Chemical Differentiation of Bilayer Surfaces in Functional Dialkylammonium Ion Vesicles: Observation of Surfactant Flip-Flop

Robert A. Moss,* Santanu Bhattacharya, and Swati Chatterjee

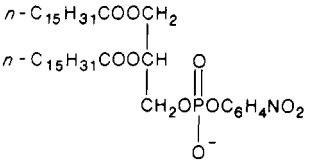
Contribution from the Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903. *Received November 4, 1988*

Abstract: Cationic *p*-nitrophenyl carbonate and *p*-nitrophenyl benzoate functionalized di-*n*-octadecylmethylammonium ion surfactants **6** and **7** were synthesized. Vesicles of **6** or covesicles of (1:9) **7** and **5**, created at pH 3.9, gave rapid, partial *p*-nitrophenylate cleavage at pH 7.9–8.0 (from **6**) or rapid, partial benzoate cleavage by external thiolate ions at pH 7.9–8.0 (from **7**), attributed to surface-specific *exovesicular* reactions of **6** or **7**/**5**. These exovesicular cleavages at pH 8 and 25 °C are apparently faster than reagent permeation across the bilayers to the endovesicular functional groups at pH 3.9. The dioctadecylammonium ion vesicles, in contradistinction to their dihexadecyl analogues, are able to maintain the indicated pH gradient long enough at 25 °C to permit the surface-specific esterolyses. Relaxation of the pH gradient and endovesicular cleavages follow upon enhancement of the fluidity of the vesicle bilayers either with the application of heat or with additives such as 1-hexanol or dioctyldimethylammonium chloride. In the surface-differentiated **7**/**5** covesicles, "flip-flop" of intact **7** from endovesicular to exoversicular sites can be promoted and visualized by experiments that involve incubation of the vesicles at 38–40 °C, pH 3.9, for 1–12 min.

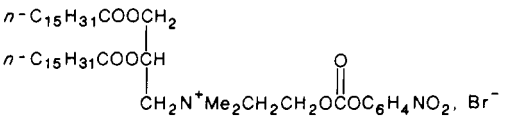
The controlled generation of dissymmetrically surface-functionalized, synthetic bilayer vesicles or liposomes is an essential step in the successful modeling of biological membranes.[1] What structural features are required at the molecular and aggregate levels of architecture to accomplish this task? The principal condition for the chemical differentiation of identically functionalized vesicular surfactants is that the differentiating reaction be faster at one surface (most often the *exovesicular* surface) than at the other. In turn, this requires that the exovesicular rate of the differentiating reaction be greater than the rate of permeation of the differentiating reagent through the bilayer. There are many variables that affect this central kinetic inequality $k_{exo} > k_{perm}$, including the nature of the key reaction and its associated reagent (especially charge type and hydrophobicity), the rapidity of the reaction, the charge type and architecture of the surfactant monomers, the tightness of their packing in the vesicle, and their head-group structure.

Several surface-differentiating reactions have been reported, including diazo coupling,[2] viologen reduction,[3] ylide protonation,[4] stilbene bromination,[5] and fluorescamine labeling.[6] We have focused on the esterolysis reaction, a fundamental biochemical process that is important in metabolic and enzymatic chemistry. The aminolysis of *p*-nitrophenyl laurate in polymeric vesicles[7] and the hydrolysis of clustered or dispersed ester-functionalized azobenzene surfactants in dialkyldimethylammonium ion vesicles[8] are early examples of intravesicular esterolytic processes that each occur as competitive, kinetically distinct pairs of reactions.

In 1986, we reported that anionic vesicles constructed at pH 5.5 from the *p*-nitrophenyl phosphate glyceryl ester surfactant **1** could be specifically cleaved at their exovesicular phosphate ester groups by hydroxide ion at an external pH of 11.8, where the exovesicular reaction was fast relative to transvesicular OH^- permeation.[9] In this case, both the initial monoanionic character of the surfactant head groups and their ultimate dianionic state (after esterolytic loss of the *p*-nitrophenylate moiety) mitigate against OH^- permeation.



1



2

A more exacting test was provided by the *cationic* vesicles constructed from the *p*-nitrophenyl carbonate surfactant **2**. Vesicles assembled at acidic pH (3.9) could be exovesicularly cleaved at pH 7.9 by hydroxide-mediated esterolysis that occurred ~700 times more rapidly than the corresponding endovesicular reaction due to rate-limiting hydroxide permeation.[10]

Surfactants **1** and **2** are closely related to the natural glyceryl diester lipids, and we can expect their long chains to pack well in vesicles.[1b,e] Our initial studies of covesicles derived from the *p*-nitrophenyl dihexadecylcholine carbonate surfactant **3** and dihexadecyldimethylammonium bromide, however, failed to demonstrate comparable surface-specific esterolysis, probably because exo → endo hydroxide permeation occurred rapidly enough to render the exo- and endovesicular cleavages kinetically unresolvable.[11] Similarly, basic cleavage of benzoate from surfactant **4** occurred without endo/exo differentiation.[11]

We suspected that the geminally constructed dialkylammonium ion chains of **3** and **4** might afford vesicles that were inherently more permeable than those incorporating the glyceryl diester architecture of surfactants **1** and **2**. Partly, the difficulty resided with the hexadecyl groups of **3** and **4**. Phase transition temper-

(1) Reviews: (a) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113. (b) Jain, M. K.; Wagner, R. C. *Introduction to Biological Membranes*; Wiley: New York, 1980. (c) Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100. (d) Fuhrhop, J.-H.; Fritsch, D. *Acc. Chem. Res.* **1986**, *19*, 130. (e) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.

(2) Fuhrop, J.-H.; Bartsch, H.; Fritsch, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 804. Moss, R. A.; Shin, J. S. *Chem. Commun.* **1983**, 1027.

(3) Baumgartner, E.; Fuhrhop, J.-H. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 550. Tundo, P.; Kurihara, K.; Kippenberger, J.; Politi, M.; Fendler, J. H. *Angew. Chem., Int. Ed. Engl.* **1982**, *104*, 5547. Patterson, B. C.; Thompson, D. H.; Hurst, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 3656.

(4) Fuhrhop, J.-H.; Penzlin, G.; Tank, H. *Chem. Phys. Lipids* **1987**, *43*, 147.

(5) Mizutani, T.; Whitten, D. G. *J. Am. Chem. Soc.* **1985**, *107*, 3621.

(6) Kunitake, T.; Okahata, Y.; Yasunami, S. *J. Am. Chem. Soc.* **1982**, *104*, 5547.

(7) Ishiwatari, T.; Fendler, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 1908.

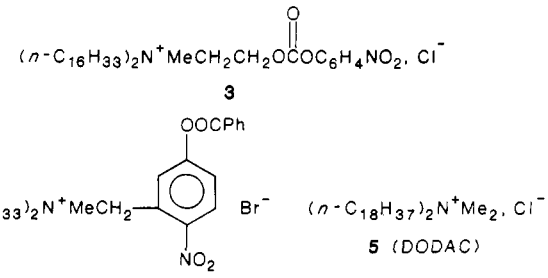
(8) Kunitake, T.; Ihara, H.; Okahata, Y. *J. Am. Chem. Soc.* **1983**, *105*, 6070.

(9) Moss, R. A.; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 5341.

(10) Moss, R. A.; Bhattacharya, S.; Scrimin, P.; Swarup, S. *J. Am. Chem. Soc.* **1987**, *109*, 5740.

(11) Moss, R. A.; Schreck, R. P. *Tetrahedron Lett.* **1985**, *26*, 6305.

0002-7863/89/1511-3680$01.50/0

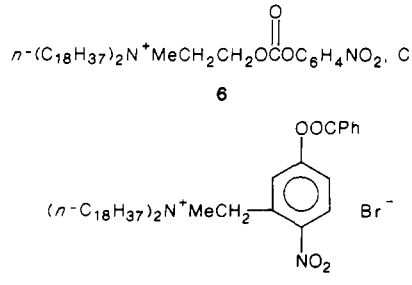


3

4 **5** (DODAC)

atures (T_c) for dihexadecylmethylammonium surfactant vesicles lie in the 25–27 °C range. At the T_c, vesicles pass from the fairly rigid "gel" phase, where the hydrocarbon chains are arranged in extended s-trans conformations and closely packed, to the less ordered, more fluid, liquid-crystalline phase that features chains containing gauche conformer "kinks".[1b,e] When vesicular kinetics are studied at the typical laboratory temperature of 25 °C, holovesicles or dihexadecyl covesicles of **3** and **4** are at or near their T_c, and both permeations and surfactant flip-flop may occur rapidly and undercut surface-specific reactions. To mitigate this problem, one might lower the reaction temperature or lengthen the surfactants' alkyl chains. However, even at 15 °C, vesicles of **4** could not be surface differentiated, so that the unfavorable k_{exo}/k_{perm} ratios for vesicular **3** and **4** involve something more fundamental than an "unfortunate" T_c.

