Dynamics of the Cleavage of Ellman's Reagent in Surfactant Vesicles

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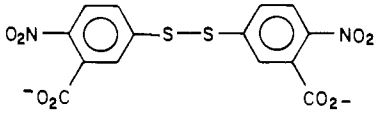
Abstract: The cleavage of Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid) (**1**)] to 2-nitro-5-thiolatobenzoate dianion, **5**, by dithionite, sulfite, and thiophenoxide ions and by zwitterion **4**, was studied at pH 8 in aqueous solution and in aqueous solutions of hexadecyltrimethylammonium ion micelles or dihexadecyldimethylammonium ion vesicles, **2**. Excepting reagent **4**, strong micellar and vesicular catalysis of the cleavage reactions was observed. When Ellman's reagent was added to the empty vesicles, followed by the addition of excess cleavage reagent, separate fast and slow reactions were observed. The relative contribution of each process varied with the aging time between the addition of the Ellman's reagent and the cleavage reagent. The results were quantitatively analyzed in terms of reversible substrate distribution between exovesicular (surface) and endovesicular (subsurface) sites. With dithionite and sulfite reagents, rapid cleavage of exovesicular substrate was followed by slow cleavage of endovesicular substrate which had permeated back to the vesicular surface to react with exovesicular dithionite or sulfite. With thiophenoxide, very rapid exovesicular cleavage was followed by slower endovesicular cleavage of substrate by rapidly permeating thiophenoxide (thiophenol). Reagent **4** was not bound by vesicular **2**, and cleavage reactions occurred only exovesicularly or off the vesicle.

The architecture of surfactant vesicles, even when they are unilamellar, is surprisingly complex. Fuhrhop and Mathieu delineate nine distinct regions in which reactions might occur in aqueous solutions of nonfunctionalized vesicles.[1] Fendler, too, has highlighted the availability of several loci for reactions occurring on or within a single vesicle.[2,3]

A number of locus-specific vesicular reactions have now been discovered. Examples include diazo coupling of aryldiazonium ion functionalized vesicles;[4] the aminolysis by ethylenediamine of *p*-nitrophenyl laurate in polymerized or nonpolymerized styrene-functionalized vesicles;[5] and the one-electron reduction of $(NH_3)_5$Ru-4-(11-dodecenyl)pyridine (3+ ion) by chromous, vanadous, or ascorbate ions in unilamellar phosphatidylcholine liposomes.[6] In these cases, the portion of the reaction occurring on the vesicular surface (exovesicular) has been kinetically differentiated from the portion of the reaction occurring at various interior sites (endovesicular).

Recently, we communicated a study of the cleavage of Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)], **1**, by dithionite ion in vesicles of di-*n*-hexadecyldimethylammonium bromide, **2**.[7] This was an initial example of a "two-site" vesicular reaction in which each reaction could not only be kinetically differentiated but the time-dependent evolution of the substrate's distribution between the sites could be quantitatively followed.



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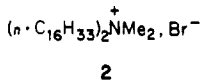

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In this full report, we extend our studies to the vesicular cleavage of **1** by sulfite and thiophenoxide ions. Analysis of all the data permits us to refine our previously offered dynamic model. Specifically, we now suggest that the dithionite and sulfite cleavages of vesicularly bound and equilibrated **1** occur in a two-step sequence that features initial rapid cleavage of exovesicularly bound **1**, followed by slower cleavage of **1** that permeates *back* from endovesicular sites to exovesicular loci. The second step is a consequence of the noncompetitive permeation of the ionic cleavage reagents; consequently, the slow cleavage of **1** appears to be rate limited by its endo → exo migration. On the other hand, cleavage of endovesicular **1** by thiophenoxide ion is much faster than the endo → exo migration of **1** and appears to represent a true endovesicular reaction.

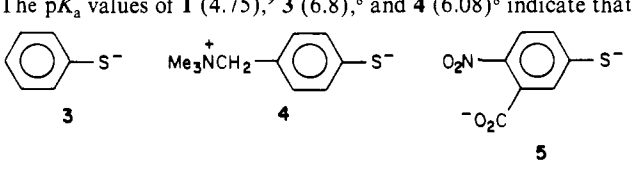
Table I. Rate Constants for Cleavage of Ellman's Reagent in Water[a]

reagent	concn, M	k_ψ,[b] s^{-1}
$S_2O_4^{2-}$	5.0×10^{-4}	$0.177 \pm 0.004_4$
SO_3^{2-}	5.0×10^{-4}	$0.190 \pm 0.008_3$
PhS^-	1.0×10^{-3}	$9.3 \pm 0.1_4$
4	1.0×10^{-3}	$15.5 \pm 0.4_3$[c]

[a] In 0.01 M Tris buffer, $\mu = 0.01$ (KCl), pH 8.0, [**1**] = 2.5×10^{-5} M. [b] Errors are average deviations of *n* (subscript) runs. [c] See text.

Results

Aqueous and Micellar Reactions. Ellman's reagent, **1**, was readily cleaved in 0.01 M, pH 8.0 aqueous Tris buffer by dithionite ion ($S_2O_4^{2-}$), sulfite ion (SO_3^{2-}), thiophenoxide (**3**, PhS^-), and the para-quaternary ammonium substituted thiophenoxide ion, **4**.[8] The pK_a values of **1** (4.75),[9] **3** (6.8),[8] and **4** (6.08)[8] indicate that



3 **4** **5**

these species are best represented as largely ionic at pH 8. The cleavage reactions were studied by rapid mixing techniques using a Durrum D-130 stopped-flow spectrometer fitted with a photomultiplier tube connected either to a Tektronix Model 5103N/D 15 storage oscilloscope or to a custom-built A/D interface, coupled to a Commodore CBM 8032 microcomputer equipped with high-resolution graphics.

