at room temperature) or by irradiation; both the *trans–cis* and *cis–trans* interconversions appear clean with little loss through several cycles.

5–8 are somewhat more soluble in water than the corresponding SPLs and it is possible to follow phase transitions by differential scanning calorimetry. Table 2 gives values for **5–8** together with values for the aggregation numbers of the compounds determined by dilution with DMPC in a manner analogous to that described above for the SPLs. The trends observed with the APLs are also followed for the SPLs: larger aggregates (probably a mosaic of unit aggregates) tend to be formed for those compounds with the chromophore relatively far from the head group, and these compounds show a higher T_c and melting parameters suggesting a more ordered layered structure. The microstructures formed from the APLs examined thus far, by light scattering and cryo-transmission electron microscopy (cryo-TEM), appear not to be closed bilayer vesicles. Light scattering (and filtration studies) suggest that the particles formed on dispersion into water to give clear solutions, are much larger than conventional phospholipid vesicles (> 100 nm diameters). The cryo-TEM studies suggest that the structures may be bilayer plates or sheets which show little curvature. A reasonable interpretation is that the aggregated chromophores resist the necessary curvature that would be required to form small unilamellar vesicles. Interestingly, when **6** in water is irradiated to convert the *trans*-azobenzene to *cis* and the photoproduct quickly subjected to

Table 2
Phase transition temperature, enthalpy and aggregate number for **5–8**

Compounds	T_m (°C) (major)	T_m (°C) (minor)	δH (kcal/mole) (major)	Aggregate number
5	74.5		11.1	42
6	40.1	36.5	7.8	3
7	35.5	39.2	2.1	3
8	45.5		6.2	3

cryo-TEM investigation, it is found that no large structures can be detected. As the photoproduct reconverts from *cis* to *trans*, some smaller, presumably spherical, structures can be detected in the intermediate ranges which could be mixed bilayer vesicles.

While the pure dispersions of **5–8** do not give stable closed vesicles that can entrap reagents, mixtures of the APLs with DPPC and other lipids which do form closed bilayer vesicles does lead to stable mixed vesicles. For example, it has been found that for mixtures of individual APLs **5–8** with DPPC, containing up to 20% APL, stable vesicles are formed that can entrap organic dyes such as carboxyfluorescein (CF). The CF can be added in sufficiently high concentration (>0.1 M) that its fluorescence is nearly totally quenched [31,32]; removal of CF from the medium surrounding the vesicles can be accomplished by filtration and the vesicles containing entrapped CF are stable towards leakage for extended periods, provided they are stored in the dark such that the azobenzene remains *trans*. Irradiation of APL–DPPC vesicles with light absorbed by the azobenzene chromophore releases the entrapped CF (as evidenced by a sharp increase in the CF fluorescence) concurrent with photoisomerization of the *trans* azobenzene to *cis*. Interestingly, it has been found that the release of entrapped CF is most effective when the APL is in the aggregated form; although the precise origin of this effect has not yet been determined, it appears reasonable that photoisomerization of an aggregated azobenzene may produce greater "damage" to the vesicle wall by catastrophic fracture (perhaps by expulsion of several APL molecules in the aggregate) than by isomerization of a single isolated azobenzene, which may either produce only a small transient "hole" by exit of a single phospholipid molecule or a persistent "defect" region in which the *cis*-azobenzene is retained but does not permit substantial leakage. Ongoing studies of the photoinduced leakage of different types of vesicles formed from mixtures of APLs with unsaturated and saturated lipids and various ternary combinations, should provide more details of the ways in which photoisomerization promotes permeation of different reagents both into and out of the bilayer walls.

We are currently investigating the possibility that similar aggregates may be formed with a wide variety of unsaturated and aromatic compounds that can be incorporated into similar amphiphilic structures, which can permit or even favor their association into clusters or aggregates in microheterogeneous media or interfaces. We are also attempting the synthesis of molecules containing the "unit aggregate" structure to more precisely define its properties as a molecular or supramolecular species.

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