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THE REACTIVITY OF SUBSTRATE FUNCTIONALIZED SURFACTANT VESICLES

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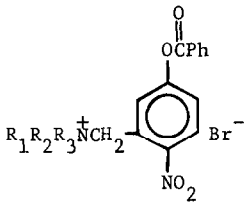
Summary. The hydrolysis and thiolysis of active ester-functionalized cationic surfactant vesicles proceed without kinetic resolution of distinct exovesicular and endovesicular reactions.

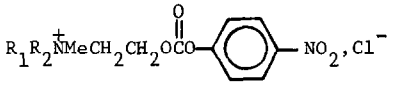
The topography of vesicles derived from even non-functional synthetic surfactants is surprisingly complex.[1,2] Fuhrhop delineated seven distinct regions (in addition to bulk and encapsulated water) that comprise a cross-sectional vesicular slice.[2] Do the local environments of these regions vary sufficiently to foster distinguishable, locus-specific chemical reactivity? There is evidence that aryldiazonium ions react differently at exovesicular and endovesicular surfaces,[3] and the cleavage of Ellman's reagent by thiophenol occurs at different rates in exovesicular and (unspecified) endovesicular sites.[4] Additionally, the aminolysis of p-nitrophenyl laurate in cationic vesicles[5] and the bromination of stilbene derivatives in anionic vesicles[6] occur biphasically, suggesting sequential reactions at different loci. We now employ the cleavage of active esters, the classic reaction studied in micellar aggregates,[7] to further probe differential reactivity at exovesicular and endovesicular surfaces.

We synthesized[8] substrate-functionalized surfactants 1S, 2S, and 2S', the corresponding short-chain model compounds 1M and 2M, and the p-nitrophenol-functionalized "indicator" surfactant, 3.[9] All intermediates[8] and final products gave appropriate NMR spectra and satisfactory elemental analyses. Covesicles of 10% 1S or 2S and 90% di-n-cetyldimethylammonium bromide (16_2Br) were prepared by rapid injection[10] of ethanolic surfactant solutions into 0.01 M aqueous KCl containing enough HCl to maintain pH ~3. These covesicles should have their substrate functionalities anchored at or near the exovesicular and endovesicular surfaces. Corresponding micellar solutions were also prepared from 1S or 2S' and cetyltrimethylammonium bromide (CTABr).

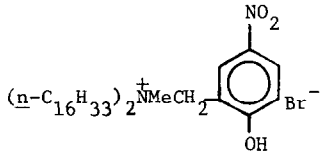
Reactions were initiated by mixing the surfactant or model solutions with either pH 8 Tris buffer (nucleophile OH^-) or 1 x 10^{-3} M thiophenoxide ions in Tris buffer. The kinetics of cleavage of the substrates could thus be determined under vesicular, micellar, or non-aggregated conditions by following the absorptions at 400 nm of the resulting p-nitrophenoxide moieties. The results appear in Table I.



1S, $R_1=R_2=\underline{n}\text{-}C_{16}H_{33}$, R_3=Me
1M, $R_1=R_2=R_3$=Et



2S, $R_1=R_2=\underline{n}\text{-}C_{16}H_{33}$
2S', R=$\underline{n}\text{-}C_{16}H_{33}$, R_2=Me
2M, $R_1=R_2$=Me



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Several unremarkable trends appear upon inspection of the rate constants. (a) The p-nitrophenyl carbonate substrates (2) are generally more reactive than the p-nitrophenyl benzoates (1). (b) The thiophenoxide cleavage reactions are much faster than the analogous hydroxide reactions, but it must be remembered that $[PhS^-]>>[OH^-]$; on a second order basis, the rates would be comparable. (c) We observe the anticipated rate enhancements associated with nucleophilic esterolyses in cationic micellar or vesicular aggregates.[1,7] These enhancements are largely attributable to strong binding of the nucleophiles (especially PhS^-)[11] to the cationic aggregates[12] and consequent reduction of the reaction volumes.[13]

More importantly, all reactions of Table I, including the vesicular reactions (cases 1 and 4), gave excellent pseudo-first-order kinetics, with quantitative generation of the p-nitrophenoxide chromophores.[14] There was no evidence for kinetically resolvable exovesicular and endovesicular reactions in either the rapid thiophenoxide or the slower hydroxide cleavages of vesicular 1S or 2S.

These reactions were initiated by combining vesicular substrate at pH 3 with buffered reagent solutions at pH 8, affording final reaction pH's of 7.8-7.9. Nevertheless, the monophasic kinetics also rule out any permeation-limited "transvesicular" reactions.[15] The pH jump of ~5 units employed here is large enough to overcome the ability of cationic vesicles to maintain pH gradients.[16] Indeed, the deprotonation of vesicular surfactant 3 (10% in 16_2Br) is "instantaneous" ($\geq$500 sec^{-1}) and quantitative when the pH increases from 3.0 to 8.5 in the stopped-flow spectrometer. There is no impediment to OH^- permeation to the endovesicular surfaces of 1S or 2S/16_2 covesicles, at least not on the time scale of the "slow" hydrolysis reactions of Table I. Nor should there be a problem with thiophenoxide (as thiophenol) permeation; this process occurs considerably faster than 3 sec^{-1}.[4]

