Bromination of Surfactant and Hydrophobic *trans*-Stilbenes in Aqueous Micelles and Vesicles. Evidence for Wide Variation of Solubilization/Reaction Sites in Microheterogeneous Media[1]

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Abstract: A comparative study of bromination rates for *trans*-stilbene and several surfactant and hydrophobic *trans*-stilbene derivatives in micellar and vesicular media is reported. Results obtained in the microheterogeneous media are compared to those for homogeneous solutions. It is found for all of the stilbenes studied that rates of bromination in SDS micelles are very rapid and monophasic; the behavior observed is consistent with the stilbene chromophore lying in all cases in a highly polar or interfacial site which is freely accessible to and relatively rich in water. In vesicles behavior is quite different and very structure dependent. In phospholipids especially, a sharp gradation in reactivity is observed with those surfactant and hydrophobic stilbenes having the chromophore at the end of a long polymethylene chain or in the middle of the chain showing slowest rates of reaction. The results show contrasting behavior of micelles and vesicles with regard to the range of solubilization sites available. They also point out striking differences in solubilization sites for vesicles which occur as a result of apparently minor changes in the structure of the solubilized reagent.

The possibility of controlling reactivity by incorporation of molecules into microheterogeneous media such as micelles, microemulsions, or vesicles has stimulated much interest. Vital to an understanding of possible modified reactivity is a knowledge of the microenvironment "provided" by the medium as well as an understanding of how the medium and its guest interact. Several studies have used numerous techniques and diverse probes to assess the structure of environments provided by different media, often with evidently correct but seemingly contradictory results.[3-14] While valid objections can be raised to the use of virtually any probe due to its possible perturbation of the environment, it is important to emphasize that the structures of "empty" micelles or vesicles are of relatively little interest for investigators using them as reaction media in which solubilized guests are involved. One of the major problems existing today involving microheterogeneous media is the elucidation of the range of solubilization sites available for different molecules and the degree to which different sites modulate reactivity.

In the present paper we report a study of bromination rates for several surfactant or hydrophobic *trans*-stilbene derivatives in micelles and vesicles. Bromination of alkenes, although a much studied reaction, has been the subject of recent concern over the question of single vs. two-electron (bromonium) transfer processes in organic chemistry.[15-17] In either case it is clear that there is considerable separation of charge as reactants proceed toward the rate-determining transition state; thus the reaction rates are highly sensitive to the polarity of the microenvironment. Our studies of bromination rates reveal a very wide variation in reaction rates for the same chromophore in micelles and vesicles which reflect the different sites available for reaction and simultaneously provide a picture of the different kinds of environment available to the quest solute.

Experimental Section

Materials. DL-α-Dipalmitoylphosphatidylcholine (DPL) and dicetylphosphate (DCP) were obtained from Sigma and used as received. Sodium dodecyl sulfate (SDS, Biorad electrophoresis grade) and dihydrocholesterol (3-β-cholestanol, Aldrich) were recrystallized from ethanol. Water was doubly distilled, once from alkaline potassium permanganate. Bromine (Fischer) was distilled. Ethanol was distilled from alkaline silver nitrate. Phosphate buffer was prepared from Na$_2$HPO$_4$ (0.64 mM), KH$_2$PO$_4$ (0.14 mM), NaCl (13.7 mM), and KCl (0.26 mM), and these reagents were Aldrich Gold Label grade. 1-Heptanol (Aldrich) was shaken with alkaline potassium permanganate three times, washed with water twice, dried over K$_2$CO$_3$, and distilled under reduced pressure.

Surfactant stilbenes (S4A, S6A, S10A, S12A, S16A, 4S6A, and 6S4A) were prepared according to the reported methods,[18] and purity was checked with NMR, UV–vis, and TLC. The hydrophobic stilbene, 4S4, was prepared from *p*-butylbenzaldehyde (Kodak) via reduction (lithium aluminum hydride), bromine substitution (PBr$_3$), and Wittig condensation with *p*-butylbenzaldehyde (mp 108–109 °C from ethanol). Anal. Calcd: C, 90.35; H, 9.65; Found: C, 90.26; H, 9.73.