Reaction rates were followed by monitoring the appearance of Ellman's anion, **5** (λ_{max} in H_2O 407 nm, log ϵ = 4.14),[10] at 400 nm. With a large excess of cleavage reagent, we observed good, monophasic, pseudo-first-order kinetics ($r > 0.999$, >90% of reaction) for the cleavage reactions with $S_2O_4^{2-}$ and SO_3^{2-}. The rate constants, derived from standard analysis of absorption vs. time data, appear in Table I. In the reactions of **1** with $S_2O_4^{2-}$

(1) Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100.
(2) Fendler, J. H. *Science* **1984**, *223*, 888.
(3) Fendler, J. H. "Membrane Mimetic Chemistry"; Wiley: New York, 1982.
(4) Fuhrhop, J.-H.; Bartsch, H.; Fritsch, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 204. Moss, R. A.; Shin, J. S. *Chem. Commun.* **1983**, 1027.
(5) Ishiwatari, T.; Fendler, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 1908.
(6) Lee, L. Y. C.; Hurst, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 7411.
(7) Moss, R. A.; Schreck, R. P. *J. Am. Chem. Soc.* **1983**, *105*, 6767.
(8) Moss, R. A.; Dix, F. M. *J. Org. Chem.* **1981**, *46*, 3029.
(9) Fendler, J. H.; Hinze, W. L. *J. Am. Chem. Soc.* **1981**, *103*, 5439.
(10) Moss, R. A.; Swarup, S.; Schreck, R. P. *Tetrahedron Lett.* **1985**, *26*, 603.

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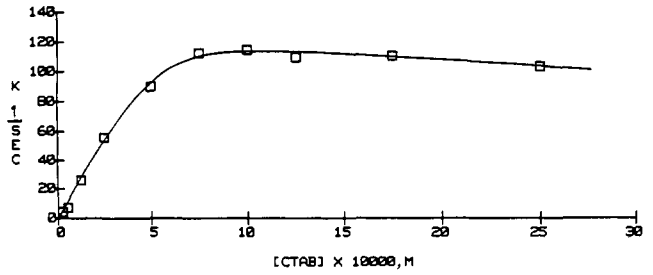


Figure 1. Rate constant (k_ψ, s^{-1}) vs. [cetyltrimethylammonium bromide] $\times 10^4$ M for the cleavage of Ellman's reagent, **1**, by dithionite ion at pH 8.

or SO_3^{2-}, measurements of the total absorbance changes showed that only 1 equiv of **5** was released, suggesting that the companion product of the disulfide cleavage was a UV-inactive (at 400 nm) arylthiosulfonate or arylthiosulfate.[11]

In reactions between **1** and excess PhS^- or **4**, 2 equiv of **5** were liberated, corresponding to normal expectations for reactions between **1** and *excess* thiol, eq 1–3.[12] The reaction of **1** with excess

$$\underset{\mathbf{1}}{\text{Ell-S-S-Ell}} + 2\text{ArS}^- \rightarrow \text{ArSSAr} + 2\underset{\mathbf{5}}{\text{Ell-S}^-} \quad (1)$$

$$\text{Ell-S-S-Ell} + \text{ArS}^- \xrightarrow{k_2} \text{Ell-S}^- + \text{Ell-S-SAr} \quad (2)$$

$$\text{Ell-S-SAr} + \text{ArS}^- \xrightarrow{k_3} \text{Ell-S}^- + \text{ArSSAr} \quad (3)$$

PhS^- showed only monophasic pseudo-first-order kinetics, but, with **1** and **4**, biphasic kinetics were observed corresponding to the "part" reactions, eq 2 and 3. Here, k_2/k_3 was sufficiently large so that reactions 2 and 3 were clearly time resolved. It is the rate constant for the slower process, eq 3, which is recorded in Table I. In contrast, for the reaction of **1** and PhS^-, the rate constant ratio (k_2/k_3) was only ~2.7,[13] so that a clean kinetic separation of the "part" reactions was not observed under the conditions of Table I.

The cleavage of **1** by $S_2O_4^{2-}$ or PhS^- is strongly catalyzed by *micellar* cetyltrimethylammonium bromide (CTAB) under the conditions of Table I. These reactions are followed at 438 nm, λ_{max} for **5** in aqueous micellar CTAB.[10] It is anticipated that reactions between anions will be accelerated by cationic micelles (or vesicles).[14] For example, the aqueous hydroxide ion cleavage of **1** is accelerated by factors of 15 or 1500 by micellar CTAB or vesicular dioctadecyldimethylammonium chloride, respectively, and the vesicular binding constant of **1** is $\sim 1.4 \times 10^4$ M^{-1}.[9] In these cases, it is very likely that most, if not all, of the apparent increase in the observed rate constants (calculated from bulk concentrations) derives from concentration of the reactants into the small reaction volume available in the micellar or vesicular polar regions and surfaces.[14b] Indeed, the "true" rate constants in the surfactant pseudophases may well be no greater, or even less, than the analogous rate constants in water.[14b]

Pseudo-first-order rate constants (monophasic, $r > 0.999$) for the CTAB-catalyzed cleavage of **1** by excess $S_2O_4^{2-}$ are shown in Figure 1 as a function of [CTAB]; a typical[14] rate constant vs. [surfactant] profile is obtained. At [CTAB] ~ 1×10^{-3} M, k_ψ reaches a maximum value of 114 s^{-1}; this represents a micellar catalysis of (114/0.177) = 644, relative to the reaction in Tris buffer (Table I).

Table II. Rate Constants for Cleavage of Ellman's Reagent in Vesicles[a]

case	cleavage reagent	mixing mode[b]	$k_\psi^{f,c}$ s^{-1}	$k_\psi^{s,d}$ s^{-1}
1	$S_2O_4^{2-}$	inverse	$176 \pm 3_3$	*e*
2	$S_2O_4^{2-}$	normal	$169 \pm 2_3$	$2.4 \pm 0.1_5$
3	$S_2O_4^{2-f}$	normal	$160 \pm 20_3$	$2.3 \pm 0.1_{12}$
4	$S_2O_4^{2-g}$	normal	$178 \pm 13_4$	$1.7 \pm 0.2_{10}$
5	SO_3^{2-}	inverse	$225 \pm 5_3$	*e*
6	SO_3^{2-}	normal	$231 \pm 12_3$	$1.46 \pm 0.06_{12}$
7	PhS^-	inverse	>500	*e*
8	PhS^-	normal	>500	$46 \pm 4_9$
9	**4**[h]	inverse	$18 \pm 1.4_5$[i]	*e*
10	**4**[h]	normal	$14.7 \pm 0.9_3$[i]	$0.64 \pm 0.06_{20}$[j]

