trans-Stilbene Aggregates in Microheterogeneous Media: Evidence for a Chiral Cyclic Supramolecular Unit

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In previous studies we have shown that stilbene fatty acids (SFAs) and related amphiphiles incorporating rodlike aromatic chromophores form aggregates in Langmuir–Blodgett (LB) films or phospholipid bilayers in water.[1-5] Interesting aspects include the tendency of surfactant mixtures to disperse largely into regions of different aggregates (at the expense of mixed aggregate formation) and the persistence of aggregate formation in SFA–saturated fatty acid mixtures or SFA phospholipid–saturated phospholipid mixtures to relatively low dilution.[1,5] Recently we reported for the SFA phospholipids S_4EPC, S_4EMPC, and S_6-EPC (see below) that the aggregates in phospholipid bilayer vesicles exhibit integral aggregation numbers of 3, 4, and 7 phospholipids, corresponding to 6, 4, and 14 stilbene units, respectively.[3] Herein we report experimental and simulation investigations and propose a novel supramolecular unit structure for the aggregate.

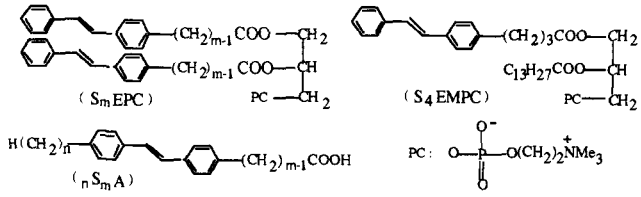

Figure 1 compares absorption and fluorescence spectra for S_4EPC in water, water with dimyristoylphosphatidylcholine (DMPC) (S_4EPC:DMPC = 1:10), and methylene chloride. The principal species are assigned to aggregate (hexamer), dimer, and monomer, respectively. Analysis of the time-resolved emission of S_4EPC in water indicates a range of lifetimes; a distribution analysis[6] shows four different lifetime distributions with center lifetimes 0.27, 1.4, 4.4, and 22.4 ns, respectively. From wavelengths associated with these distributions as well as a study of S_4EPC and S_4EMPC and other SFA derivatives,[1,7,8] these lifetimes are assigned to "nonconstrained" monomer, constrained monomer, dimer, and aggregate, respectively. Similar results have been obtained for other bis-SFA phospholipids. In each case dimer fluorescence increases at the expense of aggregate as the bis SFA phospholipid is diluted with a saturated phospholipid such as DMPC or dipalmitoylphosphatidylcholine (DPPC).

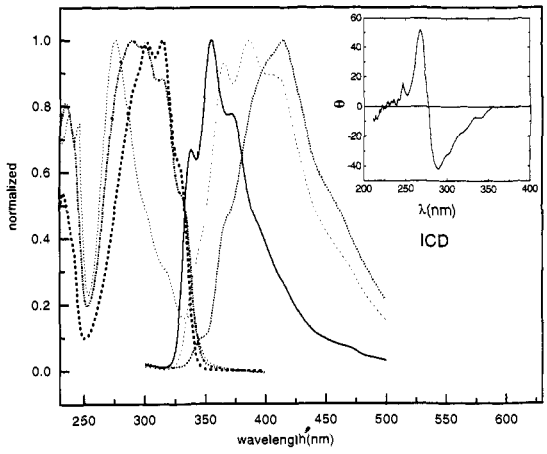

Figure 1. Absorption and fluorescence spectra of S_4EPC in CH_2Cl_2, DMPC, and water and ICD (inset) of S_4EPC aggregate in water. θ: Molecular ellipticity (1×104 deg·cm^2/dmol). —Fluorescence in CH_2Cl_2, · · ·Fluorescence of 1/10(S4EPC/DMPC) in water, •••Fluorescence in pure vesicle in water, • – •Absorption of 1/10(S_4EPC/DMPC) in water, - - -Absorption in CH_2Cl_2, · · ·Absorption of pure vesicle in water.

Although SFA phospholipids such as S_4EPC, S_6EPC, and S_4-EMPC are chiral (all *R* isomers), we see no induced circular dichroism (ICD) when the phospholipids are dissolved in organic solvents or dispersed in aqueous solutions containing excess chiral (also *R*) "host" saturated phospholipid.[9] This is reasonable since neither the chromophore nor its dimer is chiral and the chiral center in the "headgroup" is likely too far removed to induce chirality.[12] Although the "diluted" SFA phospholipids in vesicles where only dimer and/or monomer should exist give no ICD, pure and concentrated SFA phospholipids show a strong biphasic ICD (Figure 1). These ICD spectra are similar to those observed in cases where aggregates capable of "excitonic" interaction are generated from achiral molecules in the presence of a chiral host;[16-19] the biphasic ICD spans the exciton range (250–350 nm) with the crossing point near the λ_{max} of the major exciton band.[14,19] The high intensity of the ICD signal for all of the SFA phospholipids indicates that the stilbene aggregates are chiral.[16,17]