Micelles. Preparation of probe-containing SDS micellar solutions was previously described.[19] The probe-containing SDS–heptanol solutions were prepared as follows: A solution of 2.5 × 10^{-3} M stilbene in chloroform (0.1 mL) was evaporated in a 25 mL volumetric flask under N$_2$ stream. SDS micellar solution (0.06 M, 25 mL) and 1-heptanol (0.355 mL) were added and the mixture bath-sonicated (Branson Model F, power 50 W) for 1 h at 40 °C.

Vesicles. Vesicles were prepared, treated, and then used for the reaction according to several different protocols as described below. The concentration of DPL and DCP was determined by using the method described by Petitou.[20]

Method A. This is the standard preparation of vesicles, in which the mixture of vesicle-forming monomers and stilbene derivative was probe-sonicated in aqueous solutions.[21-26]

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Chloroform solutions containing appropriate amounts of vesicle-forming monomers (+ dihydrocholesterol) and 2.5×10^{-3} M stilbenes were evaporated to dryness in a round-bottomed flask. The phosphate buffer (for DPL) or sodium hydroxide solution (for DCP, equimolar NaOH to DCP) was added, and the resulting suspensions were probe-sonicated with the microtip of a Heat Systems Model W-220F (Power level 4) at 70 °C for 30-60 min until a clear solution formed. The solutions were then centrifuged to remove titanium particles released from the sonication probe.

This method was used for DPL, DCP, and DCP + dihydrocholesterol.

Method B. The probe-containing vesicular solution obtained above (Method A) was separated by Sepharose 4B gel filtration chromatography, eluted with the phosphate buffer, giving two fractions, fraction 1 (multilamellar vesicle, MLV) and fraction 2 (small unilamellar vesicle, SUV).[27,28] However, a considerable amount of stilbene was removed during the gel filtration. This was used for DPL vesicular solutions only.

Method C. In this preparation, stilbenes were added to preformed vesicular solutions, which had been purified through Sepharose 4B column.

A solution of 120 mg of DPL in 10 mL of chloroform was evaporated in a 50-mL round-bottomed flask and 20 mL of phosphate buffer was added. The suspension was probe-sonicated at 60 °C for 1 h. The solution was centrifuged at 5000 rpm for 15 min, and the supernatant was subjected to a Sepharose 4B column (2.5×47 cm), eluted with phosphate buffer at 45 °C, according to the reported method.[27] Two fractions were obtained, and the latter fraction (SUV)[27] was treated with the stilbenes as shown below.

Appropriate amounts of solutions of the stilbenes (2.5×10^{-3} M) in chloroform were evaporated in a test tube under a N_2 stream. The SUV solutions obtained above by gel filtration were added, and the mixtures were magnetically stirred at 45 °C until the stilbenes were dissolved (ca. 30 min). For the incorporation of 6S4A, 4S6A, and 4S4, probe-sonication (60 °C, 6 min) was necessary to obtain a clear solution.

Vesicular solutions of DCP were also prepared with this procedure. An aqueous dispersion of DCP (23.7 mg) in 5×10^{-3} M NaOH (10 mL) was probe-sonicated at 55 °C for 20 min. The titanium particles were removed by centrifugation (5000 rpm, 30 min), and the vesicular solution was purified by Sepharose 4B gel filtration chromatography[28] (2.5 cm $\times$ 32 cm), eluted with distilled water at room temperature. The stilbenes were incorporated in the same way as described for DPL.

Method D. The reverse-phase evaporation technique[29] for making large unilamellar vesicles (LUV) was used in this preparation.

To a solution of 16 mg of DPL in a mixture of 2.2 mL of chloroform and 2.2 mL of isopropyl ether were added appropriate amounts of stilbene solution in chloroform and 0.66 mL of the phosphate buffer. The mixture was bath-sonicated at 45 °C for 15 min. The organic solvents were removed on a rotary evaporator at 45 °C. To the solution was added 18 mL of the phosphate buffer, and the solution was stirred at 45 °C for 30 min. The solution was then centrifuged at 5000 rpm for 30 min.

Bromination Rates. The concentration of aqueous bromine stock solution was determined by iodometry.