Subsequently, we learned that vesicles of dioctadecyldimethylammonium chloride (**5**, DODAC) are relatively resistant to OH^- permeation at 25 °C at imposed exo/endo pH gradients of 3–4 units.[12,13] Now, we report that holovesicles constructed of *dioctadecyl*choline carbonate surfactant **6** ($T_c \sim 32$ °C) will indeed support hydroxide ion mediated, surface-specific esterolysis reactions at 25 °C, affording exo/endo-differentiated vesicles. Exovesicular esterolysis of vesicular **6** is rapid, relative to OH^- permeation; the latter process is rate limiting for endovesicular esterolysis, and the surface-specific reactions can be kinetically resolved.



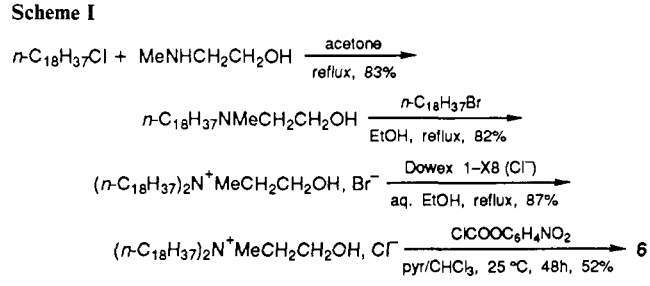
6

7

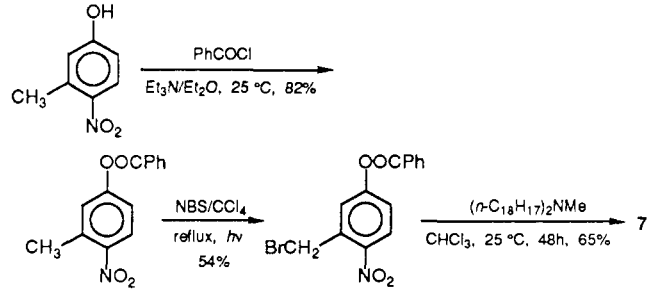
On the other hand, covesicles created from 9:1 cosonicated DODAC (**5**) and **7**, the dioctadecyl analogue of 4, have $T_c \sim 33.5$ °C but do not support kinetically differentiable exo- and endovesicular esterolysis with OH^- at 25 °C. The reason is that the *p*-nitrophenyl *benzoate* moiety of **7** is less reactive toward OH^- than is the *p*-nitrophenyl carbonate group of **6**; the exovesicular esterolysis of **7** is slower than (or comparable to) the rate of OH^- permeation, and the latter no longer limits the endovesicular cleavage, so that esterolyses occur simultaneously on both surfaces. Nevertheless, we have achieved surface-specific reactions of the **5**/**7** covesicles by employing more potent esterolytic reagents. Thus, the thiolate anions of cysteine (**8**) or glutathione (**9**) react more rapidly with the exovesicular benzoates of **7** than they permeate the vesicular bilayer at pH 8,[14] leading to surface-specific exovesicular reactions. Moreover, the resulting surface-differentiated vesicles demonstrate a particularly dramatic, chemically detected example of flip-flop of their surfactant monomers (in both directions) between endovesicular and exovesicular locations.

Our new results show that surface-differentiated bilayer vesicles are, in fact, readily preparable from synthetic, cationic, quaternary ammonium ion surfactants and that such fundamental processes as flip-flop can be easily studied. The implications for "membrane mimetic chemistry"[1e] are substantial.

Scheme I



Scheme II



Results

Synthesis. Surfactants **6** and **7** were prepared by the reaction sequences outlined in Schemes I and II, respectively. Thus (Scheme I), *n*-octadecyl chloride and *N*-methylethanolamine afforded *N*-octadecyl-*N*-methylethanolamine upon reflux in acetone. A second alkylation, this time with *n*-octadecyl *bromide*, then afforded *N*,*N*-dioctadecyl-*N*-methyl-*N*-(β-hydroxyethyl)-ammonium bromide in very good yield (82%). Ion exchange (87%) of bromide to chloride[15] followed by esterification with *p*-nitrophenyl chloroformate (52%) afforded surfactant **6**. The intermediates and final product were characterized by NMR spectroscopy and elemental analysis. The overall yield of **6** was 31% for four steps.

The synthesis of surfactant **7** (Scheme II) involved the prior preparation of 3-(bromomethyl)-4-nitrophenyl benzoate by initial benzoylation of 3-methyl-4-nitrophenol, followed by bromination of the methyl group with *N*-bromosuccinimide. The resulting benzylic bromide was then used to quaternize *N*,*N*-dioctadecyl-*N*-methylamine, affording the desired **7**. Again, the intermediates and the product were characterized.

Vesicles. Vesicles of **6** and *covesicles* of 10 mol % **7** and 90 mol % **5** were generally prepared either by sonication or by slow injection methods. In the former case, a chloroform solution of surfactant was evaporated to a surfactant film that was dispersed in aqueous HCl–0.01 M KCl solution at pH 3.9 by sonication with an immersion probe instrument (55–60 W, 6–8 min, 48–60 °C). Cooling to 25 °C and filtration through a 0.8-μm Millex filter completed the preparation.[16] Note that the sonications were carried out under acidic conditions (pH ~4) to avoid prior esterolysis of **6** or **7**, which occurred readily in neutral or basic aqueous solutions.

(12) Moss, R. A.; Swarup, S.; Zhang, H. *J. Am. Chem. Soc.* **1988**, *110*, 2914.

(13) The T_c of **5** is reported to be 36 °C in Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7. However, reaction temperature and vesicular T_c are not the only factors determining k_{perm}. Chain length and the method of vesicle preparation are also important; see below.

(14) Moss, R. A.; Swarup, S. *J. Org. Chem.* **1988**, *53*, 5860.

(15) The identical chloride could be directly prepared from *n*-$C_{18}H_{37}NMeCH_2CH_2OH$ by alkylation with *n*-octadecyl chloride in refluxing ethanol, but in only 10% yield.

(16) Holovesicles of **7** could not be prepared without substantial turbidity, but the *sonicated*, acidic solutions of covesicular **7**/**5** were optically stable for at least 2 weeks.

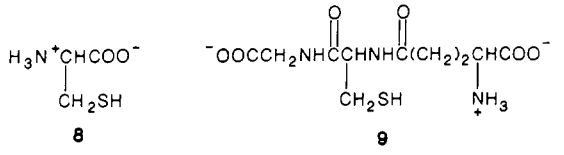
Slow-injected covesicles of **7** were prepared by controlled delivery (1 mL/90 min) of a 1×10^{-3} M chloroform solution of 1:9 **7**/**5** into 25 mL of pH 3.9 aqueous HCl–0.01 M KCl solution at 65 °C. A constant, slow stream of nitrogen was passed through the aqueous receiving solution during injection to facilitate the removal of chloroform. Cooling and filtration completed the vesicle preparation. These vesicular solutions developed turbidity after several hours and were therefore used immediately.

Dynamic light scattering studies (Ar laser, 488 nm, 90° scattering angle) gave hydrodynamic diameters of 1000–1100 Å for *sonicated* 9:1 covesicular **7**/**5** and 1200–1300 Å for sonicated holovesicular **6**, both at pH 3.9–4. Slow-injected covesicular **7**/**5** at pH 3.9 had $r = 3000 \pm 500$ Å. Light-scattering data could not be obtained at pH 8 (i.e., under the conditions of the kinetic studies) because the rapidly developing absorbance of the *p*-nitrophenylate products interfered with measurements at the 488-nm laser line.

Critical temperatures (T_c) associated with the gel to liquid-crystalline phase transitions of holovesicular **6** or 1:9 covesicular **7**/**5** were determined from discontinuities in the fluorescence polarizations (P) of covesicallized 1,6-diphenyl-1,3,5-hexatriene as a function of temperature.[17] This method has been illustrated previously;[10,14] details appear under Experimental Section. The T_c of sonicated holovesicular **6** was 32 °C, whereas that of sonicated covesicular **7**/**5** was 33.5 °C.[18] For comparisons, the T_c of holovesicular **5** was found to be 39 °C by the present method[14] or ~36 °C by several other techniques.[13,19]

Kinetic Studies. Two principal reactions were investigated: the OH^--mediated cleavage of *p*-nitrophenylate ion from the *p*-nitrophenyl carbonate moieties of holovesicular **6** and the cleavage of benzoate ion from the covalently bound *p*-nitrophenyl benzoate groups of **7** (covesicalized with 90 mol % **5**). In both cases, the appearance of *p*-nitrophenylate absorbance was followed at 400 nm. The bulk or external pH was generally adjusted to pH 7.9–8.0 with 0.01 M Tris buffer to initiate the reaction (however, the inner water pools of the vesicles remained at pH 3.9, the pH during vesicle creation). Cleavage reagents were added in excess so that the appearance of *p*-nitrophenylate was first order. Observed rate constants were generally reproducible to ±3% when duplicate experiments were carried out on the same vesicle preparation; kinetic comparisons between separate vesicle preparations gave poorer reproducibility (±10%).

