Photoreactive Supramolecular Assemblies: Aggregation and Photoisomerization of Azobenzene Phospholipids in Aqueous Bilayers

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The formation of aggregates is frequently encountered when amphiphiles incorporating chromophores such as dyes or aromatics are organized in microheterogeneous media such as thin films, bilayers, or microemulsions.[1−3] While in some cases aggregate formation may be driven by the self-organizing properties of the amphiphile, there exists the possibility that the presence of aggregates as either stable or metastable entities can have special consequences for the microscopic as well as macroscopic properties of the medium in which they are generated. This paper focuses on a demonstration of some of these effects when a fairly simple photoreactive aromatic chromophore, *trans*-azobenzene, is incorporated into a phospholipid structure which in turn can be used, either pure or in mixtures with saturated phospholipids, to form bilayer structures in aqueous media.

We have shown that phospholipids containing a *trans*-stilbene chromophore in the fatty acid portion of a phosphatidylcholine form bilayer assemblies on dispersion in water in which the *trans*-stilbene chromophore is strongly aggregated.[4,5] The aggregate is characterized by a blue shift in the absorption spectrum, a red shift in fluorescence, and a strong induced circular dichroism (ICD) spectrum; studies of the aggregate−monomer or aggregate−dimer interconversion have established relatively small, integral values for the aggregation number. From these results and molecular simulations we have proposed a chiral cyclic "unit structure" for the stilbene aggregates.[5] In other investigations a picture of a very similar structure for aggregates of squaraine dyes is emerging.[6] The overall picture is one of fairly high stability and order in a relatively small aggregate unit such that even very large arrays of aggregate may be composed of a "mosaic" of small aggregates.[5−7]

We have synthesized a series of azobenzene phospholipids (APL) (**1**−**3**) shown in Scheme 1 which form aggregates similar to those of the stilbene but are somewhat more soluble in water and photochemically active. Absorption spectra of **1** in chloroform, water, and water with excess saturated phospholipid, dipalmitoyl phosphatidylcholine (DPPC), are similar to those obtained for related stilbenes[5] and are assigned to the *trans*-azobenzene monomer, aggregate, and dimer, respectively. The isosbestic points observed upon mixing aqueous solutions of **1**−**3** with saturated phospholipids (DPPC or dimyristoyl phosphatidylcholine (DMPC)) suggest that the aggregates decompose directly into dimers; a Benesi−Hildebrand analysis[4,8] indicates that the aggregation numbers for **1**−**3** are 42, 3, and 3 azobenzene units, respectively. As was observed for the stilbene aggregates previously described,[5] the azobenzene aggregates, either in pure azobenzene phospholipid dispersions in water or in mixtures with DPPC or DMPC, show a strong biphasic induced circular dichroism spectrum (Figure 1) which suggests a chiral structure for the aggregate "unit structure".[9−11] A Monte Carlo "cooling simulation"[12,13] on the azobenzene fatty acid