The bromination of stilbenes was initiated by adding 0.02-0.1 mL of 5×10^{-3} to 3.5×10^{-2} M aqueous bromine to 2 mL of probe-containing vesicular solutions (SDS-heptanol, aqueous ethanol) at 23 °C with stirring. Reaction rates were analyzed by following the disappearance of the *trans*-stilbene absorption at 314 nm as a function of time with an IBM UV-vis spectrophotometer (9430).

For the kinetic measurement of bromination of stilbenes in SDS micellar solution, a stopped-flow spectrophotometer (Durrum, Model D-110) was used. The solution of 2×10^{-5} M stilbenes in 0.06 M SDS was mixed with an equal volume of 5×10^{-5} to 1×10^{-4} M aqueous bromine, and the decrease of adsorbance at 314 nm was analyzed.

To determine reproducibility, several (usually 5) runs were made on each sample. Thus for *trans*-stilbene in DPL, we obtained a rate constant 500 ± 140 on the basis of 5 runs with a single solution prepared according to Method C above. However, when solutions of *trans*-stilbene/DPL were prepared on three occasions and rates were determined in each case

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Table I. Bromination of Stilbenes in SDS Micelles, SDS-Heptanol, or EtOH-H_2O 1:1 (v/v)^a

| stilbene	medium	k_2 (M^{-1} s^{-1})	k_{rel}^{b}
trans-stilbene	SDS	$(5.4 \pm 1.8) \times 10^3$	1.0
4S4	SDS	$(9.3 \pm 0.9) \times 10^4$	17
S4A	SDS	$(8.9 \pm 1.0) \times 10^4$	16.5
S10A	SDS	$(7.3 \pm 0.8) \times 10^4$	13.5
4S6A	SDS	$(4.9 \pm 1.0) \times 10^4$	9.1
trans-stilbene	SDS-heptanol	300 ± 90	
4S4	SDS-heptanol	690 ± 30	2.3
S4A	SDS-heptanol	1250 ± 110	4.2
6S4A	SDS-heptanol	620 ± 160	2.1
4S6A	SDS-heptanol	1030 ± 230	3.4
trans-stilbene	EtOH-H_2O	210 ± 40	
S4A	EtOH-H_2O	490 ± 40	2.3
4S4	EtOH-H_2O	1140 ± 270	5.4

^{a} [Stilbene] = 1.0×10^{-5} M, 23 °C, [Br_2] = 4.0×10^{-5} to 1.3×10^{-4} M. [SDS] = 0.03 M (or 0.06 M for the SDS-heptanol system). [Heptanol] = 0.1 M. ^{b} Values relative to that for *trans*-stilbene.

as outlined above, results obtained were 500 ± 140, 500 ± 30, and 320 ± 100. Thus in an individual experiment, precision was generally in the range of 20-30%; however, on the basis of different preparations, a slightly lower precision is generally expected. Data shown in Tables I-III are generally for several determinations in two or three different preparations unless otherwise noted.

Solutions of surfactants used in this study, SDS, DCP, and DPL, are stable toward bromine under the conditions and time scale of these experiments.

Results

The several surfactant and hydrophobic stilbenes used in this study, abbreviated according to the structures below (S = *trans*-stilbene), were found to react rapidly with Br_2 in aqueous or polar
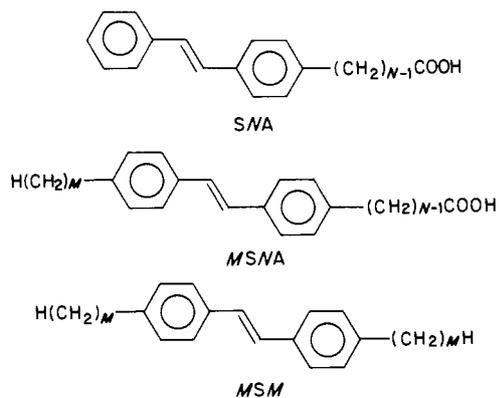
organic solvents and slowly in nonpolar aprotic solvents in accord with other studies of S.[30-35] Table I compares reactivity of several compounds in aqueous alcohol, aqueous (micellar) SDS, and aqueous SDS-heptanol. As would be anticipated, the rate for various stilbenes in homogeneous solutions follows the order, 4,4'-dialkyl-substituted S > 4-alkyl-substituted S > S. The reactions show a single rate process for all of the stilbenes and media shown in Table I. Overall the rates in SDS micelles were found to be much more rapid than those in 1:1 (v/v) aqueous ethanol while the rates in so-called "swollen" micelles[19] containing heptanol (0.1 M) in addition to SDS (0.03 M) are quite close to those measured in aqueous ethanol.