The evidence is therefore strong that the intrinsic chemical or physical variations between the exovesicular and endovesicular surfaces of simple cationic vesicles are not sufficient to generate significant kinetic differences in representative esterolysis reactions.[17,18] We attempted to alter this outcome by lowering the reaction temperature of

Table I. Kinetics of the Cleavage of Surfactant Substrates and Model Compounds[a]

Case	Substrate	Cosurfactant	Phase[b]	k_ψ(sec^{-1})[c]		k_{rel}[d]	
				OH^-	PhS^-	OH^-	PhS^-
1	1S	16_2Br	V	2.5×10^{-4}	3.3[e]	16	1650
2	1S	CTABr	M	2.2×10^{-4}	0.80[e]	14	400
3	1M	None	A	1.6×10^{-5}	0.0020	1	1
4	2S	16_2Br	V	6.1×10^{-3}	1.8[e]	81	560
5	2S'	CTABr	M	2.8×10^{-3}	1.7[e]	37	530
6	2M	None	A	7.5×10^{-5}	0.0032	1	1

[a] Conditions: 0.01 M Tris buffer, final pH 7.8-7.9, 25°C μ = 0.01 (KCl), [substrate] = 1.0×10^{-4} M, [cosurfactant] = 1.0×10^{-3} M. [b] V = vesicles, M = micelles, A = aqueous solution. [c] Pseudo-first-order rate constant for cleavage by the indicated nucleophile; [PhS^-] = 1.0×10^{-3} M. All reactions followed first order kinetics ($r \geq 0.999$) to $\geq$ 90% of completion. Reproducibilities of the rate constants were ±3%. [d] The rate constants are relative to those of the model compounds 1M or 2M. [e] Determined by stopped-flow spectroscopy.

the 1S/16_2Br thiolysis reaction to 15°C, well below the phase transition temperature of 16_2Br vesicles[19], and by stiffening[1] the vesicles with cholesterol. Although the 1S/PhS^- vesicular cleavage was slower (k_ψ = 1.75 sec^{-1}) at 15°, there was no departure from the rigorous first order thiolysis kinetics and quantitative chromophore generation that was observed at the higher temperature.

On the other hand, injected covesicles of 1S and 16_2Br, doped with 35 wt-% of cholesterol, reacted with thiophenoxide (at 25°) significantly more slowly than native covesicles (k_ψ = 0.50 sec^{-1}) and the kinetics appeared to deviate slightly from rigorous adherance to first order (r = 0.995).[20] We tentatively ascribe the rate reduction, which is proportional to the amount of cholesterol, to the competitive exclusion of thiophenoxide from vesicular binding sites occupied by the cholesterol.[21] A similar phenomenon occurs in the vesicular cleavage of Ellman's reagent by dithionite ion.[4] The departure from first order kinetics may indicate the initial incursion of permeation-limited endovesicular thiolysis, where the cholesterol has slowed the rate of thiophenoxide permeation to a value approaching the rate of thiolysis. Even so, time-resolved kinetic differentiation of the exovesicular and endovesicular thiolysis reactions was not observed.

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(8) Synthetic methods: (1S) 3-Methyl-4-nitrophenol was esterified with benzoyl chloride (Et_3N, Et_2O, 80%), brominated at the 3-methyl group (NBS, CCl_4, reflux 24h, 25%), and then quaternized with $(n\text{-}C_{16}H_{33})_2NMe$ ($CHCl_3$, 25°, 24h, 81%). (1M) The same procedure was followed except that quaternization required Et_3N (CH_3CN, 40°, 3h, 31%). (2S) was prepared by reaction of $(n\text{-}C_{16}H_{33})_2NMeCH_2CH_2OH,Cl^-$ and p-nitrophenyl chloroformate ($CHCl_3$, pyridine, 48h, 25°, 75%). (2S') A similar procedure was followed using $n\text{-}C_{16}H_{33}NMe_2CH_2CH_2OH,Cl^-$ (20h, 25°, 82%). (2M) Choline chloride was similarly esterified with p-nitrophenyl chloroformate (72h, 25°, 25%). (3) 2-Hydroxy-5-nitrobenzyl bromide (Aldrich) was quaternized with $(n\text{-}C_{16}H_{33})_2NMe$ ($CHCl_3$, 25°, 24h, 69%).

(9) $pK_a \sim 4.5$ in 1:1 covesicular $(n\text{-}C_{16}H_{33})_2NMeCH_2CH_2OH$. We thank Mr. T.F. Hendrickson for this determination.

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(18) It could be suggested that the reactions occur only at the exovesicular surfaces and that endovesicular surfactant substrates react only after "flip-flop" to exovesicular orientations. Although not impossible for at least some portion of the slow hydrolysis reactions, this mechanism is unlikely for the fast thiolysis reactions. Flip-flop rates should be much slower[1] than the observed thiolysis rates.

(19) The major phase transition temperature of vesicular 16_2Br ranges from 24°-26.5°C, depending on size: R.A. Moss, T.F. Hendrickson, S. Swarup, Y. Hui, L. Marky, and K.J. Breslauer, Tetrahedron Lett., 25, 4063 (1984).

(20) These effects are absent if the cholesterol-doped vesicles are sonicated. They are also absent in 1S/CTABr cholesterol-doped micellar reactions.

(21) The bathochromic shift of aqueous PhS^- observed upon binding to vesicular 16_2Br at pH 8 (264 to 279 nm) is reduced (to 270 nm) when the vesicles are loaded with 30 wt-% of cholesterol.

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