With **7**/**5**, cleavage was effected not only with OH^- but also with the thiolate nucleophiles cysteine (**8**) and glutathione (**9**). The pK_a values for the corresponding RSH ⇄ RS^- processes are 8.5[20] and 9.2,[21] respectively, so that significant concentrations of the nucleophilic RS^- conjugate bases will be present at pH 8.



8 **9**

The cleavage of *p*-nitrophenylate ion from 5×10^{-5} M *sonicated* vesicles of **6** (prepared at pH 3.9) in 0.01 M aqueous Tris buffer (0.01 M KCl, 25 °C) at pH 7.94 was kinetically *biphasic*, as illustrated in Figure 1. The initial fast reaction was complete within several minutes and was followed by a slower reaction that required ~1 h for completion. The total observed absorbance agreed with that calculated from the initial concentration of **6**, by use of an independently measured extinction coefficient of 17 500 for *p*-nitrophenylate under similar conditions.

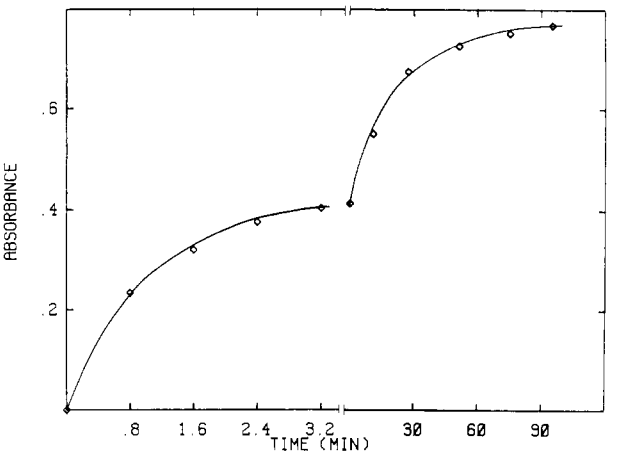


Figure 1. Absorbance at 400 nm vs time (min) for the cleavage of *p*-nitrophenylate ions from sonicated vesicular **6** at a bulk aqueous (external) pH of 7.94. Note the discontinuous time axis.

Table I. Cleavage of Benzoate from Covesicular **7**/**5** at 25 °C, pH 7.9[a]

run	10^5[7], M[b]	additive	10^5(concn), M	$10^3 k_\psi^f$, s^{-1}[c]	$10^3 k_\psi^s$, s^{-1}[d]	% fast/ % slow[e]
1	4.0	none	0.0	0.23	*f*	*f*
2	5.0	none	0.0	1.15[g]	*f*	*f*
3	3.5	**8**	17.5	22.4	0.35	49:51
4	4.0	**8**	25.0	29.8	0.41	49:51
5	5.0	**8**	50.0	39.1	0.75	49:51
6	4.0	**8**	60.0	61.6	1.54	49:51
7[h]	2.0	**8**	25.0	61.7	0.76	50:50
8[h]	2.0	**8**	30.0	64.4	1.32	50:50
9	6.0	**8**-OEt[i]	50.0	32.1	1.19	51:49
10[h]	2.0	**8**-OEt	20.0	14.7	0.44	48:52
11	5.0	**9**	5.0	34.2	0.16	45:55
12	6.0	**9**	10.0	52.3	0.44	45:55
13	6.0	**9**	20.0	109	0.75	45:55
14	5.0	**9**	25.0	150	0.94	45:55

[a] Reactions were carried out in 0.01 M aqueous Tris buffer, containing 0.01 M KCl. Vesicles were prepared by sonication, and the final pH was 7.9, unless otherwise indicated. [b] The covesicles contained 9 times as much **5**. [c] Initial "fast" pseudo-first-order cleavage. [d] Subsequent slow-cleavage reaction. [e] Distribution of the kinetic phases based on their corresponding absorbance changes. Values shown are averages of several experiments; reproducibility is ±3%. [f] Only a single, quantitative kinetic process was observed. [g] This run was at pH 8.8. [h] Slow-injected covesicles; see above. [i] Additive was cysteine ethyl ester.

From 10 reactions similar to that of Figure 1, each determined on individually prepared and sonicated vesicular solutions, the corresponding average rate constants were $k_\psi^f = (2.55 \pm 0.46) \times 10^{-2}$ s^{-1} and $k_\psi^s = (1.30 \pm 0.14) \times 10^{-3}$ s^{-1}, respectively, for the "fast" and "slow" reactions. The average distribution of kinetic phases (% fast/% slow) was ~60:40, as determined from the relative absorbance changes.[22-24]

In contrast to the behavior of **6**, sonicated holovesicles of **3**, its dihexadecyl analogue, give only monophasic, quantitative cleavage of *p*-nitrophenylate at pH 8, with $k_\psi = 0.057$ s^{-1}.[10] With vesicular

(17) Andrich, M. P.; Vanderkooi, J. M. *Biochemistry* **1976**, *15*, 1257.
(18) The T_c of the slow-injected **7**/**5** covesicles could not be determined by the fluorescence method because of the development of significant turbidity and extraneous light scattering during the latter part of the procedure.
(19) Kano, K.; Romero, A.; Djermouni, B.; Ache, H. J.; Fendler, J. H. *J. Am. Chem. Soc.* **1979**, *101*, 4030.
(20) Ogilvie, J. W.; Tildon, J. T.; Strauch, B. S. *Biochemistry* **1964**, *3*, 754. The pK_a was measured in 0.02 M phosphate/borate buffer and will be lower by ~0.5 unit in the presence of cationic vesicles.
(21) Shinkai, S.; Kunitake, T. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3219. The pK_a was determined in 2×10^{-4} M aqueous micellar cetyltrimethylammonium bromide.
(22) Interestingly, only *monophasic* cleavage was observed with *fast*-injected[23] vesicles of **6** (T_c ~32.5 °C) under comparable conditions. Here, $k_\psi = (1.67 \pm 0.15) \times 10^{-2}$ s^{-1} (three runs). Possibly, the fast-injected species are not fully closed and are leakier than sonicated or slow-injected vesicles.
(23) Batzri, S.; Korn, E. D. *Biochim. Biophys. Acta* **1973**, *298*, 1015. A solution of 2 mg of **6** in 0.5 mL of EtOH was injected in <0.5 s through a 20-gauge, 2-in. stainless steel needle into 24.5 mL of stirred, 0.01 M aqueous KCl at pH 3.9 (HCl) and 50–55 °C. After an additional 30 s at 50 °C, the solution was slowly cooled to 25 °C and then filtered through a 0.8-μm Millipore filter.
(24) Slow-injected vesicles of **6** could not be prepared due to substantial *p*-nitrophenyl carbonate lysis that occurred (even at pH 4) during the extended injection period at 65 °C.

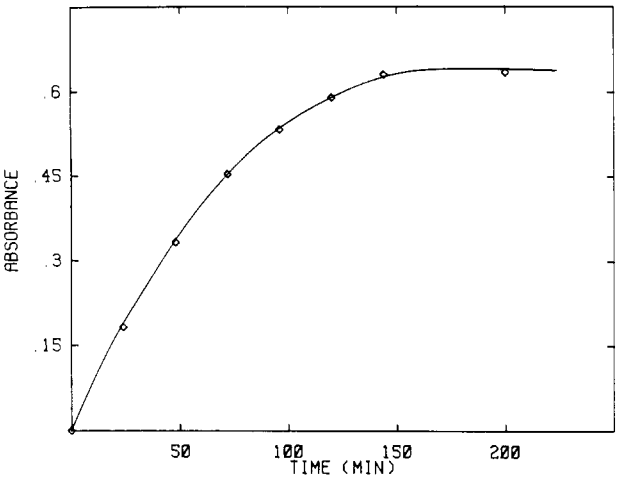


Figure 2. Absorbance at 400 nm vs time (min) for the cleavage of benzoate and the appearance of *p*-nitrophenylate absorbance from sonicated covesicular **7/5** at a bulk aqueous (external) pH of 7.94; see Table I, run 1.

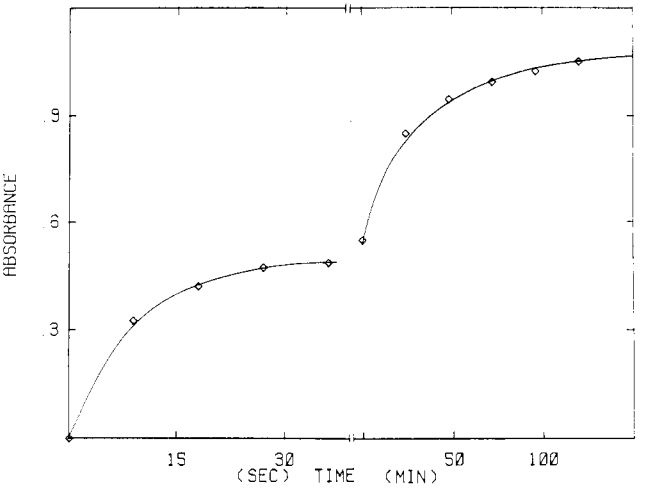


Figure 3. Absorbance at 400 nm vs time for the cleavage of benzoate and the appearance of *p*-nitrophenylate from sonicated covesicular **7/5** at a bulk aqueous (external) pH of 7.94 in the presence of 2×10^{-4} M **9**. Note the discontinuous time axis. See Table I, run 13.