[a] Conditions (after final mixing): [**2**] = 5.0×10^{-4} M, [**1**] = 2.5×10^{-5} M, [cleaving reagent] = 5.0×10^{-4} M, pH 8.0, 0.01 M Tris buffer, μ = 0.01 M (KCl), 25 ± 0.2 °C. [b] See text for definitions. [c] Observed rate constant of fast reaction; errors are standard deviations of *n* (subscript runs). [d] Observed rate constant of slow reaction; errors as in footnote *c*. [e] Only monophasic kinetics were observed. [f] Vesicles contained 20 wt % cholesterol. [g] 1 equiv of 18-crown-6 was added to the solution of substrate **1**. [h] The final [**4**] was 1.0×10^{-3} M. [i] This rate constant corresponds to k_3 in eq 3. [j] This process did not afford good first-order kinetics.

The reaction of **1** and thiophenoxide is also strongly catalyzed by CTAB. At [CTAB] = 2.5×10^{-5} M, well *below* the nominal critical micelle concentration (cmc),[15] k_ψ = 151 s^{-1} for the cleavage of 2.5×10^{-5} M, **1** by 1.0×10^{-3} M PhS^- under the conditions of Table I, a catalytic factor of (151/9.3) = 16. Thiophenoxide ion is known[16] to bind very strongly to CTAB micelles; it has been shown that 4×10^{-5} M PhS^- (pH 10, 0.01 M borate buffer) lowers the cmc of CTAB to 8×10^{-5} M.[16] Presumably, in our case, the high [PhS^-], as well as the presence of the strongly binding[9] **1**, lowers the cmc even more. At [CTAB] = 6.2×10^{-5} M, the (**1** + PhS^-) reaction becomes too fast to follow by stopped-flow spectroscopy; i.e., $k_\psi > 500$ s^{-1}.

Vesicular Reactions. Cleavage of Ellman's reagent by $S_2O_4^{2-}$, SO_3^{2-}, PhS^-, or **4**, in the presence of vesicular **2**, was studied by using a Dionex Model D-132 multimixing module retrofitted to our stopped-flow system. In this apparatus, solutions A and B were mixed (<1 ms) and, after an adjustable aging period (*minimum* aging time: 46 ± 5 ms), (A + B) was rapidly mixed (<1 ms) with solution C and passed into the stopped-flow cuvet for spectroscopic analysis. The individual solutions were each prepared in pH 8, 0.01 M Tris buffer, containing 3.5×10^{-3} M KCl. This constant concentration of background electrolyte served to minimize ionic shock to the vesicles during the mixing operations.

Vesicles of **2** were prepared by sonication (probe-type sonicator, 70 W, 15–30 min, 60–65 °C) in Tris buffer, followed by filtration through a 0.8-μm filter. Dynamic light-scattering measurements[17] gave hydrodynamic diameters of 508 and 530 Å in the absence or presence, respectively, of 2.5×10^{-5} M **1**. Cosonication of **2** with 20 wt -% of cholesterol (see below) increased the hydrodynamic diameter to ~600 Å. Our experience with electron microscopy of vesicular **2** suggests that these vesicles probably contain several lamallae. Vesicular cleavage reactions were monitored at 413 nm instead of 450 nm, the λ_{max} of **5** in vesicular **2**,[10] for reasons which will be discussed below (cf. Control Experiments).

Two methods of reagent combination were employed. In the *inverse* mixing mode, empty vesicles (A) were first combined with the cleavage reagent (B), and, subsequently, (A+B) was mixed

(11) For example, aqueous $S_2O_4^{2-}$ may provide nucleophilic HSO_2^-, which would cleave **1** to afford **5** and $ArSSO_2^-$; cf.: Castaldi, G.; Perdoncin, G.; Giordano, C. *Tetrahedron Lett.* **1983**, *24*, 2487. The reaction between **1** and SO_3^{2-} is known to produce 1 equiv each of **5** and the "Bunte" thiosulfate salt, $ArSSO_3^-$: Humphrey, R. E.; Ward, M. H.; Hinze, W. *Anal. Chem.* **1970**, *42*, 698.

(12) Whitesides, G. M.; Lilburn, J. E.; Szajewski, R. P. *J. Org. Chem.* **1977**, *42*, 332.

(13) This value was obtained by running the reaction first with [PhS^-]/[**1**] = 40, for which $k_\psi^{obsd} = 8.8 \pm 0.2_3$ s^{-1}, and then with [PhS^-]/[**1**] = 0.025, for which $k_\psi^{obsd} = 23.5 \pm 0.8_3$ s^{-1}. The former case, in which eq 3 is limiting, corresponds to the conditions of Table I. In the latter situation, eq 3 does not contribute, and we can obtain[12] an approximate value for the rate constant of eq 2.

(14) (a) Fendler, J. H.; Fendler, E. J. "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975. (b) Bunton, C. A. *Catal. Rev.-Sci. Eng.* **1979**, *20*, 1.

(15) The cmc of CTAB is $\sim 9 \times 10^{-4}$ M: Bunton, C. A.; Romsted, L. S.; Sepúlveda, L. *J. Phys. Chem.* **1980**, *84*, 2611.

(16) Chaimovich, H.; Blanco, A.; Chayet, L.; Costa, L. M.; Monteiro, P. M.; Bunton, C. A.; Paik, C. *Tetrahedron* **1975**, *31*, 1139.

(17) Dynamic light scattering measurements employed a Nicomp Model TC-100 computing autocorrelator, an argon laser (488 nm), a 90° scattering angle, and a Hazeltine microcomputer fitted with the cumulant program.

