Anatomy of an Aggregate. Use of Functionalized Phospholipids To Investigate Stoichiometry and Structure of "H" Aggregates Formed from Amphiphilic *trans*-Stilbene Derivatives

Xuedong Song, Cristina Geiger, Inna Furman, and David G. Whitten*

Department of Chemistry
University of Rochester
Rochester, New York 14627

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Amphiphiles such as *trans*-stilbene fatty acids (SFAs) with an extended chromophore embedded in a fatty acid backbone form stable films at the air–water interface (with mechanical properties very similar to those of the saturated fatty acids (FAs) of similar length) and Langmuir–Blodgett (LB) multilayer assemblies.[1-5] Characteristic of both films on water and the LB assemblies is the prominence of aggregates characterized by relatively sharp blue-shifted absorption and red-shifted fluorescence. Similar phenomena occur for 4-donor, 4′-acceptor-substituted SFAs and the corresponding diphenyl-1,3-butadiene and diphenyl-1,3,5-hexatrienes; aggregation persists even until relatively low dilution in mixed SFA–FA layers.[4,5] The spectral shifts can be ascribed to exciton interactions which could arise by "packing" the *trans*-stilbene chromophores into an "H" aggregate or "card pack" array.[1,6,7] The near-constancy of aggregate spectra over a wide concentration range and their persistence even to relatively low dilution suggest the aggregate may be of relatively small size and high stability. Here we report an investigation using specially synthesized dimeric "building blocks", in this case SFA-substituted phospholipids, which readily form similar aggregates both in water and in LB assemblies. The ability to study these aggregates systematically in aqueous solution has led us to determine that the SFA aggregate is indeed an energy minimum in which an integral number of stilbene units combine to form relatively stable supramolecules.

Although we have prepared a number of mono- and bis-SFA phospholipids with either the same or different SFAs in the case of the latter compounds,[8,9] the present discussion will focus on the three relatively short-chain SFA phospholipids whose structures and acronyms are shown in Chart 1. All three compounds dissolve in organic solvents such as chloroform or methylene chloride, giving solutions which show absorption, fluorescence, and fluorescence lifetimes characteristic of *trans*-stilbene or the SFAs in dilute solution; clearly the observation of only monomeric stilbene even in S_6EPC and S_4EPC where the "local concentration" of *trans*-stilbene is very high indicates no evident tendency toward association in either the ground or the excited state.

Quite different behavior is observed when the three compounds are dispersed (with or without sonication) in water or water containing a saturated fatty acid phospholipid such as dimyristoylphosphatidylcholine (DMPC). In water alone, all three of the substituted phospholipids give blue-shifted absorption (Figure 1) and structured red-shifted fluorescence nearly identical to those obtained earlier for LB assemblies of SFAs.[1,4] Using treatment which leads to small unilamellar vesicles or liposomes with DMPC,[10] clear solutions for the SFA PCs are obtained which scatter light but cannot be extruded through filters which readily pass vesicles of DMPC. Light-scattering data for S_4EPC in water (diameter 198 ± 1 nm)[11] and other bis-SFA phospholipids indicate a relatively narrow distribution of sizes for the vesicles formed from the SFA phospholipids but significantly larger diameters than those from saturated phospholipids.[12-14] For S_4EPC, a discontinuity in the fluorescence efficiency and light-scattering intensity with temperature near 23–24 °C suggests a phase transition; this is consistent with an increase in equilibration rates

