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Surface-Specific Phosphate Cleavage of a Substrate-Functionalized Vesicular Surfactant

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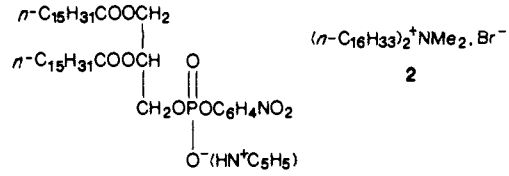
One goal of vesicular chemistry is the control of reaction locus, for example, by the development of reactions specific to exo- or endovesicular surfaces.[1] Not only are such reactions intrinsically interesting, but they differentiate these surfaces, creating further opportunities for locus-specific chemistry. Examples involving reactions of functional surfactant vesicles include diazo coupling,[2] imine formation,[3] viologen reduction,[4] stilbene bromination,[5] and fluorescamine labeling.[6]

The hydrolytic or nucleophilic cleavage of esters or phosphates, an extensively studied reaction in aggregate chemistry,[1d,7] has not been successfully surface-restricted,[8] although two kinetically distinct, competitive cleavages were observed in the aminolysis of *p*-nitrophenyl laurate in polymeric vesicles[9] and in the hydrolysis of clustered or dispersed ester-functionalized, azobenzene surfactants in dialkyldimethylammonium ion vesicles.[10] Here we report the first example of a surface-specific vesicular hydrolytic reaction, one that is dependant on the maintenance of a substantial pH gradient[11] by the vesicle.

1,2-Dipalmitoyl-3-glyceryl *p*-nitrophenyl phosphate (**1**, DPGPNPP) was prepared by reaction of *p*-nitrophenyl phosphorodichloridate (Aldrich) with 1,2-dipalmitoyl-*rac*-glycerol (Sigma) in ether/pyridine (4 h, 25 °C), followed by hydrolysis (H_2O, pyridine, 1 h, 25 °C). The product was isolated as its pyridinium salt in 80% yield, mp 68–70 °C, after several recrystallizations from EtOAc, and was characterized by NMR spectroscopy and elemental analysis. Treatment of a sample with ethanolic KOH released the theoretical quantity of *p*-nitrophenoxide ion ($PNPO^-$).



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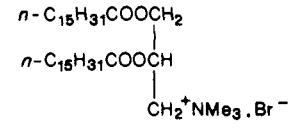
1 (DPGPNPP)



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Vesicles of **1** were created at pH 5.5 by sonication (immersion probe, 80 W, 55–60 °C, 15 min), cooling to 25 °C, and filtration through a 0.8 μM Millipore filter. Dynamic light scattering[12] revealed vesicles of 1500-Å diameter at pH 11.8 (under reaction conditions, see below.) Differential scanning calorimetry[12,13] detected a phase transition (T_c) at 29.5 °C, with ΔH = 14.8 kcal/mol.

Vesicular **1** is *anionic* and resistant to phosphorolytic cleavage at pH 8. At pH 11.8, however, it releases 48–50% of the theoretical $PNPO^-$ in a pseudo-first-order process characterized by $k_\psi = 4.68 \times 10^{-4}$ s^{-1} (Table I, run 1),[14] appropriate to vesicular cleavage of an anionic phosphate.[15] A more significant observation is the *partial* nature of $PNPO^-$ cleavage, even after 24 h. If, however, the vesicles are "damaged" by the addition of excess cetyltrimethylammonium chloride (CTACl), the residual $PNPO^-$ is released within 6 min (Figure 1, curve 1).[16a]

Partial $PNPO^-$ cleavage of DPGPNPP vesicles persists at 35 °C, above T_c (run 2), and is unaffected by added K^+ or hydrophobic Bu_4N^+ cations (runs 3 and 4). However, incorporation of cationic CTACl during vesicle formation at pH 5.5 profoundly affects subsequent behavior at pH 11.8 (runs 5–11). When [DPGPNPP] > [CTACl] (run 5), k_ψ^f is enhanced, but still only ~55% of the $PNPO^-$ is cleaved. When [CTACl] slightly exceeds [DPGPNPP] (run 6; Figure 1, curve 2), the reaction becomes kinetically biphasic, with a slow, pseudo-first-order process, k_ψ^s accounting for the residual ~45% of $PNPO^-$ over ~22 h. When [CTACl] exceeds [DPGPNPP] by 1.6 times or more (runs 7–11; Figure 1, curve 3), all the $PNPO^-$ cleaves in *one* exponential process and k_ψ^f increases with increasing [CTACl].[16b]