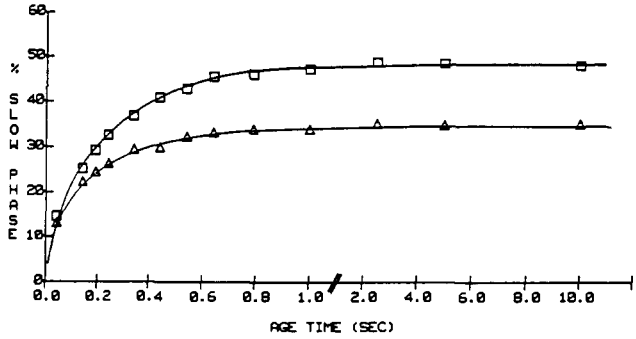


Figure 2. Percent of slow kinetic phase vs. aging time (s) for the vesicular **2** + substrate **1** + $S_2O_4^{2-}$ multimixing reaction: (□) native vesicles, (△) vesicles contain 20 wt % cholesterol.

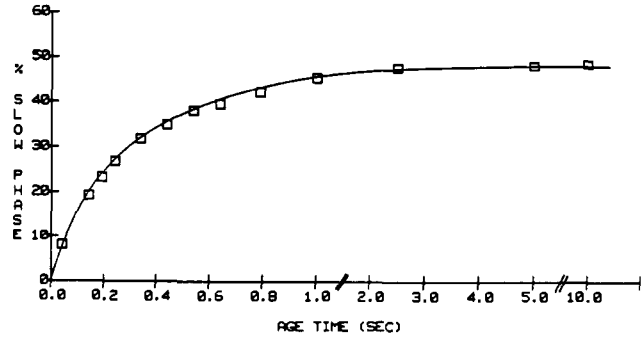


Figure 3. Percent of slow kinetic phase vs. aging time (s) for the vesicular **2** + substrate **1** + SO_3^{2-} multimixing reaction.

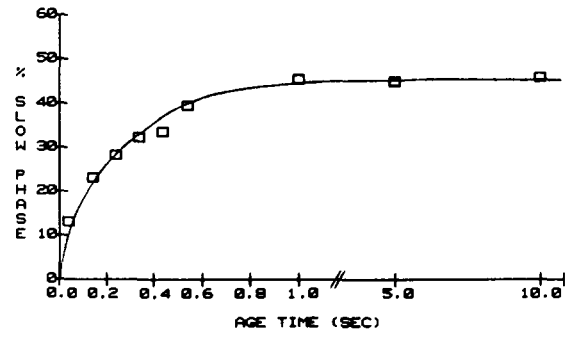


Figure 4. Percent of slow kinetic phase vs. aging time (s) for the vesicular **2** + substrate **1** + PhS^- multimixing reaction.

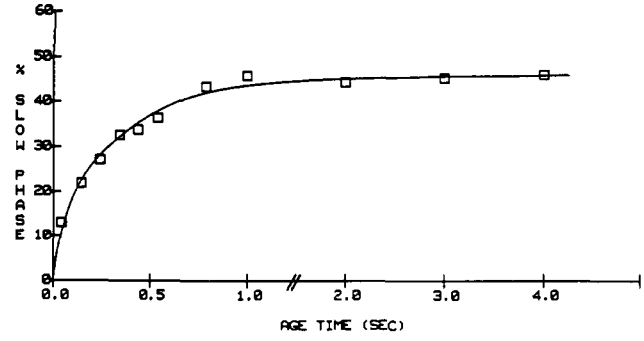


Figure 5. Percent of slow kinetic phase vs. aging time (s) for the vesicular **2** + substrate **1** + reagent **4** multimixing reaction.

with substrate **1**. In the *normal* mixing mode, vesicles and substrate were combined first, and this mixture was then exposed to cleavage reagent. Table II records rate constants for the observed vesicular cleavages of **1** under the two different multimixing regimes.

Examination of this table indicates that reactions carried out under inverse conditions generally exhibit clean monophasic pseudo-first-order kinetics with very marked catalysis relative to the analogous reactions in buffer alone. The catalytic factors observed for $S_2O_4^{2-}$, SO_3^{2-}, and PhS^- (Table II, cases 1, 5, 7 vs. Table I, first three entries) are 994, 1180, and >54, respectively, at [**2**] = 5×10^{-4} M. With the quaternary ammonium ion substituted thiophenoxide, **4**, catalysis is not observed; the rate constant for cleavage of **1** is essentially identical in the presence or absence of vesicular **2** (cf. Tables II and I).

Anionic substrate **1** should be strongly bound to cationic vesicular **2**. Thus, Fendler and Hinze report a binding constant of $1\text{–}4 \times 10^4$ M^{-1} for **1** and dioctadecyldimethylammonium chloride vesicles.[9] Assuming that our dihexadecyldimethylammonium bromide vesicles bind **1** about as strongly as the di-C_{18} vesicles,[9] and taking $K \sim 2 \times 10^4$ M^{-1}, ~90% of substrate **1** would be bound to vesicular **2** at [**2**] = 5×10^{-4} M (the concentration maintained in our experiments). Dianionic reagents such as $S_2O_4^{2-}$ and SO_3^{2-}, as well as thiophenoxide ion,[16] will also be strongly bound to cationic vesicular **2**; for example, similar anions ($S_2O_3^{2-}$, SO_4^{2-}) are known to bind more strongly than bromide to CTAB micelles.[18] Because the reagent anions are also used in 20-fold excess over substrate, we can be confident that an excess of reagent over substrate will also be maintained at the vesicular surface.

Whereas both anionic substrate **1**[9] and the anionic cleavage reagents should be strongly bound to cationic vesicular **2**, zwitterionic reagent **4** is not strongly bound,[19] and its reaction with bound[9] **1** is not catalyzed. Note, too, that the charge type of the vesicle is important. Although cationic vesicular **2** strongly catalyzes the reactions between anions **1** and $S_2O_4^{2-}$ or PhS^-, zwitterionic vesicular dipalmitoylphosphatidylcholine does not catalyze these reactions.

When the vesicular cleavages are carried out under "normal" multimixing conditions (i.e., **2** + **1**, *followed* by cleavage reagent), *two* sequential reactions are observed (Table II, cases 2–4, 6, 8, and 10). The faster processes, k_ψ^f, are kinetically identical with the monophasic reactions observed under inverse mixing conditions (cases 1, 5, 7, and 9). The slower processes, k_ψ^s, are much slower than the "fast" reactions, but (except in case 10) they are still modestly catalyzed relative to the analogous buffer reactions: viz., factors of 14 ($S_2O_4^{2-}$), 7.7 (SO_3^{2-}), and 4.9 (PhS^-). With reagent **4**, however, k_ψ^s is 24 times *less* than the rate constant of the buffer reaction. Cases 3 and 4 (Table II) reveal that pairs of sequential reactions are also observed when the vesicles are doped with 20 wt % of cholesterol, or when an equivalent of 18-crown-6 is added to the solution of **1**.