6, the interior water pools are initially at pH 4 (the condition during vesicle preparation); adjustment of the external pH to 8 initiates rapid exovesicular carbonate cleavage, but the endovesicular functionalities remain intact until the internal pH rises due to inward OH^- permeation. Vesicular **6** can hold the transmembrane pH gradient long enough to permit exovesicular cleavage to occur, but vesicular **3** is more permeable and cannot long maintain the gradient. Consequently, permeation of OH^- into vesicular **3** is faster than the exovesicular esterolysis reaction, and both exo- and endovesicular carbonates cleave together.

Kinetic results with 10:90 covesicular **7/5** are summarized in Table I. Runs 1 and 2, where OH^- at pH 7.9 or 8.8 is the only effective nucleophile, display strictly pseudo-first-order kinetics in the appearance of the (covalently bound) *p*-nitrophenylate product absorbance. Run 1 is illustrated in Figure 2, where the contrast with Figure 1 (OH^- cleavage of **6**) is obvious. The rate constant for esterolysis of **7** at pH 8 is $\sim 2.3 \times 10^{-4}$ s^{-1}, about 100 times less than the analogous value for vesicular **6**. Since the apparent pseduo-first-order rate constant for OH^- permeation into DODAC vesicles (**5**) under comparable pH 8 conditions is $\sim 4 \times 10^{-4}$ s^{-1},[12] the lack of biphasic kinetics in the pH 8 esterolysis of covesicular **7/5** is most reasonably attributed to an "unfavorable" k_{exo}/k_{perm} rate constant ratio; permeation is faster than exovesicular cleavage, both exo- and endovesicular reactions occur at once, and k(observed) is sensitive to [OH^-] (cf. runs 1 and 2). This line of reasoning also implies that the rate constants for the exo- and endovesicular cleavage reactions must be quite similar.

A more favorable k_{exo}/k_{perm} for the surface-specific cleavage of **7/5** could be achieved with the reactive thiolate nucleophiles **8** and **9**, as shown in runs 3–8 and 11–14 of Table I. In these cases, the cleavages are kinetically biphasic. Run 13 is illustrated in Figure 3; note that the initial fast reaction between **9** and exovesicular **7** is complete within ~30 s at pH 7.94, whereas ~100 min are required for completion of the subsequent, slow, endovesicular reaction.

The exovesicular rate constants (k_ψ^f) for the thiolysis of **7** range from ~0.02 to 0.06 s^{-1} with **8** and from 0.03 to 0.15 s^{-1} with **9**, depending on nucleophile concentrations. These values are equivalent to apparent second-order rate constants of ~100 M^{-1} s^{-1} (**8**) and ~600 M^{-1} s^{-1} (**9**). The endovesicular reactions also appear to respond to the initial external [nucleophile], but they are considerably slower. The apparent rate constant for the permeation of **8** into *DODAC* vesicles, under conditions analogous to those employed here, is $\sim 1 \times 10^{-3}$ s^{-1},[14] so that $k_\psi^{exo} > k_{perm}$ for **7/5** and surface-specific exovesicular cleavage can be effected.[25]

(25) The observed increase in k_ψ^s with increasing [RS^-] is attributable to increasing k_{perm} at higher exo/endo RS^- concentration gradients.

Table II. Temperature Dependence of Cleavage Reactions[a]

run	surf	additive[b]	temp, °C	$10^2 k_\psi^f$, s^{-1} [c]	$10^3 k_\psi^s$, s^{-1} [c]	% fast/ % slow[c]
1	**6**	none	25.0	2.55	1.30	64:36
2	**6**	none	28.5	3.65	2.36	75:25
3	**6**	none	30.0	4.00	5.78	75:25
4	**6**	none	36.0	8.71	*d*	*d*
5	**6**	none	36.5	10.0	*d*	*d*
6	**7(5)**	Cys-OEt	21.0	3.17	0.395	45:55
7	**7(5)**	Cys-OEt	25.0	3.21	1.19	51:49
8	**7(5)**	Cys-OEt	33.0	3.90	6.9	72:28
9	**7(5)**	Cys-OEt	34.5	4.20	8.5	82:18
10	**7(5)**	Cys-OEt	40.0	8.94	*d*	*d*
11	**7(5)**	Cys-OEt	42.5	9.26	*d*	*d*

[a] Conditions as in Table I, except for indicated temperature; pH 7.9–8.0. Concentrations of surfactants were as follows: [**6**] = 5×10^{-5} M; [**7**] = 6×10^{-5} M (with 9-fold excess covesicular **5**). [b] [Cys-OEt] = 5×10^{-4} M. [c] See Table I for definitions. [d] Only one kinetic phase was observed.

Runs 7 and 8 of Table I show that the large, slow-injected **7/5** covesicles behave similarly to the smaller sonicated variety. Runs 9 and 10 suggest that the additional charges on the cysteine (carboxylate) and its associated cation are not essential, because cysteine ethyl ester also serves to bring about biphasic cleavage.

Table II records the temperature dependence of the esterolytic kinetics of vesicular **6** and covesicular **7/5** at pH 8. As the temperature increases, both k_ψ^f and k_ψ^s increase for OH^-/**6** reactions. Between 30 and 36 °C (runs 3 and 4), kinetic resolution disappears, and only rapid, monophasic, quantitative esterolysis can be observed. The T_c of **6** is 32 °C, so that the loss of biphasic kinetics at these temperatures is most reasonably associated with enhanced vesicular fluidity above T_c, an increase in k_{perm}, and an unfavorable ratio of k_{exo}/k_{perm}. Additionally, enhanced flip-flop (see below) of endovesicular **6** at or above T_c could also contribute to its rapid cleavage.

Similar results were obtained in the reactions of covesicular **7/5** with Cys-OEt. As the temperature increased from 21 to 34.5 °C, both k_{exo} and k_{endo} increased, and the contributions of the two kinetic processes (measured by their corresponding absorbances) shifted from ~50:50 toward 100:0. Between 34.5 and 40 °C, kinetic resolution was lost and replaced by a monophasic reaction. The independently determined T_c for covesicular **7/5** (33.5 °C) is consistent with these observations, so that the suggestions offered to account for the loss of surface-specific cleavage with vesicular **6** are also likely to apply to **7/5**.

Toward an Endovesicular Chemistry. The foregoing results demonstrate that we can readily prepare chemically unsymmetrical vesicles of **6** or **7/5** by modifying their exovesicular functional groups, while leaving their endovesicular reactive ester moieties

intact. We next carried out several initial experiments with these unsymmetrical vesicles in attempts to control or utilize their surface differentiation.

We have shown that incorporation of 0.2 wt % 1-hexanol into sonicated DODAC vesicles greatly increases their fluidity, lowers their T_c to ~25 °C, enhances their permeability toward simple reagents, and destroys kinetic resolution between exovesicular and endovesicular reactions of Ellman's reagent with thiolate ions.[14] We now observe an apparently related effect with surface-differentiated **6** or **7**/**5**.

At the completion of the fast, exovesicular kinetic phase of the pH 8 esterolysis of vesicular **6** (see Figure 1), the modified vesicles carry choline ($N^+CH_2CH_2OH$) functionalities on their exovesicular surfaces (exposed to pH 8 buffer) but retain the original *p*-nitrophenyl carbonate functional groups at their endovesicular surfaces (where the pH is still ~4). If, at this point we increase the fluidity of the bilayer membranes, then we should enhance the normally permeation-limited k_ψ^s or k_{endo} for vesicular **6**. Indeed, addition at this point of 0.1 wt % of 1-hexanol brought about the cleavage of the residual endovesicular carbonates with $k_\psi = 0.021$ s^{-1}, about 16 times faster than that normally observed with **6**. Referring to Figure 1, the second reaction was completed in ~5 min when the hexanol was added, as opposed to ~60 min in the absence of hexanol.

A similar result was obtained with the surface-differentiated covesicles of **7**/**5**. For example, in run 7 of Table I, the *endovesicular p*-nitrophenyl benzoate cleavage by **8** occurred with $k_\psi^s = 0.76 \times 10^{-3}$ s^{-1}. Addition of 0.1 wt % 1-hexanol at the end of the initial fast kinetic phase of run 7 increased k_ψ for the endovesicular cleavage to 7.4×10^{-3} s^{-1}, a factor of ~10.