The DCP[21-23,28] and DPL[24-26,27] vesicular solutions have been widely studied and well characterized in recent research. The DCP vesicle prepared by probe-sonication is reported to be about 600 Å in diameter and homogeneous in size.[21] The vesicular solution of DPL is somewhat complicated because aggregation of vesicles is reported to occur below the phase-transition temperature (37 °C), to change the diameter from 300 to 850 Å.[26] For these experiments the DPL solutions were prepared just before use and kept at 45 °C until subjected to kinetic measurements.

Table II summarizes bromination rates measured for aqueous dicetylphosphate (DCP) vesicles prepared by probe-sonication; both unchromatographed (Method A) and sepharose-filtered

Table II. Bromination of Stilbenes in DCP Vesicles or DCP–Dihydrocholesterol Vesicles[a]

stilbene	vesicle	preparation method	k_f ($M^{-1} s^{-1}$)[c]	k_s ($M^{-1} s^{-1}$)[c]	k_{rel}(slow)	k_f/k_s
trans-stilbene	DCP	A	3500 ± 600 (36%)	380 ± 180 (64%)	1.0	9.2
4S4	DCP	A	2000 ± 700 (56%)	300 ± 40 (44%)	0.8	6.7
S4A	DP	A	4900 ± 1100 (58%)	670 ± 280 (42%)	1.8	7.3
4S6A	DCP	A	2500 ± 500 (52%)	330 ± 70 (48%)	0.9	7.6
trans-stilbene	DCP–DHC	A[b]	890 ± 140 (52%)	5 ± 10 (48%)	1.0	180
S4A	DCP–DHC	A[b]	4500 ± 1200 (69%)	740 ± 320 (31%)	150	6.1
4S6A	DCP–DHC	A[b]	1280 ± 190 (68%)	86 ± 10 (32%)	17	15
trans-stilbene	DCP	C	2700 ± 330 (22%)	510 ± 130 (78%)	1.0	5.3
S4A	DCP	C	7200 ± 300 (46%)	2500 ± 100 (54%)	4.9	2.9
4S6a	DCP	C	2100 ± 100 (66%)	250 ± 10 (34%)	0.5	8.4

[a] [Stilbene] = 1.0×10^{-5} M, [DCP] = 1.0×10^{-3} M, [Br$_2$] = 4.0×10^{-5} to 1.5×10^{-4} M, 23 °C. [b] [Dihydrocholesterol] = 5.0×10^{-4} M. [c] The rate constants were calculated by assuming the following equation: $-d[\text{stilbene}]/dt = pk_f[\text{Br}_2][\text{stilbene}_1] + (1 - p)k_s[\text{Br}_2][\text{stilbene}_2]$, i.e., $(A(t) - A_\infty)/(A_0 - A_\infty) = pe^{-k_f[\text{Br}_2]t} + (1 - p)e^{1k_s[\text{Br}_2]t}$ where $k_f > k_s$. The weights, $100p$ (%) and $100(1 - p)$ (%), are shown in parentheses.

Table III. Bromination of Stilbenes in DPL Vesicles[a]

stilbene	preparation method	k_f ($M^{-1} s^{-1}$)	k_s ($M^{-1} s^{-1}$)	k_f/k_s
trans-stilbene	A	7200 ± 1200 (55%)	520 ± 250 (45%)	13.8
S10A	A	530 ± 200 (43%)	50 ± 13 (57%)	10.6
6S4A	A	220 ± 80 (40%)	23 ± 18 (60%)	9.6
trans-stilbene	C		470 ± 130	
4S4	C		2 ± 1	
S4A	C		2600 ± 1300	
S6A	C		920 ± 230	
S10A	C		30 ± 6	
4S6A	C		5 ± 3	
6S4A	C[b]		11 ± 5	
trans-stilbene	D[c]		190 ± 90	
4S4	D		19 ± 12	
S6A	D[d]		160 ± 80	
6S4A	D		11 ± 8	