A striking observation is that the relative amounts of the fast and slow components (but *not* the observed rate constants) of the kinetically biphasic reactions are strongly dependent on how long the vesicles of **2** and substrate **1** are permitted to *age before* the addition of the cleavage reagent. In Figure 2, this dependence is illustrated by a plot of the percent of slow reaction[20] vs. aging time. At the minimum aging time (~46 ms), about 90% of the **1** + $S_2O_4^{2-}$ reaction is rapid and only ~10% is slow (Figure 2, upper curve). As the aging time is extended, however, the percent slow reaction smoothly increases at the expense of the fast component. After about 1 s, an "equilibrium", time-independent distribution of ~52% fast and 48% slow is obtained. With 20 wt % of cholesterol in the vesicles (Figure 2, lower curve), neither

(18) Cf.: Lissi, E. A.; Abuin, E. B.; Sepúlveda, L.; Quina, F. H. *J. Phys. Chem.* **1984**, *88*, 81 and references therein.

(19) That **4** is not strongly bound is suggested by its UV behavior; λ_{max} = 283 nm in the presence or absence of 1×10^{-3} M vesicular **2** at pH 8.0. Contrast this with thiophenoxide ion, which exhibits strong bathochromic shifts from 264 nm in pH 8 Tris buffer, to 273 nm in 1×10^{-3} M micellar CTAB, to 279 nm in 1×10^{-3} M vesicular **2**. Cf., also, ref 16.

(20) The percent of slow reaction was calculated from the absorbance change due to the slow process relative to the *total* absorbance change (slow + fast). The total ΔA was constant, as long as reagent concentrations and monitoring conditions were unchanged; it was also independent of the multimixing mode (normal or inverse). In our original report,[7] a portion of the fast reaction was not accounted for because some of the fast reaction occurred in the cuvet prior to the start of data acquisition. Percent slow was therefore overemphasized. The present data have been corrected for this problem by taking as "fast reaction" all of the absorbance change not due to the slow reaction.

the rate constants (k_ψ^f, k_ψ^s) nor the time course of the approach to equilibrium change, but the distribution of fast and slow kinetic phases at equilibrium is displaced toward the fast process.[21] The addition of 18-crown-6 (not shown in Figure 2) had little effect on the course of the normal mode (**2** + **1** + $S_2O_4^{2-}$) biphasic reaction.[22]

Percent slow reaction vs. aging time profiles for the vesicular reactions of **1** with SO_3^{2-}, PhS^-, and **4** are shown in Figures 3–5, respectively. In each case, we observed time dependencies analogous to those of the **1** + $S_2O_4^{2-}$ experiment: percent slow reaction smoothly increased from ~10% at the 46 ms minimum aging time to ~40–50% at ≥1–2 s aging, i.e., at equilibrium.

Control Experiments. Multimixing combination of vesicular **2** and (e.g.) $S_2O_4^{2-}$ under the experimental conditions of Table II led to no time-dependent spectroscopic (413 nm) phenomena within several lifetimes of either the fast or slow processes associated with the cleavage of **1**. Therefore, there are no light-scattering changes associated with ionic shock which could be mistaken for slow reactions.[23]

Multimixing experiments in which **1** was first combined with *micellar* CTAB (in place of vesicular **2**), and then with $S_2O_4^{2-}$, led only to fast, *monophasic* cleavage of **1**. The biphasic kinetics observed when analogous reactions were run in the presence of **2** must therefore be associated with the vesicles.

When anion **5** was combined with vesicular **2** and $S_2O_4^{2-}$ under the "normal" multimixing conditions of Table II (order of mixing: **2**, **5**, then $S_2O_4^{2-}$), with monitoring at 450 nm (λ_{max} for **5** in vesicular **2**), we observed an initial "instantaneous" absorption increase due to the admission of **5** to the observation cuvet, followed by a small (3–4% of the total ΔA), time-dependent absorbance increase. The latter is due to permeation of **5** from solution (λ_{max} ~438 nm, if this site is similar to micellar CTAB), to either unspecified endovesicular sites or (more likely) an equilibrium distribution of exovesicularly and endovesicularly bound **5**, where λ_{max} = 450 nm.[10] The difference in extinction coefficients for λ at these sites (slightly lower in water or in CTAB micelles than in vesicles of **2**) leads to a net gain in absorbance at 450 nm as aqueous or surface-bound **5** permeates the vesicles of **2**. This process, followed by stopped-flow spectroscopy at 450 nm, leads to an apparent rate constant of 1.7 s^{-1},[10] similar to k_ψ^s for the reaction of **1** + $S_2O_4^{2-}$ in vesicular **2**.

Although the secondary ΔA_{450} due to the permeation of **5** *generated* on the surface of vesicular **2** by the fast cleavage of **1** by $S_2O_4^{2-}$ would only be ~10% of ΔA due to the subsequent slow cleavage reaction, described in Table II, it was possible to eliminate the absorbance change due to permeation by adjusting the monitoring wavelength. Control multimixing experiments with **2** and **5** showed that ΔA due to permeation decreased as λ decreased from 450 to 413 nm. At 413 nm, ΔA_{perm} was nil. For this reason, the cleavage experiments reported in Table II and Figures 2–5 were monitored at 413 nm and should be free of permeation effects.

Discussion

The most novel and significant aspect of our results is the observation of two, sequential cleavage reactions of Ellman's reagent, **1**, in vesicles of **2**, and the time-dependent "evolution" of the initial "fast" reaction toward the much slower but (except for reagent **4**) still catalyzed "slow" reaction, cf., Table II and Figures 2–5.