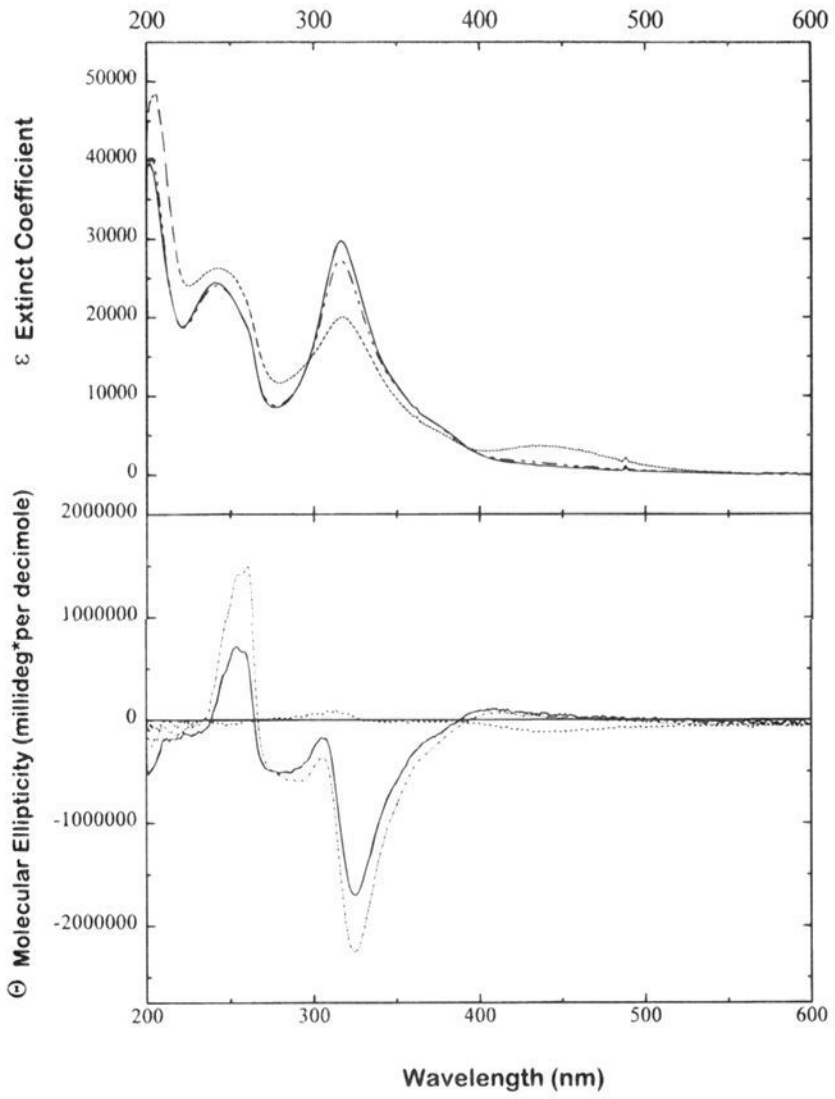
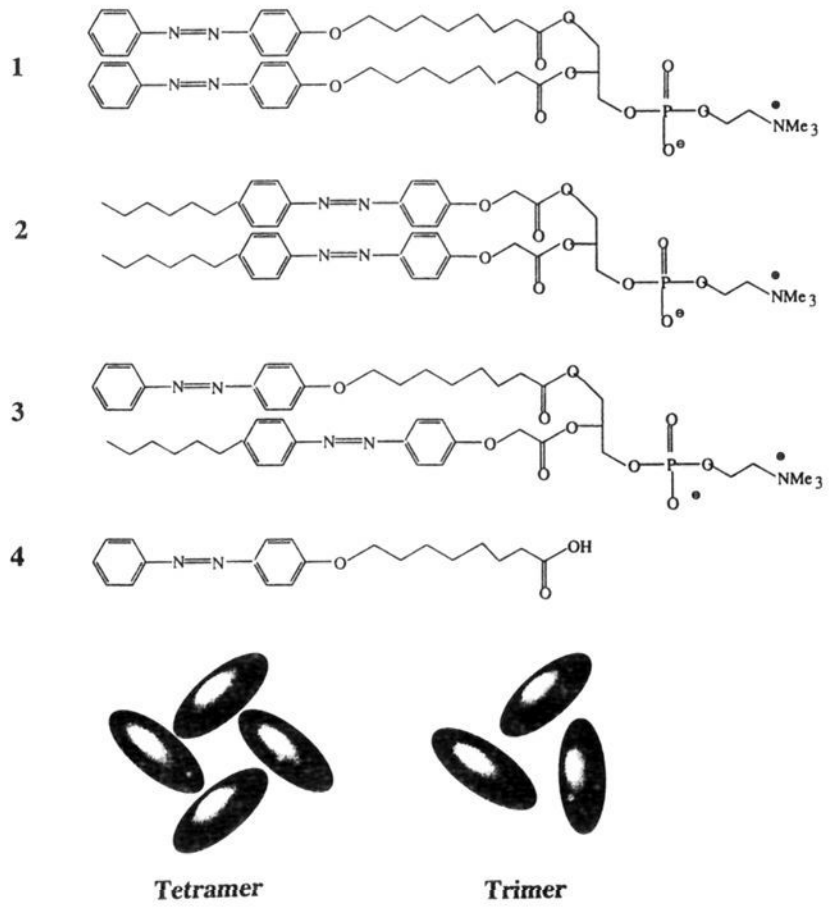


Figure 1. Absorption and ICD of **1** in aqueous dispersion: —, before irradiation; - - -, after irradiation at 365 nm for 20 min; - - -, after reirradiation by a tungsten lamp.