[a] [Stilbene] = 1.0×10^{-5} M, [DPL] = 1.0×10^{-3} M, [Br$_2$] = 5×10^{-5} to 4×10^{-4} M, 23 °C. [b] A faster component, $k = 81 \pm 45$ (27%), was detected. [c] A faster component, $k = 4700 \pm 2700$ (37%), was detected. [d] A faster component, $k = 1000 \pm 300$ (51%), was measured.

(method C) vesicle solutions were used as well as vesicle solutions to which dihydrocholesterol had been added. In each case the bromination process shows two components to the rate profile that are first order in [Br$_2$]; the faster component generally accounts for somewhat more than 50% of the total reaction. The fast component of the reaction is about tenfold slower than the rate constant obtained for SDS micelles while the slower component is generally a bit less than another tenfold slower.

Table III lists rate constants measured for several stilbene derivatives in phospholipid (dipalmitoyllectithin (DPL)) vesicles prepared and treated by various techniques. For vesicle solutions of DPL prepared by the "standard" probe-sonication techniques[21-26] it was found that the bromination shows biphasic kinetics with the faster component generally accounting for ca. 40% of the total. The ratio between fast and slow components for the entire range of stilbenes studied (several data are not included in Table III, vide infra) in this medium was found to be 10 ± 1.6. When DPL vesicles prepared by probe-sonication were subjected to Sepharose filtration either before or after incorporation of the stilbene derivative (method C or B, respectively) and only the small unilamellar vesicle (SUV) fraction studied,[28] it was found that *only* the slow component for bromination could be observed. The Sepharose 4B separated fraction consisting of multilamellar vesicles[28] was also examined under the same conditions. Although we do not report tabulated data for these solutions, it was found that the same rates as those obtained for SUV's in Table III (within experimental error) were measured. DPL vesicles prepared by probe sonication with the stilbene followed by Sepharose filtration were found in almost every case to contain no fraction exhibiting the "fast" bromination rate. The SUV's containing stilbene derivatives were found (Tables III and IV) to give a single rate component in bromination, and the measured rate constants were found to be the same as the "slow" component obtained for vesicles prepared by probe-sonication alone. The rate constants obtained for DPL vesicles prepared by method D (this gives large unilamellar vesicles according to Szoka

Table IV. Bromination of Stilbene Derivatives in Homogeneous Small Unilamellar Vesicles of DPL[a]

stilbene derivative	k_2 ($M^{-1} s^{-1}$)	no. of determinations	k_{rel}
S	470 ± 130	11	1.0
4S4	2 ± 1	2	0.004
S4A	1780 ± 200	6	3.8
S6A	920 ± 230	15	1.96
S10A	30 ± 6	9	0.06
6S4A	11 ± 5[b]	24	0.02
4S6A	5 ± 3	8	0.01

[a] Vesicles prepared and separated according to method C. [b] For 6S4A a faster component, $k_2 = 81 \pm 45$, was observed in 13 of 24 determinations; this component accounted for 20–40% of the observed reaction.

and Papahajopoulos)[29] are in general close to those obtained for SUV's, especially for the surfactant stilbenes. In general, for DPL vesicles the reaction rates of all stilbene derivatives are slow compared to those for aqueous ethanol, SDS micelles, and DCP vesicles. Especially noteworthy are the rate constant variations observed across the series of stilbenes studied in the small vesicles of DPL (Table IV) and the contrasts in k_{rel} between micelles (Table I) and DPL vesicles.

Several experiments were carried out to determine possible "aging" effects[26] on DPL vesicle solutions. Although it has been reported that DPL vesicles aggregate rapidly below T_C,[26] it was found that there was no difference in rate constants measured over a period of 10 h at room temperature or for samples subjected to heating to 73 °C for 5 h prior to bromination at room temperature.