A different approach to modifying the properties of vesicular **6** involves cosonication of a second surfactant during the construction of the vesicle. If the cosurfactant modifies the rate of OH^- access to endovesicular surfactant, then the kinetic resolution of the endo- and exovesicular esterolysis reactions will be altered. For example, coaggregates of 5×10^{-5} M **6** and 2.5×10^{-4} M CTACl (a 16-carbon, single-chain, cationic surfactant), prepared as above at pH 4, are much more accessible than holovesicular **6**, fail to exclude exovesicular OH^- at pH 8, and cleave with $k_\psi = 2.25 \times 10^{-2}$ s^{-1} in a single, quantitative, monoexponential reaction. The observed rate constant is about the same as that for exovesicular cleavage of holovesicular **6** [$(2.5 \pm 0.5) \times 10^{-2}$ s^{-1}]. Indeed, dynamic light scattering indicates an apparent hydrodynamic diameter of 750–850 Å for the sonicated **6**/CTACl coaggregates used in these experiments. This significant size reduction from the 1200–1300-Å holovesicles of **6** suggests that the **6**/CTACl coaggregates may have a very different construction.

A somewhat more controllable but related effect was observed with covesicles of **6** and dioctyldimethylammonium chloride (**10**).

$(n\text{-}C_8H_{17})_2N^+Me_2,\ Cl^-$

10

The twin chains of the latter are too short to support a bilayer structure,[26] so that **10** should function as a "structure breaker" in **6**/**10** covesicles and therefore as a permeable "flaw". The data in Table III record experiments in which the rate constants and kinetic resolution of the "fast" exovesicular and "slow" endovesicular esterolytic cleavages of *p*-nitrophenylate from vesicular **6** are examined as a function of added covesicular **10**. The initially observed ~1:1 fast/slow kinetic phase distribution and the 44-fold $k_\psi^f > k_\psi^s$ rate constant inequality of holovesicular **6** (run 1) are steadily compromised by covesicallized **10**. In the 1:1 covesicles (run 4), k_ψ^f/k_ψ^s has dropped to 14.5, and the apparent "fast" reaction now comprises ~85% of the total esterolysis. Continued increase of **10** finally results in the loss of meaningful kinetic resolution (runs 6 and 7). Dynamic light-scattering measurements indicate the persistence of vesicle-sized aggregates, even at 1:9 **6**/**10**, although the structures may be modified.

(26) Kunitake, T.; Okahata, Y.; Tamaki, T.; Kumamaru, F.; Takayanagi, M. *Chem. Lett.* **1977**, 387; Kunitake, T.; Okahata, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1877.

Table III. Cleavage of *p*-Nitrophenylate from Covesicular **6**/**10**[a]

run	10^5[**10**], M	[**6**]/[**10**][b]	$10^2k_\psi^f$, s^{-1} [c]	$10^3k_\psi^s$, s^{-1} [d]	% fast/ % slow[e]	diam, Å[f]
1	0		3.7[g]	0.84[g]	1:1	1300
2	1.25	4:1	3.3	2.4	4:1	1550
3	2.5	2:1	3.0	1.5	3:1	1400
4	5.0	1:1	3.5	2.4	6:1	1350
5	10	1:2	2.9	1.7	6:1	*h*
6	25	1:5	2.3	*i*	*j*	1050
7	45	1:9	1.8	*i*	*j*	950

[a] Conditions: 0.01 M aqueous Tris buffer, pH 7.9–8.0, 0.01 M KCl, and 25 °C. Vesicles were prepared by cosonication of **6** and variable quantities of **10** at pH 4. [**6**] was 5×10^{-5} M in all cases. [b] Molar ratio. [c] Fast, exovesicular cleavage. [d] Slower, endovesicular cleavage. [e] Distribution of kinetic phases based upon the corresponding absorbance changes at 400 nm. [f] Apparent hydrodynamic diameter (±100 Å) of the aggregates by dynamic light scattering. [g] The rate constants observed within the kinetic series from this set of vesicular preparations were somewhat faster (k_ψ^f) and slower (k_ψ^s) then those described above; e.g., $k_\psi^f = 0.025$ s^{-1} and $k_\psi^s = 0.0013$ s^{-1}. Such deviations between different vesicle preparations are not uncommon. [h] Not determined. [i] Only one major kinetic phase (>90%) was observed. [j] <10% residual slow reaction.

Obviously, the **6**/**10** covesicles become increasingly permeable, or damaged and leaky, as their content of **10** increases. We assume that k_{perm} must correspondingly increase until it exceeds k(esterolysis), and kinetic resolution is lost.

Flip-Flop Studies. The transverse or "flip-flop" motion of a surfactant molecule from one interface of a bilayer to the other is a relatively slow process because it requires energetically costly transient interactions of the ionic surfactant head groups with the hydrocarbon bilayer interior and of the hydrocarbon chains with water.[27] McConnell devised an elegant ESR method to follow the time course of flip-flop in egg lecithin phosphatidylcholine vesicles that depended on observing the reduction of a spin-labeled dipalmitoylphosphatidylcholine molecule as it flipped from an endo- to an exovesicular location where it could react with an external ascorbate ion.[28] The half-time for the resultant decay of initially endovesicular probe molecules was 6.5 h at 30 °C.[28]

We now report a measure of flip-flop in the surface-differentiated **7**/**5** covesicles that is designed to closely follow molecules of **7** as they move from endo- to exovesicular locations. The experimental protocol was as follows. (1) Covesicles of 1:9 **7**/**5** were prepared as previously by sonication in 0.01 M aqueous KCl at pH 3.9. (2) The external pH was adjusted to 7.9, and exovesicular thiolysis was carried out with excess cysteine (see Table I, runs 3–6). (3) After completion of the fast, exovesicular benzoate cleavage,[29] the pH was reduced to 3.9 with HCl. (4) The vesicular solution was warmed to 38–40 °C (i.e., above T_c ~ 33.5 °C) for 1–12 min and then cooled back to 25 °C over ~2 min (ice bath). (5) The external pH was readjusted to 7.9 with NaOH, and *p*-nitrophenylate appearance was monitored.

The principal finding made in this series of experiments is that heating the cysteine-differentiated **7**/**5** covesicles (step 4) primes *an additional fast cleavage process* that becomes observable upon readjustment of the external pH to 7.9 (step 5). Table IV collects relevant experimental data. In run 1 (identical with run 6 of Table I), "normal" biphasic kinetic behavior is observed as cysteine rapidly cleaves the exovesicular benzoate esters of **7** (k_ψ^f), uncovering the chromophoric *p*-nitrophenylate moieties. Protocol steps 3–5 are omitted, so that the exovesicular cleavage is followed by the slower, endovesicular esterolysis that accompanies the decay of both cysteine and pH gradients across the vesicular bilayer at 25 °C. In runs 2–5, the acidification, heating, cooling, and rebasification steps 3–5 are imposed, and in each case, an *additional* k_ψ^f process appears immediately upon readjustment of the pH to 7.9.[30] Run 6 shows that a similar process occurs when glutathione,

(27) For discussions, see ref 1b, pp 110*f*, and ref 1e, pp 145*f*.
(28) Kornberg, R.; McConnell, H. M. *Biochemistry* **1971**, *10*, 1111. See also McNamee, M. G.; McConnell, H. M. *Biochemistry* **1973**, *12*, 2951.
(29) The absorbance of the product *p*-nitrophenylate-bearing surfactant molecules was continuously monitored at 400 nm.

Table IV. Flip-Flop Kinetics with Covesicular **7**/**5**[a]

run	10^5[**7**], M[b]	10^5[**8**], M[c]	heat[d]	$10^3 k_\psi^f$, s^{-1}[e]	$10^3 k_\psi^{new}$, s^{-1}[f]	$10^3 k_\psi^s$, s^{-1}[g]	phase dist, %[h]
1	4.0	60	0[i]	61.6	*i*	1.54	49:0:51
2	2.5	40	1	70.6	79.6	1.10	45:15:40
3	2.5	50	2	88.2	81.3	1.28	50:20:30
4	4.0	60	6	62.0	54.0	1.54	46:27:27
5	2.0	50	12	97.9	93.9	1.81	45:29:26
6	5.0	25[j]	6	140	120	1.16	48:27:25

[a] See text for experimental protocol. [b] The covesicles included a 9-fold excess of **5**. [c] Cysteine (**8**) was the thiolytic nucleophile. [d] Incubation time (min) of the surface-differentiated vesicles at pH 3.9 and 38–40 °C; see protocol steps 3 and 4. [e] Initial thiolytic rate constant for exovesicular benzoate cleavage, corresponds to k_ψ^f in Table I. [f] Rate constant for additional fast cleavage observed upon readjustment of the pH of the heat-treated vesicles to 7.9; see protocol step 5. [g] Rate constant of slow, residual, endovesicular benzoate cleavage, observed after the readjustment of pH; see protocol step 5. This corresponds to k_ψ^s in Table I. [h] Relative distribution of processes corresponding to $k_\psi^f/k_\psi^{new}/k_\psi^s$ as measured by ΔA at 400 nm; reproducibility ~±4%. Total observed absorbance equaled the theoretical absorbance calculated from the initial concentration of **7**. [i] For reference; this is run 6 of Table I. Steps 3–5 of the protocol were omitted; only k_ψ^f and k_ψ^s occurred. [j] Glutathione (**9**) was used as the thiolytic nucleophile; compare with run 14, Table I.

rather than cysteine, is the thiol reagent.