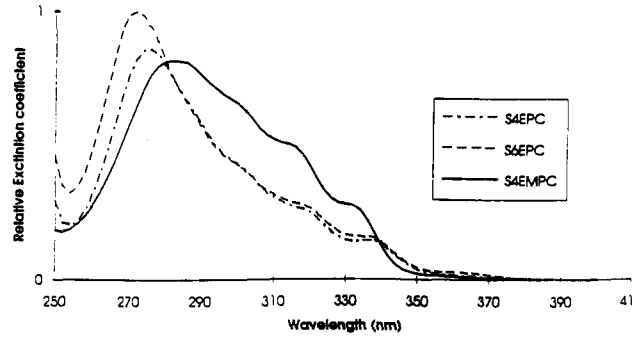
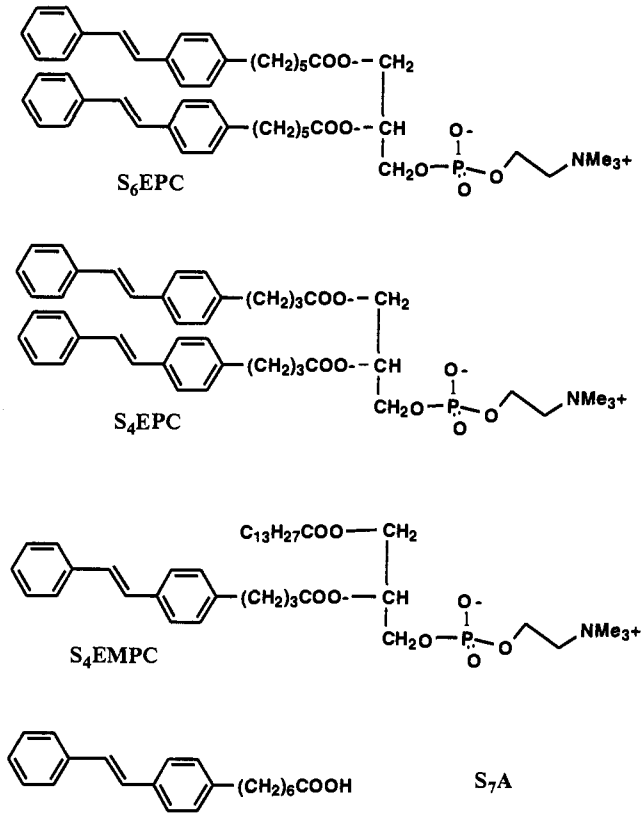


Figure 1. Absorption spectra of SFA phospholipid in water. The spectrum of S_4EMPC shows a trace of monomeric component.

Chart 1



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(11) We thank Dr. Thomas Whitesides of Eastman Kodak Co. for obtaining this value for us.
(12) Other SFA phospholipids give diameters in the range 240–520 nm;[13] these may be compared with our measured value for DPPC (93 nm) and the literature values for DMPC (96.8 ± 2.9 Å at 15 °C and 127 ± 2.8 Å at 30 °C).[14]
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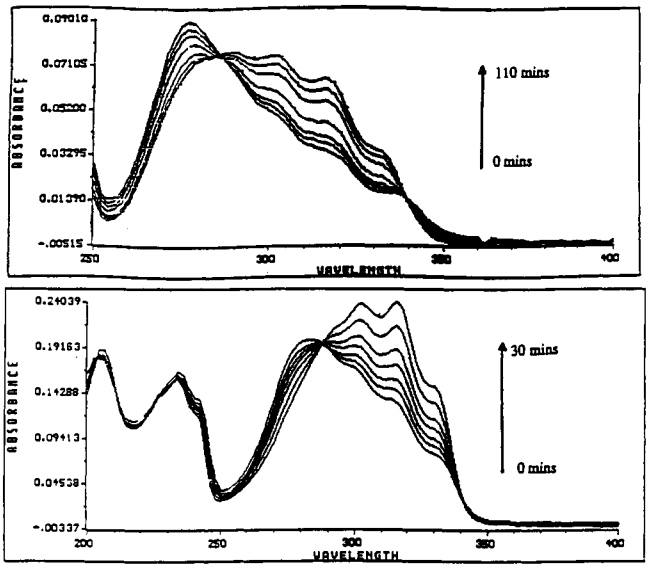

Figure 2. (a) (upper trace) Dilution of aqueous solution of S_4EPC with DMPC as a function of time. (b) (lower trace) Dilution of aqueous solution of S_4EMPC with DMPC as a function of time.

observed above this temperature (*vide infra*). For aqueous solutions of SFA phospholipid with excess saturated phospholipid (either DPPC or DMPC), absorption spectra show either stilbene monomer (S_4EMPC) or a spectrum slightly broadened from monomer (S_6EPC and S_4EPC). That the latter are actually "dimeric" is suggested by our finding of nearly identical spectra when S_4EPC or water-soluble SFAs such as S_7A are treated with high concentrations of γ-cyclodextrin (in contrast, only sharp monomer spectra are obtained when any of these compounds are treated with α- or β-cyclodextrin under identical conditions.).[15,16]