Experiments were also carried out with both DPL and DCP vesicles to determine whether bromine can penetrate a bilayer assembly and if the experiments as described above employed "sealed" vesicles. DPL vesicles were prepared by probe-sonication in the presence of 0.1 M sodium ascorbate. After filtration through

a 3.0-μm membrane filter and passage through a gel filtration column (AcA 202, Ultrogel) to remove ascorbate from the outer aqueous phase, the ascorbate-containing DPL vesicles were studied by independent treatment with bromine and potassium permanganate. Addition of bromine (8 × 10^{-3} M) was found to cause disappearance of the characteristic absorbance of ascorbate at 265 nm within 5 s. In contrast, the same DPL vesicles were found to give no reaction of the ascorbate within several minutes upon treatment with KMnO$_4$ (2.8 × 10^{-4} M). In contrast, DCP vesicles treated under the same conditions showed rapid bleaching of the ascorbate with both Br$_2$ and KMnO$_4$. Thus, while it seems clear that the DPL vesicles studied are closed bilayer liposomes through which Br$_2$ can rapidly pass, the DCP solutions probably do not consist of stable closed liposomes.

Discussion

Bromination in Aqueous Micelles. The rates in SDS micelles are much faster than those in 50% (v/v) aqueous ethanol as shown in Table I. While the rates in micelles are slightly slower than those observed in pure water,[30] it is clear that all of the derivatives studied react rapidly in SDS micelles with a reactivity order in accord with what would be expected on the basis of electronic effects (dialkylstilbene > monoalkylstilbene > S). There is little evidence of any "sequestering" of any of the surfactant stilbenes even for the "hydrophobic" 4S4 or the stilbene decanoic acid, S10A. The results obtained are clearly in accord with an "average" reaction site that is polar and readily accessible to Br$_2$.

The stilbenes in SDS–heptanol (alcohol-swollen micellar) solution react with Br$_2$ one or two orders of magnitude more slowly than those in SDS (Table I). The comparison of rates shows that the microenvironment around the stilbenes in SDS–heptanol is similar to that of 50% (v/v) aqueous ethanol. The difference in reactivity among stilbenes is small, however, suggesting that the structure of SDS–heptanol is somewhat random, or at least that on the time scale of these experiments all of the stilbenes studied may access a relatively polar environment. The only significant effect to be noted in the micelles (both normal and "swollen") is the slightly lower than expected (on the basis of dialkyl substitution) reactivity of the "internal" stilbenes 4S4 and 4S6A relative to the terminal stilbenes S4A and S10A. Such an effect, though somewhat more pronounced, has been noted by Menger and Doll in the permanganate oxidation of surfactant alkenes in micelles.[12] The finding of rapid rates, consistent with a quite polar reaction microenvironment for the stilbene bromination in both micelles and swollen micelles, is consistent with a growing number of experimental and theoretical studies indicating that molecules solubilized in micelles and, indeed, the hydrocarbon chains of the surfactant component molecules themselves experience a largely "interfacial" environment in which frequent contact with water occurs and in which there is little, if any, sequestering of hydrophobic from hydrophilic reagents.[36]

Bromination in Vesicles. In contrast to the bromination rates measured in micelles, bromination rates for the various stilbenes in DPL and DCP vesicles are substantially slower. Moreover, there is a more pronounced difference between various stilbene derivatives that frequently runs counter to the relative rates expected on the basis of electronic effects. The results obtained with the two different bilayer-forming surfactants are quite different and offer a contrasting picture of the two media.

The results obtained with the anionic DCP vesicles are clearly more complicated than those obtained with DPL. The observation of biphasic kinetics in each case, regardless of method of preparation or subsequent treatment of the vesicles, suggests that there