Moreover, the new k_ψ^f phase derives from that portion of the total cleavage that originally constituted k_ψ^s. The redistribution of k_ψ^s into a new k_ψ^f and a residual k_ψ^s depends directly on the incubation time at 40 °C/pH 3.9, with % k_ψ^f (new) increasing from 15% (1 min of incubation), to 20% (2 min), to ~28% at 6 or 12 min of warming. There are corresponding decreases of k_ψ^s. After ~6 min of warming, the extents of the k_ψ^f and residual k_ψ^s kinetic phases become comparable. The rate constants observed for these processes identify k_ψ^f (new) with the original k_ψ^f (i.e., exovesicular thiolysis) and k_ψ^s with the normally observed endovesicular reaction.

An example of these "triphasic" kinetics experiments appears in Figure 4. This experiment is similar in [**7**] and in heating time (6 min) to run 4 of Table IV, except that the concentration of cysteine has been increased to 1×10^{-3} M, so that k_ψ^f (0.12 s^{-1}), k_ψ^f (new) (0.11 s^{-1}), and k_ψ^s (5.6×10^{-3} s^{-1}) are all greater than those in run 4. When the heating step is omitted,[30] the additional k_ψ^f is not observed; only the normal biphasic behavior is seen (dotted line in Figure 4). Indeed, the surface-differentiated **7**/**5** covesicles can be incubated at pH 3.9 and 25 °C for 2 h (i.e., ca. four half-lives of the k_ψ^s process under normal conditions) *without displaying any new fast process upon rebasification.* This means that flip-flop of endovesicular surfactant molecules *is slow at 25 °C.*

The most reasonable interpretation of these results involves flip-flop of **7** as the key, *heat-induced* step. Thus, the initial k_ψ^f represents the usual exovesicular thiolysis of benzoate ester groups by cysteine at pH 8, and it is complete in ~48 s under the conditions of Figure 4. At this point, *p*-nitrophenyl benzoate (PNPB) moieties remain only on endovesicular molecules of **7**, where they confront the endovesicular aqueous pool at pH ~4. Exovesicular **7** has been modified and bears chromophoric *p*-nitrophenylate groups at pH 8. Acidification of the external water pool to pH 3.9, followed by heating to 38–40 °C (above T_c), now enhances surfactant flip-flop and brings endovesicular PNPB groups into exovesicular sites. Of course, exo → endovesicular flips also occur. (The latter flips maintain *protonation* at pH 3.9 of the newly endovesicular *p*-nitrophenylate groups of the modified **7**, so that absorbance at 400 nm is initially *reduced* after the heating step; cf. point A in Figure 4.)

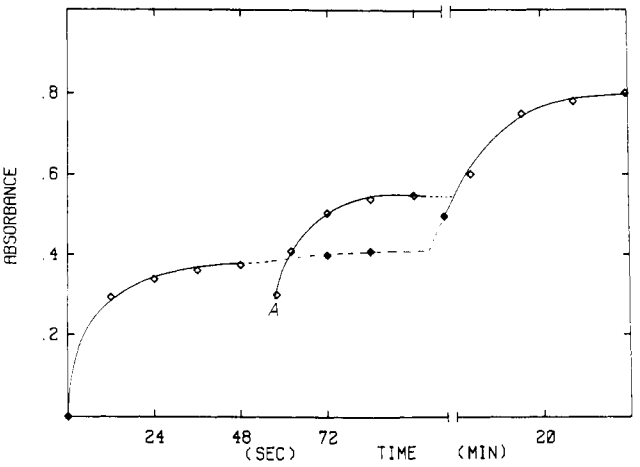


Figure 4. Absorbance at 400 nm vs time for the cleavage of benzoate and the appearance of *p*-nitrophenylate from sonicated covesicular **7**/**5** under the flip-flop protocol described in the text; see Table IV. The dotted line refers to a control experiment in which the heating step was omitted.[30] The reduced absorbance at point A is discussed in the text.

The extent of exo → endo and endo → exo surfactant flip-flop will depend on the incubation time at 40 °C, and if this is long enough (≥6 min), an equal redistribution of the originally endovesicular PNPB functional groups will occur. Readjustment of the external water pool to pH 8 then brings about rapid cleavage (k_ψ^f, new = k_ψ^f) of the restored exovesicular PNPB groups and is later followed by k_ψ^f cleavage of the residual endovesicular PNPB groups, as well as (permeation-limited) deprotonation of *p*-nitrophenol moieties that had flipped to endovesicular sites.

Note that when the **7**/**5** covesicles are heated to 40 °C at pH 3.9, cysteine undoubtedly crosses into the vesicles. However, at this pH, it is protonated and not thiolytic. After the external pH of the cooled vesicle solution is readjusted to 8, external cysteine is deprotonated at SH and begins to cleave the new exovesicular PNPB groups. But the restored exo pH 8/endo pH 4 gradient must at least partially decay before endovesicular cysteine can cleave residual endovesicular PNPB groups. The complicated, permeation-limited endovesicular processes have an apparent $k \sim 10^{-3}$ s^{-1}. From the time dependence displayed by the kinetic data in Table IV, we estimate that the half-time for decay of the surface-differentiated **7**/**5** covesicles is ~2 min at 40 °C. Note that our analysis assumes throughout that the **7**/**5** covesicles are unilamellar. All *observed* kinetic behavior is consistent with this idea. If the covesicles do contain several lamellae, no distinct kinetic phenomena arising at the "inner" lamellae are resolved by our experiments.

Discussion

The central result of this study is that the exovesicular and endovesicular surfaces of head group functionalized dioctadecylmethylammonium ion surfactant vesicles can readily be surface differentiated by hydrolytic or thiolytic esterolysis reactions. The key to chemical differentiation of this type is selection of the surfactant structure, the functional head group, and the reagent such that the rate of the exovesicular reaction is >10 times faster than that of reagent permeation to endovesicular sites. In the case of vesicular **6** (Figure 1), $k_\psi^{exo} \sim 2.6 \times 10^{-2}$ s^{-1} at pH 7.9, whereas the apparent k_{perm} (which controls k_{endo}) is $\sim 1.3 \times 10^{-3}$ s^{-1}, at an exo/endo pH gradient of 8 vs 4.[31]

Studies with dihexadecylmethylammonium surfactants **3** and **4** failed to exhibit kinetic resolution of exo- and endovesicular cleavage reactions, most probably because the vesicles were quite

(30) A control experiment, in which the heating step (4) was omitted, showed that heating, not the acidification and rebasification operations (or incubation at pH 3.9 and 25 °C), was necessary to induce the additional fast kinetic process. Only the normal 50% of k_ψ^s was observed upon reestablishment of pH 7.9 when the heating step was omitted. It is also known from the method of vesicle preparation (at 55 °C, pH 3.9) that heating covesicular **7**/**5** at *acidic* pH leads to minimal benzoate cleavage.

(31) We believe that the decay of the pH gradient across *cationic* bilayers is best represented as exo → endo permeation of OH^-, followed by endo → exo permeation of Cl^- to maintain charge neutrality. Alternative scenarios featuring endo → exo out-migration of H^+, followed by exo → endo movement of K^+ to maintain charge balance, would require two cations to cross the catonic bilayer. On this point, see Okahata, Y.; Nakamura, G.-i.; Hachiya, S.; Noguchi, H.; Lim, H.-j. *Chem. Commun.* **1983**, 1206.

leaky and reagent permeation was faster than exovesicular cleavage; e.g., for **3**, $k_{perm} \geq 0.06\ s^{-1}$ (k_ψ, observed) at an initial pH gradient of 8 vs 4 at 25 °C.[10,11] This problem was bypassed with surfactant **2**, where the glyceryl diester backbone provided better packing for the vesicle bilayers, so that the same *p*-nitrophenyl carbonate hydrolysis reaction, which failed with **3**, here permitted surface-specific exovesicular cleavage; the permeation of OH^- ($k_{perm} \sim 5 \times 10^{-5}\ s^{-1}$) was much slower than the exovesicular cleavage ($k_\psi^{exo} = 3 \times 10^{-2}\ s^{-1}$ at pH 8), at the usual exo/endo pH gradient.

The present results show that surfactants with the dioctadecylmethylammonium architecture (**5**,[12] **6**, or **7**) can pack sufficiently well in either sonicated or slow-injected vesicular bilayers to slow OH^- permeation enough to kinetically resolve exovesicular and endovesicular reactions. These effects are not simple functions of vesicular T_c and the reaction temperature. Thus, dihexadecylmethylammonium ion vesicles of **3** ($T_c \sim 25$–27 °C) are still too permeable to OH^- at 15 °C to sustain surface-specific exovesicular esterolysis.[10] In contrast, dioctadecylmethylammonium ion vesicles of **6** ($T_c = 32$ °C) are readily surface differentiated by exovesicular hydrolysis at 25 °C, although this kinetic resolution disappears at temperatures above T_c (Table II). Still more resistant are the glyceryl diester vesicles of identically functionalized surfactant **2** ($T_c \sim 34$ °C), where the surface-specific exovesicular esterolysis observed at 25 °C *persists* at 40 °C.[10] There are thus intrinsic differences with vesicular **3**, **6**, and **2** that are specific to their vesicular architectures. It may be that some of the differences we observe between these vesicular systems are related to the relative ease of surfactant flip-flop at certain temperatures, rather than to reagent permeation, although (as we have seen above) flip-flop in surface-differentiated covesicles of **7**/**5** is very slow and apparently not competitive with the permeation processes at 25 °C (below T_c). The details of these processes are still largely unknown but are clearly of central importance to the rational design of functional, surface-differentiated vesicles.