The results point to moderately fast exovesicular $PNPO^-$ cleavage from vesicular **1**, whereas native vesicles are not attacked at endovesicular sites. However, such cleavage does occur in the presence of cationic CTACl, which also charge neutralizes anionic **1**, enhancing k_ψ due to OH^- attack. CTACl could either convert the vesicles to micelles, thus exposing all the *p*-nitrophenyl phosphate head groups, or it could "insert" into the vesicle providing regions permeable to OH^-, facilitating endovesicular attack.

We favor the latter explanation because (1) dynamic light scattering shows that 2000-Å species persist under conditions corresponding to run 9, Table I; (2) quantitative, single-exponential $PNPO^-$ release occurs from *covesicles* of **2** and DPGPNPP, where surfactant **2** is structurally related to CTACl (run 12); (3) the fluorescence at 530 nm of entrapped riboflavin[11a] in vesicular DPGPNPP is maintained for at least 5 h at pH 11.8 but is quenched by OH^- immediately upon the addition of excess CTACl; and (4) vesicles of **2** and analogues are readily permeable to hydroxide[8] and other anions.[12,17]

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(16) (a) A typical rate constant for CTACl-induced cleavage of residual $PNPO^-$ was 55×10^{-4} s^{-1} at [CTACl] = 3.6×10^{-4} M. (b) The similarity of the preceding rate constant[16a] and that of run 8, Table I, suggests that OH^- transit of CTACl-damaged vesicles is not rate-limiting and that $PNPO^-$ cleavage occurs at comparable rates at exo- or endovesicular sites; see below.

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Table I. Cleavage of *p*-Nitrophenoxide from DPGPNPP Vesicles at 25 °C[a]

run	additive	concn, 10^4 M	$10^4 k_\psi^{f,b}$ s^{-1}	$k_\psi^{s,c}$ s^{-1}
1	none		4.68	$\ll 1 \times 10^{-5}$
2[d]	none		7.08	$\ll 1 \times 10^{-5}$
3	KCl	50	6.52	$\ll 1 \times 10^{-5}$
4	n-$Bu_4N^+Br^-$	8	5.92	$\ll 1 \times 10^{-5}$
5	CTACl	1.25	8.15	$\ll 1 \times 10^{-5}$
6	CTACl	1.80	20.0	4.3×10^{-5}
7	CTACl	2.40	23.1	*e*
8	CTACl	3.60	24.4	*e*
9	CTACl	4.56	27.6	*e*
10	CTACl	5.55	32.1	*e*
11	CTACl	6.48	100	*e*
12	**2**	6.00	130	*e*
13	**3**	2.25	70[f]	$\ll 1 \times 10^{-5}$

[a] Conditions: [DPGPNPP] = 1.35×10^{-4} M in all cases, additives were cosonicated with DPGPNPP at pH 5.5. Reaction pH 11.8 ± 0.1 after addition of 0.01 M NaOH. [b] Initial "fast" pseudo-first-order cleavage of *p*-nitrophenolate ion (400 nm), ~50% of theory. [c] Subsequent "slow" cleavage; see text. [d] At 35 °C. [e] No slow reaction, the fast reaction accounted for quantitative *p*-nitrophenoxide release in a single exponential process. [f] ~60% of *p*-nitrophenoxide release was observed.