When the cleavage reagent is added to the vesicles *before* substrate **1**, the substrate is very rapidly cleaved at the vesicular surface before it can permeate to endovesicular sites. In this case, we observe monophasic kinetics characterized by large rate constants, similar in magnitude to those observed in analogous reactions in the Stern layers of CTAB micelles (see text and Table II, cases 1, 5, and 7). In contrast, when the substrate is first added to the vesicles, very rapid binding ($\tau_{1/2}$ < 1 ms) is followed by slower permeation. About 1–2 s is required for permeation to establish an equilibrium between **1** at exovesicular and endovesicular sites. Subsequent addition of cleavage reagent, after "aging" times ranging from 46 to 1000 ms, then results in separate fast and slow reactions with distributions which increasingly favor the slow process as the aging time increases. After 1–2 s, site equilibration of substrate is complete; further aging before the addition of cleavage reagent no longer affects the fast/slow distribution.[24]

Table III. Rate Constants for Vesicular Processes[a]

case	cleavage reagent	K_{eq}[b]	k_{app},[c] s^{-1}	k_{in},[d] s^{-1}	k_{out},[d] s^{-1}	k_ψ^s,[e] s^{-1}
1	$S_2O_4^{2-}$	0.92	3.85	1.85	2.00	2.4
2[f]	$S_2O_4^{2-}$	0.54	3.67	1.29	2.38	2.3
3	SO_3^{2-}	0.95	2.81	1.37	1.44	1.5
4	PhS^-	0.85	3.37	1.55	1.82	46
5	**4**	0.85	2.57	1.18	1.39	0.64

[a] Experimental conditions are described in Table II, note footnote *a*. See text for definitions of constants. [b] Determined from A_{slow}/A_{fast} for cleavage of equilibrated **1** + **2**; see text. [c] From analysis of the data in Figures 2–5; see text. [d] From eq 4 and 5; estimated errors are ±7%. [e] From Table II. [f] The vesicles contained 20 wt % of cholesterol.

We can now analyze this scheme more closely, identify and assign rate constants to the rate-limiting permeations, and, more importantly, differentiate true endovesicular reactions from slow cleavage reactions which are rate limited by the reverse permeation of endovesicular substrate.

The time-dependent fast/slow distribution data for each of the four cleavage reagents (Figures 2–5) give good fits to the integrated equations describing the approach to equilibrium of the system $\mathbf{1}_{exo} \rightleftharpoons \mathbf{1}_{endo}$, where "exo" and "endo" refer to the vesicular site occupied by **1**.[25,26] Plots of ln [% $slow_{eq}$/(% $slow_t$ − % $slow_{eq}$)] vs. time afford linear relations (r > 0.99), the slopes of which give k_{app}, the first-order rate constants for the approach to site equilibrium of **1**.[25] Moreover, k_{app} is equal to the *sum*[25] of the rate constants for the exo → endo (k_{in}) and endo → exo (k_{out}) permeations, eq 4. We can experimentally determine K_{eq}, the equilibrium constant, from the ratio of absorbance changes corresponding to the slow and fast cleavage reactions after **1** has been equilibrated (aging time > 2 s). We then set K_{eq} equal to the quotient of k_{in} and k_{out}, eq 5. From the experimental values of k_{app} and K_{eq}, together with eq 4 and 5, we calculate values of k_{in} and k_{out}.

$$k_{app} = k_{in} + k_{out} \quad (4)$$

$$K_{eq} = \Delta A_{slow}/\Delta A_{fast} = k_{in}/k_{out} \quad (5)$$

Table III includes all of these experimental and calculated constants for the cleavages of **1** by each of the four reagents, as well as k_ψ^s observed for the multimixing reactions of **1** (cf., Table II). The rate constants k_{app}, k_{in}, and k_{out} describe the permeation of **1** within vesicular **2** investigated by a kind of chemical "stop-action" technique. Insofar as the various cleavage reagents do not greatly perturb the vesicles and change their properties toward the permeation of **1**, the rate constants (and K_{eq}) should be independent of the reagent. In fact, for the four cases of Table III, mean values (± average deviation) of k_{app}, k_{in}, and k_{out} are 3.2 ± 0.4, 1.4 ± 0.2, and 1.8 ± 0.3 s^{-1}, respectively. The mean value

(21) This result differs from that in the original report,[7] where cholesterol appeared to speed the attainment of equilibrium without inducing much change in its position. Recalculation of the original data in accord with the method described in ref 20 gives results which agree with the present observations.

(22) This is in contrast to the initial study,[7] where 18-crown-6 appeared to speed the attainment of equilibrium. Again, recalculation of the original data in accord with the method described in ref 20 gives results which agree with our present observations.

(23) Cf.: Moss, R. A.; Swarup, S.; Hendrickson, T. F.; Hui, Y. *Tetrahedron Lett.* **1984**, *25*, 4079.

(24) Sonicated (i.e., "infinitely aged") samples of **1** in vesicular **2** afford fast/slow distributions similar to normal mode multimixing experiments in which **2** + **1** are aged ≥2 s before the addition of the cleavage reagent.

(25) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; 2nd ed.; Wiley: New York, 1961; pp 185–186, eq 71.

(26) The "endovesicular" site, in particular, probably represents a range of distinct sites separated by very low permeation barriers.

of K_{eq} is 0.89 ± 0.04. The 10–20% variance over a wide variety of cleavage reagents appears satisfactory.

The mean value for k_{app}, 3.2 ± 0.4 s^{-1}, which measures the approach to a "two-site" equilibrium, is similar to k_{app} for the permeation-controlled equilibrations in vesicular **2** of 8-anilinonaphthalenesulfonate (ANS) where k_{app} = 3.5 s^{-1} as determined by fluorescence spectroscopy[27] and of anion **5**, for which k_{app} = 1.7 s^{-1} as measured by absorption spectroscopy.[10] Each permeation probe, **1**, ANS, and **5**, is an hydrophobic anion or dianion. The similar magnitude observed for k_{app} in cationic vesicular **2** with three different molecular probes, using three different methods, is comforting and indicates related redistribution processes for each probe.