are two non- or slowly (slower than the time scale of bromination) interconverting environments for the stilbenes in the various DCP or DCP–dihydrocholesterol vesicles. Whether these sites coexist within a single vesicle or they are due to different kinds of aggregation forming from DCP is not clear. Results obtained with DPL (vide infra) might tend to support the latter possibility. In any case, the fast component of the rate is quite rapid and for *trans*-stilbene itself only slightly slower than in SDS micelles. The substituted and surfactant stilbenes react with rates closer to stilbene (compare k_{rel} values in Tables I and II) for both fast and slow components; clearly electronic effects are competing with other factors in determining the overall rate. The slow component is 1–2 orders of magnitude slower than that observed in micelles and is consistent with the stilbenes occupying a generally less-polar site. The generally lower reactivity of the disubstituted 4S4 and 4S6A in DCP alone suggests that the stilbene chromophores in these compounds in DCP vesicles are situated in a more hydrophobic site than is *trans*-stilbene. The sharp decrease in the rate for stilbene bromination upon adding dihydrocholesterol to DCP suggests the stilbene is in a much more hydrophobic site in this medium. Even if attention is focused only on the "slow" reaction component in DCP vesicles, the results suggest that the chromophores of all the stilbene derivatives studied reside in relatively hydrophilic sites. This is in some respects not too surprising. Thus even though the temperatures used in these studies (23–25 °C) are well below the phase transition temperature of DCP so that the vesicles are supposedly in an ordered or gel phase, other studies have shown that organic ions bind to DCP by "hydrophobic" effects very similar to those active in micelle solubilization.[37,38] It is thus reasonable that there is a relatively disordered "interface" region for DCP vesicles which is accessible to the stilbenes used in this study such that their reaction is rapid, consistent with the bromination occurring in a polar environment.

The results obtained in DPL vesicles (Table IV) are perhaps the most interesting and significant part of this study. Although simple probe-sonicated solutions of DPL–stilbenes give biphasic kinetics for reaction with bromine, Sepharose-filtered preparations—both SUV and multilamellar vesicles—as well as larger vesicles (Table III) show only a single component equal to the slower rate in the biphasic behavior. It is clear that these experiments show the two kinetic phases here are due to two different kinds of aggregates. Since the ratio k_{fast}/k_{slow} is fairly constant for stilbenes showing quite different rate behavior, it seems reasonable to infer that the "fast" component is associated with a smaller aggregate which has some similarity to DPL vesicles but provides a generally more hydrophilic environment. Since it has not proved possible to isolate or characterize such aggregates, it is not instructive to discuss them further.[39] The "slow" component is ascribed to stilbene derivatives incorporated into the DPL vesicles.

The rate constants for the single ("slow") component in SUV's listed in Table IV show a remarkable variation in reactivity for the different stilbenes; the spread of a factor of 900 between the fastest and slowest stilbene is even more pronounced if the aqueous ethanol rates are used to provide a "correction" based on electronic effects. The difference in rates could be attributed in part to such factors as concentration gradients of bromine across the vesicle as well as to differences in the effective polarity of the specific reaction microenvironment. It is somewhat difficult to assess the importance of the former; bromine is known to be quite soluble in nonpolar solvents such as alkanes and aromatic hydrocarbons.[40]

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However, the solubility or reactivity in an ordered, semicrystalline region of a vesicle might be anticipated to be lower, since these experiments were carried out well below T_C. The observation that DPL-entrapped ascorbate is rapidly oxidized by bromine indicates that passage of Br_2 through the gel-phase bilayer is facile and rapid on the time scale of the reaction rates listed in Table IV. It appears most consistent, in any case, to conclude that the major differences in the second-order rate constants listed in Table IV can be attributed to substantially different "average" (on the time scale of these reactions!) microenvironments for the stilbene chromophore in the different molecules.

An interesting comparison may first be made between the nonsurfactant molecules S and 4S4. The reactivity of *trans*-stilbene in DPL is about an order of magnitude slower than that in SDS micelles and roughly comparable to its reactivity in 1:1 aqueous ethanol, DCP vesicles, and SDS-heptanol swollen micelles. This clearly suggests that *trans*-stilbene experiences a polar environment and is most consistent with a solubilization site for S in an interfacial region of the vesicle.[41] In sharp contrast the "hydrophobic" 4S4, which reacts much faster than stilbene in micelles and aqueous ethanol, is *less* reactive by a factor of 235 in DPL. The most evident explanation for this difference is that 4S4 is solubilized away from the interface in a hydrophobic portion of the vesicle interior. It is rather remarkable that two such relatively similar *hydrocarbon* solutes can experience such dissimilar environments upon solubilization in an aqueous surfactant assembly; these results suggest a caveat against simple assumption of a solubilization site in the hydrocarbon phase for nonpolar solutes incorporated into bilayer vesicles or membranes.[42-44]