Although aqueous solutions of longer chain SFA phospholipids undergo only slow changes when treated with aqueous solutions of excess saturated phospholipid such as DMPC or DPPC, solutions of S_4EPC, S_6EPC, and S_4EMPC undergo fairly rapid equilibration upon mixing with DMPC solutions without any perturbation such as sonication. Figure 2a shows the time evolution of a mixture of S_4EPC and DMPC from aggregate to "dimer" at 30 °C; the two isosbestic points suggest that the aggregate decomposes directly to give dimer. Similar behavior is observed for S_6EPC and S_4EMPC, although for the latter the end product exhibits the monomer spectrum as indicated above (see Figure 2b). Thus the equilibration can be described by eq 1. Using a modified Benisi–Hildebrand[17] approach, the equili-

$$\mathrm{agg}_n \overset{K}{\rightleftarrows} n \text{ dimer (or monomer for } S_4EMPC) \quad (1)$$

$$\ln K = n \ln [\text{dimer}] - \ln [\mathrm{agg}_n] \quad (2)$$

$$\ln A_{agg} = n \ln [A_{agg}^{\circ} - A_{agg}] + \text{constant} \quad (3)$$

bration is described by eqs 2 and 3 in terms of the absorbance of aggregate and dimer. Plots according to eq 3 allow determination of n over a range of concentrations for each of the SFA phospholipids, where A_{agg} is the absorbance of the aggregate in the equilibrium mixture and A_{agg}° is the initial absorbance for the aggregate prior to mixing with DMPC (monitored at 276, 272, and 282 nm for S_4EPC, S_6EPC, and S_4EMPC, respectively). For S_4EPC, we obtain $n = 3.0 \pm 0.3$, while S_6EPC gives $n = 7 \pm 1$ and S_4EMPC gives $n = 4 \pm 0.3$, in each case at 30°C; consequently, the aggregate sizes in terms of *trans*-stilbene units are respectively 6, 14, and 4. For equilibration of S_4EPC with DMPC over the temperature range 30–50 °C (above the phase transition temperature for both phospholipids), we obtain $\Delta H = 30 \pm 1$ kcal/mol and $\Delta S = 90 \pm 1$ cal/mol deg.[18] The kinetics of the dilution with excess DMPC are first order in SFA phospholipid in each case, and for the case of S_6EPC, the rate is independent of [DMPC]. This suggests that aggregate dissociation may procede by extrusion of a single phospholipid unit from vesicles of pure SFA phospholipid.[19] Measured rate and equilibrium constants for reaction 1 for the three SFA phospholipids are listed in Table 1.

Table 1. Rate and Equilibrium Constants for Reaction 1 for S_4EPC, S_6EPC, and S_4EMPC at Different Temperatures

compound	t (°C)	K^a	k^b (min^{-1})
S_4EPC	23		1×10^{-2}
	30	3×10^{-3}	2×10^{-2}
	40	8×10^{-3}	
S_6EPC	30		1×10^{-3}
	40	8×10^{-8}	
	42		5×10^{-3}
S_4EMPC	23		3×10^{-2}
	30	5×10^{-1}	4×10^{-2}

[a] K is the equilibrium constant for reaction 1; units depend upon n. [b] k is the first-order rate constant for aggregate dissociation.

The observed results suggest that aggregate formation can be attributed to relatively strong apolar association[21–23] and not to simple packing or hydrophobic interactions. The fact that the aggregation numbers have clear integral values,[24] the lack of evident stability of the dimer, and the sharp absorption and fluorescence spectra for the aggregates together with the decrease in both dissociation rate and equilibrium constants with increasing size of the aggregate suggests that the structures consist of discrete, perhaps cyclic arrays of the stilbene chromophores, which can fairly be described as supramolecules, all point to this conclusion. The behavior observed in this study with *trans*-stilbene units may well be generalizable to other chromophores and the basis for rich new chemical and photochemical reactivity.

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(15) The size of the γ-cyclodextrin should permit inclusion of two SFA molecules (area/molecule, 24–25 Å^2)[1,4] or one SFA phospholipid (area/molecule, ~50 Å^2).[13]

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(19) Interestingly, in other cases of vesicles formed from functionalized phospholipids where similar dilution experiments are performed, the reaction rate is clearly dependent on [DMPC].[20]

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(24) We cannot exclude the possibility that the integral values measured reflect a distribution of sizes averaging to these values; however, the isosbestic points and constancy of aggregate spectra for different SFA phospholipids would then indicate that such a distribution must remain constant over a wide range of SFA phospholipid/DMPC concentrations.