Cholesterol-doped vesicles display rate constants similar to those of native vesicles for the permeation of **1** (Table III, cases 2 vs. 1), but the cholesterol significantly reduces the equilibrium contribution of the endovesicular component. We interpret this to mean that the cholesterol occupies endovesicular sites which might otherwise be open to **1** and thus excludes some of the substrate, forcing more of it to remain in exovesicular sites. In phosphatidylcholine liposomes, cholesterol appears to intercalate among the first 7–10 methylene units of the acyl chains[28] (Fuhrhop's sites 4–5[1]). In vesicles of **2**, it may be that cholesterol and substrate **1** occupy analogous loci relative to the quaternary nitrogen atoms.

We come now to the detailed mechanism responsible for biphasic kinetics in these vesicular cleavages. We can think of two "extreme" alternative models. In the first model (i), the cleavage reagent reacts rapidly (k_ψ^f) with exovesicularly-bound substrate and then permeates to endovesicular or shallow "subvesicular" sites where it again reacts (k_ψ^s) with substrate. Here, the rate constant for reagent permeation would have to be ≫1.8 s^{-1}, the mean rate constant for in → out "reverse permeation of substrate" (see above). In the second model (ii), we imagine that exovesicularly-*bound* cleavage reagent rapidly reacts with similarly bound substrate **1** (k_ψ^f), and that *excess* reagent *subsequently* reacts (k_ψ^s) with additional substrate as the latter undergoes reverse permeation from endovesicular sites to the vesicular surface. In this case k_{out} for **1** will be rate limiting.

We suggest that the vesicular reactions of **1** with $S_2O_4^{2-}$ and SO_3^{2-} correspond to model ii. Note that the observed k_ψ^s values in these cases are experimentally identical with the values of k_{out} derived from the equilibrium and approach to equilibrium data (Table III, cases 1–3). That is, k_{out} appears to determine k_ψ^s, as required for model ii. This conclusion, at variance to our previous assignment of model i to the $S_2O_4^{2-}$ cleavage,[7] is in better agreement with independent evidence which suggests that $S_2O_4^{2-}$ (and SO_3^{2-} by analogy) do not permeate cationic or anionic surfactant vesicles and should remain at exovesicular sites during the short time courses of our reactions.[29]

On the other hand, the vesicular cleavage of **1** by PhS^-, Table III, case 4, appears to follow model i. Here $k_\psi^s \gg k_{out}$, so that reverse permeation of **1** is clearly not limiting. It is, however, unclear whether the observed k_ψ^s represents the rate constant for the cleavage of **1** by PhS^- at an endovesicular site or the effective rate constant for a rate-limiting exo → endo permeation of PhS^- (perhaps as PhSH), followed by a rapid endovesicular reaction. Although we cannot differentiate these two possibilities with the present evidence, we conclude that the slow reaction of **1** and PhS^- is indeed endovesicular.

The reactions of **1** and zwitterion **4** are curious (Table III, case 5). Here, there is spectroscopic evidence that **4** does not strongly bind to the exovesicular surface;[19] the observed "fast" reaction is therefore not catalyzed relative to the reaction in buffer alone. More interestingly, k_ψ^s is about 2 times smaller than k_{out}. We suggest that the mechanism here conforms to a modified version of model ii: after exovesicular **1** has been cleaved by **4** in a region near, but not on, the exovesicular surface (k_ψ^f), endovesicular **1** must permeate back to the surface and then exit the vesicle to react with excluded, excess **4**. An additional, small activation barrier must be postulated to account for the observation that $k_\psi^s < k_{out}$. Perhaps this barrier arises in a necessary desorption of the permeating **1** from the product-modified vesicular surface before it can react with **4**.

Table IV. Temperature Dependence of the Ellman's Anion–$S_2O_4^{2-}$ Reaction[a,b]

temp, °C	K_{eq}	k_{app}, s^{-1}	k_{in}, s^{-1}	k_{out}, s^{-1}	k_ψ^s, s^{-1}
20	0.84	0.38	0.17	0.21	0.27 ± 0.03$_7$
25[c]	0.92	3.85	1.85	2.00	2.4
30	0.84	10.[d]	4.5	5.5	5.8 ± 0.2$_9$

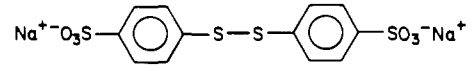
[a] Experimental conditions are described in Table II, footnote *a*. [b] See text and Table III for definitions of the constants. [c] From Table III, case 1. [d] Estimated value; equilibrium is established in ~350 ms.

A close analogy to our observations is found in the ethylenediamine aminolysis of *p*-nitrophenyl laurate in cationic vesicles.[5] The ester substrate is rapidly distributed between exo- and endovesicular sites, and the observed biphasic cleavage kinetics are attributed to sequential exovesicular and endovesicular reactions. It is recognized, however, that k_ψ^s could represent either the rate constant of an endovesicular laurate/amine reaction or the rate-limiting permeation of ethylenediamine to "buried" laurate.[5]

We also note recent reports that **1** does not readily permeate phosphatidylcholine/phosphatidylglycerol liposomes.[30] In our case, vesicles of **2** are quite permeable to **1**. In part, this difference reflects the generally greater resistance to permeation by hydrophobic anions of phosphatidylcholine liposomes, relative to **2**, particularly below the phase-transition temperature (T_c) of the former.[27,31]

The principal T_c for small vesicles of **2** is ~25 °C[27] so that the rate and equilibrium constants given for our cleavage reactions in Table III refer to vesicles that are just at T_c. In Table IV, we present data for the **1**/$S_2O_4^{2-}$ vesicular cleavage determined either 5 °C above or below T_c. Below the T_c (20 °C), all of the rate constants (but not K_{eq}) are decreased ~10-fold, relative to their values at 25 °C. However, the previously described phenomena persist, and our mechanistic analysis is unchanged. Similarly, 5 °C above T_c (30 °C), the rate constants are all enhanced by factors of 2–3, but the biphasic kinetics and the similarity between k_{out} and k_ψ^s persist. The two-site vesicular reactions described in this report therefore occur in both gel and liquid crystalline phases of vesicular **2**; they are not restricted to either phase. The persistence of the two-site reactions in gel-phase vesicular **2** may again be related to the generally greater permeability of **2** compared to liposomes of (e.g.) dipalmitoylphosphatidylcholine (DPPC). For example, 1-anilino-8-naphthalenesulfonate rapidly permeates vesicular **2** at temperatures well below T_c, whereas it begins to permeate DPPC liposomes only at temperatures near T_c.[27]