For the surfactant stilbenes incorporated into DPL the rates listed in Table IV show some rather clear trends. The pronounced decrease in reactivity in the series S4A > S6A > S10A is in accord with what one would expect for a gradually decreasing polarity of the "average" site for the stilbene chromophore as it is moved away from the polar head group in the molecule and presumably deeper into the hydrocarbon "interior" of the bilayer assembly. Whether the decrease in rate can be attributed to a gradient of water concentration across the bilayer or to increasingly less facile migration of the stilbene chromophore from less polar to more polar regions within the assembly cannot be definitively determined from the present results. The water-insoluble surfactant stilbenes S10A, 6S4A, and 4S6A as well as other derivatives not included in this study all show surface pressure-area isotherms in Langmuir-Blodgett films that indicate similar packing behavior, either pure or with fatty acid cosurfactants, to conventional straight-chain surfactants.[18] This would suggest that incorporation of the surfactant stilbenes into the vesicle should produce relatively little disordering and that the "probe" molecules should behave relatively similarly to any single-chain surfactant incorporated into the bilayer. It is interesting, however, that the intrachain derivatives 6S4A and 4S6A show considerably lower reactivity than the terminal stilbenes having corresponding intrachain distances between the carboxyl and aromatic groups, S4A and S6A. Photophysical studies of the intrachain stilbenes 6S4A and 4S6A[45]

suggest these molecules are firmly anchored in the bilayer; fluorescence efficiencies near unity for these molecules in DPL contrast with lower efficiencies and correspondingly greater mobility for S4A and S6A.[45] This might be taken as supporting evidence for a mechanism in which the more rapid rates for S4A and S6A can be attributed to a greater ease of migration to the vesicle interface.

Since we observe only a single rate constant for second-order bromination with each of the stilbene derivatives studied in SUV DPL, it is reasonable to conclude that the reactivity of specific stilbenes in inner and outer leaflets of the bilayer is comparable. Comparable (or single phase) reactivity could be attributed to several factors among which could be rapid equilibration of the stilbenes between inner and outer leaflets, unsealed or open bilayers in which there is no true inner-outer distinction, or simply comparable microenvironments for the stilbenes coupled with equal $[Br_2]$ for both sites. The studies with DPL-entrapped ascorbate showing unreactivity toward $KMnO_4$ on the time scale of these reactions as well as experiments indicating no reaction of Br_2 with DPL alone on this same time scale infer that the reactions involve assemblies that are indeed closed bilayer vesicles. The question of rapid exchange between inner and outer leaflets is less easily decided. Several previous studies have indicated that exchange of surfactant molecules between inner and outer leaflets of vesicles, liposomes, or biomembranes occurs on the order of days.[46,47] On the other hand, it is possible that the surfactant stilbenes, being single-chain reagents, may "flip-flop" or otherwise exchange somewhat more rapidly.

The results of this study provide several interesting observations. First of all the several different stilbenes studied show no important differences in reactivity in micelles that could be attributed to different microenvironments for the reaction. In contrast, reactivity in vesicles—especially the small unilamellar DPL vesicles—shows remarkable variation which is most easily attributed to different average microenvironments for the reaction. The results obtained in DPL vesicles clearly suggest *at least* two limiting environments for solubilized hydrocarbon substrates in the bilayer. One of these is clearly a hydrophobic, hydrocarbon-like environment such as would be expected for the bilayer interior. The other is clearly more polar and can be ascribed to an interfacial region near the polar head groups. An interesting question remaining is to what extent there is a gradient between these two regions. For DPL our studies suggest that there may be a significant gradient between a polar, perhaps disordered interface and the nearly crystalline interior. The results obtained with DCP vesicles suggest a much more disordered aggregate in which the "interface" may extend much more deeply into the bilayer interior. These results emphasize the differences between micelles and vesicles and indeed between different types of vesicles. They indicate that vesicles, unlike micelles, may provide a relatively varied array of "average" solubilization sites with a correspondingly rich range of reactivities.

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