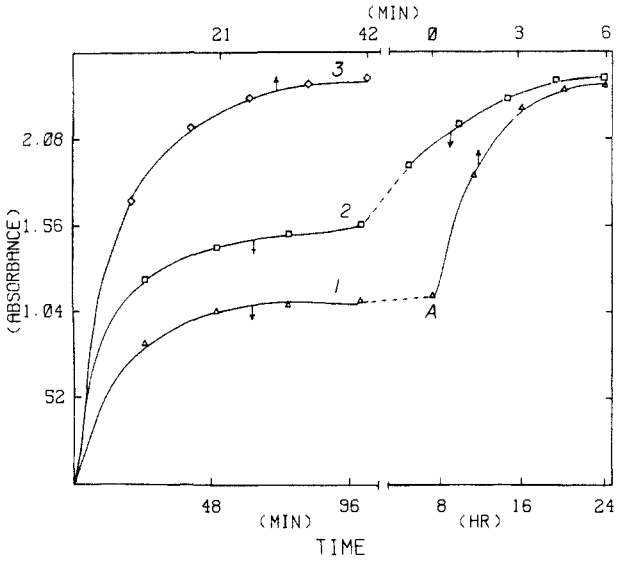

Figure 1. Absorption at 400 nm of *p*-nitrophenoxide cleaved from 1.35×10^{-4} M vesicular **1** at pH 11.8 vs. time; see text and Table I. Arrows refer to appropriate time scales. Curve 1, native vesicles; 4×10^{-3} M CTACl added at A. Curve 2, 1.8×10^{-4} M CTACl included in vesicle; note discontinuous time scale. Curve 3, 2.4×10^{-4} M CTACl included in vesicle.

In vesicles of **1** doped with CTACl or **2**, enhanced hydroxide permeation does not solely depend on the cationic head groups provided by the "foreign" surfactant. Rather, disruption of the glyceryl ester surfactant packing makes the vesicles porous:[17] note that covesicles of anionic **1** and cationic (but glyceryl ester based) **3**[18] do *not* under endovesicular $PNPO^-$ cleavage (run 13). Moreover, fluorescence from riboflavin entrapped in vesicular **3** is not quenched by OH^- at pH 11.8 after 5 h.

The present work provides clear evidence for surface-specific reactions of vesicular **1**, demonstrates how the vesicles can be chemically differentiated at exo- and endovesicular sites, and suggests that glyceryl ester functional surfactants should be suitable for surface-specific vesicular chemistry.[19,20]

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(18) The synthesis of surfactant **3** will be described elsewhere. It was prepared in this laboratory by Dr. P. Scrimin and S. Bhattacharya.
(19) Maintenance of chemical diferentiation is dependent on a slow rate of exchange ("flip-flop") of surfactant monomers from endo- to exovesicular surfaces and vice versa; cf. ref 11b, pp 110-114.

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(20) A referee points out that vesicles of **1** are particularly well suited to the chemical differentiation described here because (a) the initial cleavage of $PNPO^-$ from the monoanionic disubstituted phosphate leaves behind a less reactive, dianionic monoalkylphosphate ester; (b) $PNPO^-$ is a better leaving group than a primary aliphatic alkoxide, so that the cleavage occurs at the desired P–O bond; and (c) the residual phosphatidic acid anion surfactants form stable vesicles.

Nature of the 190-nm Transition in Carbonyls: CD Measurements of Camphor Using Synchrotron Radiation

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The CD of the 190-nm transition of ketones was recently the subject of an extensive study.[1-3] The measurements extended the wavelength region to 185 nm and solvents such as hexane, acetonitrile, and trifluoroethanol were used. The second transition in simple ketones was assigned as the n → 3s Rydberg.[4] This assignment was based on the behavior of this transition under increasing pressure of a perturbing gas[5] or in solution[6] where vibronic structure is lost and substantial blue shifts are observed. Fluorine-containing solvents are especially known as strong shifters.[7]

The problem of whether a separate n valence transition exists or is being mixed with the Rydberg state was addressed in Kirk's study.[2] However, a decisive answer was not found. A second excited transition was identified in Kirk's investigation when 2,2,2-trifluoroethanol (TFE) was used.[2] This solvent causes a large blue shift to the n → 3s and exposes a second transition which was red-shifted in the latter solvent.

In this study we have measured the VUVCD of camphor in solution and in the gas phase. Two factors motivated this research. The first was Kirk's remark that it was unfortunately not possible to examine the region of the spectrum below 183 nm with available instrumentation, so the presence of a blue-shifted component of the CD could not be directly confirmed.[2] The second is that for a comprehensive characterization of excited states it is essential to compare gas and solution spectra and the lack of gas-phase data might lead to a wrong assignment. Camphor was chosen as our target molecule because of its rigid skeleton. Synchrotron radiation has been previously[8,9] employed in VUVCD studies and its ad-

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