Hydrolytic cleavage of the PNPB moieties of dioctadecyl covesicular **7**/**5** is too slow at pH 8 ($k_\psi \sim 2 \times 10^{-3}\ s^{-1}$, Table I, run 1, and Figure 2) vs k_{perm} against the usual pH gradient to permit kinetic resolution of exo- and endovesicular hydrolytic reactions. However, use of the more reactive cysteine or glutathione thiolytic reagents enhances k_ψ^{exo} by at least 2 orders of magnitude, while leaving the apparent k_{perm} at 10^{-3}–$10^{-4}\ s^{-1}$, so that surface-specific exovesicular thiolysis can be readily achieved (Table I and Figure 3).

The surface-differentiated **7**/**5** covesicles are useful in chemically monitored studies of surfactant flip-flop (Table IV and Figure 4), whereby the dynamics of endovesicular/exovesicular surfactant exchanges can be visualized. From the observed half-time for decay of the surface-differentiated **7**/**5** covesicles (~2 min at 40 °C), we imagine that flip-flop in liquid-crystalline (i.e., $T > T_c$) covesicles containing 90% of dioctadecyldimethylammonium chloride (**5**) is faster than the comparable process in glyceryl diester vesicles of egg lecithin or dipalmitoylphosphatidylcholine ($T_c \sim -5$ °C),[32] where the half-time for flip-flop and the decay of spin-labeled probe surfactants is ~6.5 h at 30 °C.[28] Allowing for head-group differences, it is tempting to conclude that packing, even above T_c, is better in the glyceryl diester vesicles than in the dioctadecylmethylammonium vesicles. A similar conclusion follows from our observation of persistent surface-specific hydrolysis *above* T_c with glycerol diester vesicles of **2**[10] but not with dioctadecylmethylammonium vesicles of **6** (this work).

In the future, we will be able to use several effects to modulate or fine tune the relative rates of exovesicular reactions, reagent permeation, and surfactant flip-flop. Important variables will include surfactant architecture, head-group functionality, covesiculation of "softening" components or "higher tech" pore compounds, and more sophisticated design of surface-specific reagents, perhaps incorporating elements of molecular recognition.

(32) Huang, C.; Mason, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 308.

Experimental Section

Materials. *p*-Nitrophenyl chloroformate (Aldrich) was recrystallized from CCl_4 and then sublimed. (Methylamino)ethanol (99%, Aldrich) and 3-methyl-4-nitrophenol (98%, Aldrich) were used as received. Also obtained from Aldrich and used as received were *n*-octadecyl chloride and *n*-octadecyl bromide. Capillary GC (SE-30, 230 °C) indicated purities of >98% for the chloride and >97% for the bromide. Each contained several unidentified ~2–3% impurities of similar retention times, possibly higher or lower homologues. Dioctyldimethylammonium chloride (**10**) was a gift from Dr. D. P. Cox (Lonza, Inc.) and was 98% di-C_8; 2% mixed *N*-octyl-*N*-decyl-*N*,*N*-dimethylammonium chloride was also present.

***N*,*N*-Dioctadecyl-*N*-methylcholine *p*-Nitrophenyl Carbonate Chloride (6).** To 10 g (0.035 mol) of *n*-octadecyl chloride was added 26.2 g (0.35 mol) of *N*-methylethanolamine and 50 mL of acetone. The mixture was refluxed in a 100-mL round-bottom flask for 18-20 h. After cooling, the upper, oily layer of the product mixture was stripped of acetone on the rotary evaporator, washed (2 × 50 mL) with aqueous $NaHCO_3$ solution, and extracted with 2 × 50 mL of ether. The ethereal extract was backwashed with 20 mL of 10% aqueous NaCl and then dried over anhydrous K_2CO_3. The drying agent was filtered, and ether was stripped to give a viscous oil that was crystallized from ether at 0 °C to afford 9.5 g (0.029 mol, 83%) of *N*-octadecyl-*N*-methyl-β-hydroxyethylamine, mp 31–32 °C. NMR[33] ($CDCl_3$): 0.85 (crude t, 3 H, CH_2CH_3), [s, 30 H, $(CH_2)_{15}$], 1.45 (m, J = 6 Hz, 2 H, NCH_2CH_2R), 2.21 (s, 3 H, NCH_3), 2.35 (t, J = 6 Hz, 2 H, $NCH_2C_{17}H_{35}$), 2.50 (t, J = 6 Hz, NCH_2CH_2OH), 3.55 (t, J = 6 Hz, 2 H, NCH_2CH_2OH). Anal. Calcd for $C_{21}H_{45}NO$: C, 77.0; H, 13.9; N, 4.28. Found: C, 77.1; H, 14.0; N, 4.25.

The tertiary amine (1.2 g, 3.67 mmol) and 1.5 g (4.5 mmol) of *n*-octadecyl bromide were stirred and refluxed for 48 h in 50 mL of dry ethanol containing 50 mg of Na_2CO_3. Cooling, filtration, and removal of most of the ethanol on a rotary evaporator afforded a milky white solid that was crystallized from 100 mL of anhdyrous ether. The solid product was recrystallized 5 times from minimal quantities of dry acetone to give 2.0 g (3.0 mmol, 82%) of *N*,*N*-dioctadecyl-*N*-methyl-*N*-(β-hydroxyethyl)ammonium bromide, mp 73–75 °C (softens) and 138–140 °C (melts). Without further purification, the bromide was ion exchanged to the chloride. Thus, 1.0 g (1.5 mmol) of the bromide was dissolved in 100 mL of 40:60 (v/v) aqueous ethanol and stirred and refluxed with 40 g of prewashed and dried Dowex 1-X8 (chloride form) for 12 h. The resin was filtered, and the solvents were stripped. The residue was lyophilized to dryness to give 0.80 g (1.3 mmol, 87%) of the quaternary chloride, mp 78–79 °C (softens) and 143 °C (melts); TLC (silica gel, 4:1 $CHCl_3$/MeOH) R_f 0.56, 1 spot. NMR ($CDCl_3$): 0.85 (crude t, 6 H, 2 × CH_2CH_3), 1.25 [br s, 60 H, 2 × $(CH_2)_{15}$], 1.7 (br s, 4 H, 2 × NCH_2CH_2R), 3.3 (s, 3 H, NCH_3), 3.4 (m, 4 H, 2 × $NCH_2C_{17}H_{35}$), 3.65 (m, 2 H, NCH_2CH_2OH), 4.15 (m, 2 H, NCH_2CH_2OH). [Resolution was poor, probably due to aggregation of the surfactant and inappropriate relaxation times.] Anal. Calcd for $C_{39}H_{82}ClNO{\cdot}0.5H_2O$: C, 74.9; H, 13.2; N, 2.24. Found: C, 75.0; H, 13.0; N, 2.22.

The quaternary chloride (500 mg, 0.80 mmol) and 700 mg (3.4 mmol) of *p*-nitrophenyl chloroformate were dissolved in 50 mL of dry $CHCl_3$; 3.4 mmol of dry pyridine was added, and the reaction mixture was stirred at 25 °C for 48 h (protected from moisture). Solvent was stripped, and the gummy residue was further dried under high vacuum (2 h) to remove traces of pyridine. Trituration and washing with warm ether gave solid **6**, which was purified by three recrystallizations from acetone. We obtained 330 mg (0.42 mmol, 52%) of the title quaternary salt *p*-nitrophenyl carbonate **6**, as a white solid, mp 100–101 °C (softens) and 127 °C (decomp to yellow liquid). NMR ($CDCl_3$): 0.85 (crude t, 6 H, 2 × CH_2CH_3), 1.25 [br s, 60 H, 2 × $(CH_2)_{15}$], 1.75 (m, 4 H, 2 × NCH_2CH_2R), 3.45 (s + m, 7 H, NCH_3 + 2 × $NCH_2C_{17}H_{35}$), 4.35 (m, 2 H, NCH_2CH_2O), 4.9 (m, 2 H, NCH_2CH_2O), 7.42, 7.48, 8.22, 8.28 (AA′BB′, aromatic). Anal. Calcd for $C_{46}H_{87}N_2O_5Cl{\cdot}0.5H_2O$: C, 69.7; H, 11.1; N, 3.54. Found: C, 69.5; H, 10.8; N, 3.39.