To determine possible aggregate structures that could account for the strong ICD in phospholipid vesicles, Monte Carlo cooling simulations were made on monolayer clusters of the SFAs S_4A and $_4S_6A$, whose LB film aggregates are similar to those for the phospholipids in vesicles.[1,2] Translation and glide stack local minima were first found in stage 1 using the Kitaigorodskii aufbau principle (KAP),[20] and these were then used in stage 2 of KAP to find the global and local layer minima.[21] Throughout the simulation all single bonds (except the OH group) were allowed to rotate (6 for S_4A and 12 for $_4S_6A$). The surface areas/molecule

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Table 1. Predicted Unit Cell, Surface Area/Molecule, and Spectral Shifts for $_4S_6A$ and S_4A Monolayers

		unit cell dimensions[a]							
	layer type	*a*	*b*	γ	surface area[b]	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 angstroms; angle in degrees. [b] Surface area/molecule in Å^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 strengths $f(\lambda_2)/f(\lambda_1)$ 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.

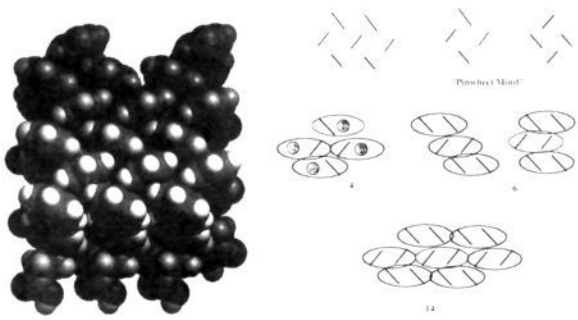

Figure 2. Left: "Overhead" view of simulated monolayer assembly of S_4A (17 molecules) in most stable glide layer arrangement. Right: Schematic representations of (top) possible cyclic chiral unit structures within a glide layer arrangement (overhead view) and (bottom) possible arrangements of four (S_4EMPC), three (S_4EPC) and seven (S_6EPC) SFA phospholipid units in glide aggregates.

were computed from unit cell dimensions of the local minima structures. Exciton shift spectra were computed using the extended dipole approximation of Czekkely, Forsterling, and Kuhn.[22] For this computation a transition moment of 9.07 D was estimated from the oscillator strength of the monomer in solution at 320 nm and the classical dipole length was estimated from the carbon–carbon end–end distance for trans-stilbene of 9.43 Å. A dielectric constant of 2.5 was assumed. The computation converged for a layer structure of 14 × 14 unit cells. The glide layers show a Davydov splitting whose oscillator strength ratio was estimated from the resultant vector sum and difference of the classical transition moments.[23] Table 1 lists results for the most stable structures in order of increasing energy above the apparent global minimum (the glide layer structure) and compares them with experiment. The lowest energy structures, which show close agreement with the experimental surface area and exciton band shifts, are the global minimum glide layer of $_4S_6A$ and the first local minimum for S_4A. Other structures showing close correlation are local minima that also correspond to glide layer structures for both compounds.

An "overhead view" of the simulated glide layer structure for the S_4A monolayer is shown in Figure 2 along with a schematic representation of this structure. While we have not calculated structures (monolayer or bilayer) for the phospholipids containing two SFA units, the similarity of spectra and photophysics[1,3–5] suggests similar structures for the aggregates of both SFAs and phospholipids in the LB films and vesicles. Thus we propose that a glide layer structure is the extended arrangement for the stilbene chromophores in the aggregates of the SFA phospholipids in vesicles. The strong ICD for the SFA phospholipids indicates that the aggregate structure is chiral; while it is clear that both a simple dimer in the glide aggregate and an infinite aggregate of the glide layer should be achiral, smaller units within the glide layer structure can be chiral. Recent reports suggest that overall symmetry can be broken by component molecule domains of differing chirality within Langmuir–Blodgett films,[24] an effect that may be operative in the bilayer vesicle.[25] The observation of multiple fluorescent lifetimes supports the idea of different "domains" in both layer structures. In fact the smallest chiral units or domains are the two cyclic structures diagrammed in Figure 2. Of these the "pinwheel" consisting of four stilbenes in a cyclic array appears most attractive to explain the induced chirality and the observed aggregation numbers of 4, 6, and 14. The mono-SFA phospholipid S_4EMPC can give the simplest "pinwheel" structure, while any bis SFA phospholipid requires at least three units combined. Aggregates such as the pinwheel exist as *R* or *S* isomers; since the phospholipid headgroups are chiral, the *R* and *S* aggregates from a single chiral phospholipid are diastereomers and hence of differing energies.

It appears reasonable that the "pinwheel" unit may be a general supramolecular energy minimum for a variety of aromatics and conjugated compounds topologically similar to *trans*-stilbene such as azobenzenes, diphenylbutadienes, diphenylhexatrienes, and diphenylacetylenes, since many of these structures give evidence for formation of aggregates having very similar absorption spectra under the same conditions.[1,5,26] This structure appears closely related to other stabilized aromatic clusters and can be viewed as a dimer of the "T"-shaped species which has been indicated to be the most stable dimer for benzene (in both the gas phase and crystal)[27–34] and related aromatics.

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