Finally, it is worth pointing out that the biphasic kinetics exhibited in the vesicular dithionite cleavage of **1** are not observed when the cleavage reagent is hydroxide ion. Reaction of **1**, in vesicular **2**, with OH^- (pH 11.0) afforded anion **5** with K_ψ = 0.15 s^{-1} in a strictly monophasic reaction. Similar results were reported previously for this reaction in dioctadecyldimethylammonium ion vesicles.[9] Additionally, only monophasic kinetics are observed in the normal mode, multimixing, sulfite ion cleavage of the dianionic diaryl disulfide **6**[32] under the vesicular conditions described above (cf. Table II). Here, the reaction is followed at 254 nm, and it



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gives $k_\psi = 2.85\ s^{-1}$ ($2.7 \times 10^{-2}\ s^{-1}$ in the absence of vesicles). In these cases, we believe that the catalyzed cleavage reactions are still too slow to effectively compete with substrate or reagent (OH^-) permeations, so that site equilibria, if established, are "drained" by slow, probably exovesicular, cleavage reactions.

Experimental Section

Materials. Di-*n*-hexadecyldimethylammonium bromide, **2**, was prepared by the reaction of *n*-hexadecyl bromide with *N*-*n*-hexadecyl-*N*,*N*-dimethylamine[33] and purified by repeated crystallization from ethyl acetate, mp 145–155 °C. A satisfactory microanalysis (C, H, Br) was obtained. Cetyltrimethylammonium bromide (Sigma) was purified by the method of Perrin et al.,[34] mp 227–235 °C (lit.[34] mp 227–235 °C dec). *N*,*N*,*N*-Trimethyl-*N*-(*p*-mercaptobenzyl)ammonium bromide (protonated form of **4**) was prepared by the procedure of Moss and Dix.[8] Thiophenol, **3** (Aldrich, >99%), was used without further purification. Also used as received were cholesterol (Sigma, >99%), sodium dithionite (J. T. Baker, purified), sodium sulfite, (Fisher, ACS certified), 5,5′-dithiobis(2-nitrobenzoic acid), **1** (Aldrich), and 18-crown-6 (Aldrich). Solvents were of ACS reagent grade quality and were dried before use. Buffer solutions were prepared from N_2-purged "Steam-Distilled" water (distilled, U.S.P., Electrified Water Co., East Orange, NJ).

Methods. UV spectra were obtained on a Cary Model 17D spectrophotometer. pH measurements were made with a Radiometer Type PHM 25 pH meter. Cmc determinations employed a Fisher "Tensiomat" and were determined from the "break points" of observed surface tension vs. log [surfactant]. Vesicular solutions were prepared by sonication with a Braun Sonic Model 1510 probe-type sonicator; the conditions appear in Results. The vesicle solutions were filtered through 0.8-μm Millipore "Millex-PF" filters before use.

Kinetic Studies. Slower reactions were followed with use of a Gilford Model 250 spectrophotometer equipped with a Gilford Model 6051 recorder. Faster reactions were monitored with the stopped-flow or multimixing units described in Results. All reactions were carried out at 25.0 ± 0.2 °C, except as noted. Rate constants were obtained from computer-generated correlations of log $(A_\infty - A_t)$ vs. time. Conditions, errors, monitoring wavelengths, and other details appear in Results, cf., especially, Tables I and II.

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Registry No. 1, 69-78-3; **2**, 70755-47-4; **3**, 108-98-5; **4**, 98541-71-0; **5**, 77874-90-9; sodium dithionite, 7775-14-6; sodium sulfite, 7757-83-7; CTAB, 57-09-0.

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Carbometalation Reaction of Alkynes with Organoalane–Zirconocene Derivatives as a Route to Stereo- and Regiodefined Trisubstituted Alkenes[1]

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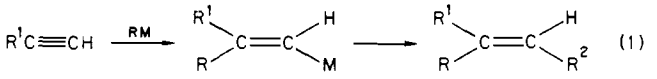
Abstract: The reaction of alkynes with a methylalane, such as Me_3Al, and a zirconocene derivative, such as Cl_2ZrCp_2, provides a convenient and selective route to (*E*)-2-methyl-1-alkenylalanes in high yields. The reaction is catalytic in Zr. The stereoselectivity is generally >98%, and the regioselectivity observed with terminal alkynes is typically ca. 95%. Although the reaction of alkynes with trialkylalanes containing β-hydrogens and Cl_2ZrCp_2 is complicated by competitive hydrometalation and diminished (70–80%) regioselectivity, the hydrometalation reaction can be avoided by using chlorodialkylalanes and Cl_2ZrCp_2. The Zr-catalyzed carboalumination reaction of typical alkynes, such as phenylacetylene and 1-octyne, most likely involves direct Al–C bond addition assisted by Zr.

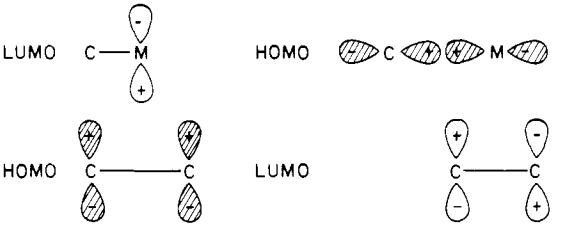
Controlled carbometalation of alkynes as shown in eq 1 provides a conceptually attractive route to trisubstituted alkenes. Con-



(1)

sideration of the frontier orbitals of alkynes and organometals (RM) suggests that the key requirement for concerted carbometalation, which is expected to involve cis addition of the R—M bond to the C≡C bond, is the presence or the ready availability of a low-lying metal orbital (Scheme I) and that this requirement is essentially the same as that for concerted hydrometalation. In reality, however, the greater steric requirements of the M–C bond

Scheme I



relative to the corresponding M–H bond are expected to limit the scope of carbometalation. Thus, for example, whereas hydroboration[3] is facile and general, simple alkylboranes, such as BEt_3, do not readily add to alkenes and alkynes.[4] Similarly, the scope

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