***N*,*N*-Dioctadecyl-*N*-methyl-*N*-[4-nitro-1-(benzoyloxy)-3-benzyl]ammonium Bromide (7).** In 150 mL of anhydrous ether at 10 °C were dissolved 7.65 g (50 mmol) of 3-methyl-4-nitrophenol and 5.0 g (49 mmol) of Et_3N. Freshly distilled benzoyl chloride (7.0 g, 50 mmol) was added with stirring over 15 min. The reaction mixture was then stirred for 4 h at 25 °C. The precipitate of $Et_3NH^+Cl^-$ was filtered, and the filtrate was stripped to give a white solid that was recrystallized from 100 mL of ether (freezer, 12 h). The resulting white crystalline product was filtered, washed with 3 × 10 mL of cold EtOAc, and dried in air to give

(33) All proton NMR spectra were recorded at 200 MHz on a Varian VXR-200 instrument. All chemical shifts are reported in δ units, relative to TMS at δ 0.0. However, the actual lock signal was $CHCl_3$.

10.6 g (41 mmol), 82%) of 3-methyl-4-nitrophenyl benzoate, mp 72.5–73.5 °C (lit.[34] mp 74 °C). NMR ($CDCl_3$): 2.65 (s, 3 H, CH_3), 7.2, 7.5, 7.65, 8.2 (4 m, 8 H, aromatic).

The benzoate (6.4 g, 24.9 mmol) and 4.6 g (25.8 mmol) of freshly purified *N*-bromosuccinimide (mp 182–183 °C) were added to 40 mL of CCl_4 (distilled from P_2O_5). About 100 mg of benzoyl peroxide was added as an initiator, and the mixture was stirred, refluxed, and irradiated with a Ge 250-W infrared heating lamp for 10–12 h. At this point, the hot mixture was filtered free of succinimide. The latter was leached with 2 × 20 mL of hot CCl_4. Combined CCl_4 (filtrate and leachings) was stripped to afford a solid product that was recrystallized three times from anhydrous acetonitrile to give 4.5 g (13.4 mmol, 54%) of 4-nitro-3-(bromomethyl)phenyl benzoate as colorless crystals, mp 114–115 °C. NMR ($CDCl_3$): 4.85 (s, 2 H, CH_2Br), 7.4, 7.55, 7.7, 8.2 (4 m, 8 H, aromatic). Anal. Calcd for $C_{14}H_{10}BrNO_4$: C, 50.0; H, 3.00; Br, 23.8. Found: C, 49.7; H, 2.97; Br, 23.6.

The bromomethyl ester was used to quaternize *N,N*-dioctadecyl-*N*-methylamine.[35,36] The tertiary amine (1.08 g, 2.0 mmol) and 740 mg (2.2 mol) of the bromomethyl ester were dissolved in 25 mL of purified $CHCl_3$ and then stirred in the dark at 25 °C for 48 h. Solvent was removed on the rotary evaporator, and the gummy residue was dried under high vacuum to remove traces of $CHCl_3$. The solid was recrystallized from 15 mL of anhydrous ether. The product was subjected to final purification by preparative TLC on predried (60 °C) 2000-μm Analtech silica gel plates, with 30:1 $CHCl_3$/MeOH as eluent. Fractions with R_f ~0.45–0.5 were collected and recrystallized several times from dry EtOAc. We obtained 1.1 g (1.3 mmol, 65%) of title compound **7**, mp 56–80 °C (softens) and 85 °C (melts). NMR ($CDCl_3$): 0.90 (crude t, 6 H, 2 × CH_2CH_3), 1.3 [br s, 60 H, 2 × $(CH_2)_{15}$], 1.7 (m, 4 H, 2 × NCH_2CH_2R), 3.25–3.7 (s + m, 7 H, NCH_3 + 2 × NCH_2R), 5.8 (s, 2 H, NCH_2Ar), 7.4–7.7 (m, 5 H, C_6H_5), 8.2 (m, 3 H, $C_6H_3NO_2$). Anal. Calcd for $C_{51}H_{87}BrN_2O_4$: C, 70.2; H, 10.1; N, 3.21. Found: C, 70.1; H, 10.2; N, 3.35.

Generation of Vesicles. (A) Sonicated Vesicles. Typically, 13.0 mg of DODAC and 2.2 mg of surfactant **7** were combined with 0.1 mL of spectroscopic grade $CHCl_3$ in a 50-mL beaker. Solvent was removed under a N_2 flow, followed by evacuation at high vacuum for 2 h. The resulting surfactant film in the beaker was then covered with 25 mL of pH 3.9 aqueous HCl that was 0.1 M in KCl. The liquid was placed in a water bath at ~25 °C and sonicated with the 108 mm × 19 mm (diameter) immersion probe of the Braunsonic Model 2000 sonicator at 55–60 W for 7–8 min. During sonication, the temperature of the solution rose to 55–60 °C. The solution was then cooled to 15–20 °C (ice–water bath) and filtered through a 0.8-μm Millex (millipore) filter. Sonications of **6** were carried out on 2-mg (0.1 mmol) samples, in a similar fashion, with 6–7 min of sonic irradiation (final temperature 48 °C). The immersion probe tip was lowered 0.5 in. into the solution during sonication. Sonicated solutions were allowed to cool naturally to 25 °C.

(B) Injected Vesicles. A similar **5**/**7** mixture (see above) was dissolved in 1 mL of pure $CHCl_3$ and slowly injected via a Sage Instruments Model 341A syringe pump into 25 mL of 0.01 M aqueous KCl solution at pH 3.9. The receiving solution was kept at 65 °C during injection and continually sparged with a gentle stream of nitrogen. The flow rate of the injection was adjusted so as to deliver the 1 mL of solution over 90 min. After injection, the solution was cooled naturally to 25 °C and then filtered as above. Physical characterizations of sonicated and injected vesicles appear in the text.

T_c Measurement. To 13.0 mg of DODAC and 2.2 mg of **7** was added 100 μL of a 4×10^{-3} M THF solution of 1,6-diphenyl-1,3,5-hexatriene (Fluka) and 0.5 mL of $CHCl_3$. This solution was then used to prepare sonicated **7**/**5** covesicles exactly as described above. Final concentrations were [**5**] = 9×10^{-4} M, [**7**] = 1×10^{-4} M, and [hexatriene] = 4×10^{-5} M. Fluorescence polarization measurements were carried out on 2–3-mL samples as described in ref 17, with a Perkin-Elmer Model MPF-3L fluorescence spectrometer and Polacoat 4B polarizers for both excitation and emission beams. Temperature (±1 °C) was regulated by a thermostated circulating water bath. Results of these measurements appear above. Similar procedures were used to determine the T_c of vesicular **6**.

Light Scattering. Light-scattering data were collected at 25 °C and a 90° scattering angle with a Nicomp Model TC-100 computing autocorrelator, an argon laser light source (488 nm), and a Hazeltine microcomputer that used the cumulant program. The channel width was adjusted to produce a decay of 1.5–2.0 s. Vesicular solutions of **6** or **7**/**5** were studied at total surfactant concentrations of 1×10^{-4} or 5×10^{-4} M, respectively. Variance in the observed hydrodynamic diameters ranged from 0.4 to 0.8, where a variance of <1.0 is indicative of a unidisperse system.

Kinetic Studies. Reactions were normally initiated by mixing vesicle solutions at pH 3.9 with aqueous Tris buffer at pH 8. Reaction rates were followed at 400 nm on a Gilford Model 250 spectrophotometer and a Gilford Model 6051 recorder. Reactions were normally done at 25 °C, with temperature controlled to ±0.2 °C by a circulating, thermostated water bath. Other reaction temperatures are indicated in the tables.

Buffer solutions were prepared from "steam-distilled" water (distilled, USP, Electrified Water Co., East Orange, NJ). We used 0.01 M, pH 8 (or 9) Tris buffers, adjusted to 0.01 M in KCl. Buffer solutions were purged with N_2 prior to preparation of thiolate solutions. pH measurements on buffers and reaction solutions employed a Radiometer PHM 25 pH meter.

Pseudo-first-order rate constants were obtained from absorbance vs time data by computer analysis. Kinetic runs and conditions are summarized in Tables I–IV.

Acknowledgment. We are grateful to the U.S. Army Research Office and to the Charles and Johanna Busch Memorial Fund at Rutgers University for financial support. We thank Professor Paolo Scrimin (Universita di Padova) for a helpful discussion.

(34) Gibson, G. P. *J. Chem. Soc.* **1923**, *123*, 1269.

(35) The tertiary amine was prepared from 24 mmol of methylamine (Matheson), 45 mmol of *n*-octadecyl chloride, 52.5 mmol of NaOH, 80 mg of KI (catalyst), and 3.6 mL of H_2O in a sealed tube heated at 120 °C for 6 days. After purification by recrystallizations from EtOAc and acetone, we obtained about 80% of dioctadecylmethylamine, mp 40–40.5 °C (lit.[36] mp 40 °C).

(36) Staudinger, H.; Rössler, K. *Chem. Ber.* **1936**, *69*, 49; cf. pp 59–60.