Scheme 1. Pinwheel Representation of Tetramer and Trimer Unit Aggregates



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(**4**) aggregates in a monolayer shows a glide or herringbone structure as the lowest energy configuration and leads us to suggest a "pinwheel" structure for the aggregate unit structure similar to that proposed for the stilbenes with either 3 or 4 monomers per pinwheel (Scheme 1).[5]

For the azobenzene phospholipids it is possible to investigate the relationship between aggregate structure and the properties of the assemblies formed from these aggregates. Since the oxygenated azobenzene phospholipids show reasonable solubility in water, phase transition temperatures (T_c) and enthalpies can be measured; T_c's were found to be 74.5°, 40°, and 35° for **1**, **2**, and **3**, with molar enthalpies of 11, 7.8, and 2.1 kcal/mol, respectively. Since the APLs are comparable in hydrophobic chain length to DMPC, the relatively high T_c values suggest that aggregate incorporation may increase the rigidity of the phospholipid bilayer. The large difference in measured aggregation number for **1** and the higher T_c are attributed to a greater ease in forming an extended aggregate when the chromophore is located far from the head group (compared to **2**) or where the phospholipid structure (as in **3**) precludes formation of an extended aggregate. The clear solutions of pure APL in water exhibit light scattering showing dispersity similar to the dispersities of DMPC or DPPC but with larger apparent diameters (355, 86, and 71 nm, respectively, for **1**−**3**).[14,15] A preliminary cryo transmission electron microscopy (TEM) study of **1** in aqueous solution shows very flat bilayer structures which appear to be extended sheets.[14] We find that pure aqueous solutions of **1**−**3** do not entrap dyes such as carboxyfluorescein (CF) as would be anticipated if closed stable bilayer vesicles were formed.[16,17] However, mixtures of APLs with excess DPPC or DMPC do form good mixed vesicles which are only slightly different in size from pure saturated phospholipid and which can entrap CF without detectable leakage for extended periods at 4 °C; since the equilibrium for aggregate formation is favorable, it is possible to form stable mixed vesicles in which there is a moderate concentration of aggregate.

The *trans*-azobenzene in aggregated, dimeric, or monomeric forms has been found to photoisomerize upon irradiation into the main 365 nm absorption band; not surprisingly, the efficiency is variable, with the photoisomerization being somewhat slower when the azobenzene is aggregated in aqueous solution either pure or in mixtures with DPPC. The resulting *cis*-azobenzene reconverts back to *trans* either optically (5 min exposure with a tungsten lamp, cutoff filter at 430 nm) or thermally (24 h at 23 °C). In contrast to the *trans*-APLs, aqueous solutions of the *cis* show no spectral evidence of aggregation; when the photoisomerization cycle of **1** is followed by ICD (Figure 1), a reversible loss of the ICD is observed as the *cis* is formed. Interestingly we find that while aqueous solutions of **1** (*trans*) cannot be filtered through 100 nm nucleopore polycarbonate membranes, irradiated solutions with near total conversion to *cis* are easily filtered; after photoregeneration of *trans* the solutions once again cannot be extruded. Inspection of aqueous **1** by cryo TEM following irradiation shows much smaller, curved structures indicating major morphological changes accompanying the photoisomerization.

The *trans*−*cis* photoisomerization of the azobenzenes in mixed aqueous solutions with DPPC where bilayer vesicles are formed is especially interesting. Controlled reagent release from vesicles has been the subject of several investigations using hyperthermia, photoreactivity, or pH-sensitive polymer surfactants;[18−23] it has been proposed that photoisomerization of certain azobenzene amphiphiles could also enhance leakage.[11,21] Vesicles containing entrapped CF can be prepared containing relatively high [CF] (>0.1 M) such that most of the CF fluorescence is self-quenched; leakage of the CF from the vesicles is easily monitored by an increase in the CF fluorescence.[16,17] When a 1:10 mixture of **2**/DPPC (under conditions where ca. 30% of the APL is aggregated) is prepared with entrapped CF,[24] irradiation of the vesicles at 365 nm for 40 s[25] results in near total release of the CF (as measured by comparison with the leakage induced by Triton-X-100 addition) concurrent with *trans*−*cis* isomerization of the azobenzene and its deaggregation. In contrast, when vesicles containing monomer at the same concentration (a 1:5 mixture of **4**/DPPC) are irradiated, there is only a small amount of leakage, even when most of the azobenzene has been isomerized to *cis*. These results indicate that photoisomerization-induced deaggregation of the APLs is much more effective in promoting reagent release than an isolated photoisomerization process in a nonaggregated azobenzene.[24] This rather striking result might be attributed to a number of factors. One possibility is that photoisomerization of a single azobenzene unit in an environment of relatively flexible hydrocarbon chains may result in only a small area perturbation of the bilayer wall that can be repaired fairly readily by an "annealing" with the hydrocarbon chains. In contrast, within the more rigid aggregate (or an extended mosaic of the aggregate) the isomerization may provide a much greater perturbation which can lead either to a larger "hole" by transient loss of additional molecules (such as an entire "unit aggregate") beyond the isomerized component or to a defect which is not as easily self-repaired.[26] The possibility of using photoreactive APLs such as **1**−**3** and related compounds with natural phospholipids suggests a number of possibilities for constructing photoregulatable membranes and related materials.

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