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IODOSOBENZOATE-FUNCTIONALIZED SURFACTANT VESICLES: ADJUSTABLE REACTIVITY IN REACTIVE PHOSPHATE CLEAVAGE

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Summary. The reactivity of vesicular iodosobenzoate surfactant 3 toward p-nitrophenyldiphenyl phosphate is strongly potentiated in covesicles with dihexadecyldimethylammonium bromide, 2.

The imidazole-functionalized, zwitterionic phosphatidylcholine surfactant 1 displayed unusual kinetic behavior.[1] Native vesicles of 1 were quite unreactive in the cleavage of (e.g.) p-nitrophenyl hexanoate, whereas 1:2 covesicles of 1 and N,N-dihexadecyldimethylammonium bromide, 2, were more than 200 times as reactive. We suggested that the reactivity of vesicular 1 was controlled by the accessibility of its imidazole groups to the substrate. In native vesicles, strong $N^+/^-O$-P electrostatic interactions between adjacent surfactant head groups "pinned" them and their pendant imidazole moieties to the vesicular surface, mitigating their reactivity. Covesicallization with cationic 2, however, "neutralized" these interactions, freed the imidazole moieties, and potentiated their reactivity.

Now we examine the generality of these effects. We report that the iodosobenzoate-functionalized vesicles constructed from zwitterionic surfactant 3 manifest analogous adjustable reactivity. Moreover, in view of the importance of iodosobenzoate reagents for the catalytic cleavage of toxic phosphates,[2] we demonstrate the action of covesicular 3/2 against the simulant substrate, p-nitrophenyldiphenyl phosphate (*PNPDPP*).

Surfactant 3 was prepared as shown in Scheme I. The bromoethyl ether 4[2c] was reacted with excess alcoholic Me_2NH, affording the tertiary amine,5, that was purified by chromatography over silica gel (EtOAc).[3] Quaternization with a slight excess of 3-bromopropylene glycol then gave ammonium salt diol 6 (mp 174-176°C, recryst. CH_2Cl_2/MeOH/Ether). Diol 6 was esterified with 3 equivalents of palmitic anhydride and 2 equivalents of 4-dimethylaminopyridine in CH_2Cl_2. All reagents and solvents were carefully dried and the reaction was conducted under nitrogen. The product mixture was acidified (dil. HCl), extracted with $CHCl_3$, and chromatographed (silica, $CHCl_3$ to elute palmitic acid, then 8:1 $CHCl_3$/MeOH) to afford dipalmitate 7 (phase trans. 84°C, mp 104°C). Finally, the ethyl iodobenzoate moiety of 7 was oxidized to the iodosobenzoate by chlorination/hydrolysis,[4] affording the desired surfactant 3 (phase trans. 139°C, mp 148°C) after $CHCl_3$ extraction and chromatography on silica with 4:1 $CHCl_3$/MeOH. In addition to an appropriate 200 MHz ^1H nmr spectrum, 3 gave 88% of theoretical

iodoso activity in titrimetric analysis.[4b,5]

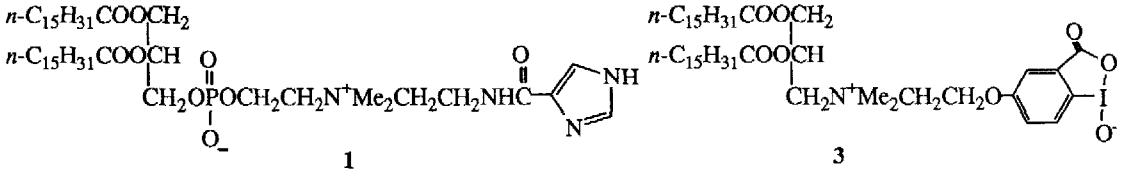
Vesicles of 3 were prepared by immersion sonication (Braunsonic 2000, 65 W, 15 minutes, 65-70°C) of a $CHCl_3$-cast film of 3.2 mg (0.35 mmol) of 3 in 10 ml of (standard) 0.01 M, pH 8 Tris buffer (μ=0.01, KCl),[6] followed by cooling to 25°C and filtration through a 0.8 μM Millex (Millipore) filter. These vesicles had a sharp phase transition, T_c at 36°C,[7] a unimodal size distribution, and an apparent diameter of 1600 ± 20 A by dynamic light scattering.[8] In constrast, T_c and diameter were reduced to 28°C and 1360 ± 10 A in 1:2 covesicular 3/2.

Native vesicular 3 is quite unreactive toward PNPDPP in the standard pH 8 Tris buffer; at [3] = 2.5×10^{-4} M and [PNPDPP] = 1×10^{-5} M, k_ψ for the cleavage of PNPDPP and the release of p-nitrophenoxide ion, monitored by uv at 400 nm, was 1.0×10^{-4} s^{-1} (k_2 = 0.42 $M^{-1}s^{-1}$), only ~7 times greater than in buffer alone. This contrasts with the behavior of iodosobenzoate itself in cetyltrimethylammonium ion micelles, which cleaves PNPDPP with $k_\psi = 6.4 \times 10^{-2}$ s^{-1}, more than 600 times faster.[2b] (All rate constants were measured at 25°C.)

The reactivity of 3, however, is strongly potentiated in covesicles with nonfunctional, cationic surfactant 2 (Figure 1). As with covesicular 1/2[1] a 1:2 blend of 3 and 2 appears optimal. At [3] = 1.9×10^{-4} M and 2 = 3.8×10^{-4} M, k_ψ for the cleavage of 1×10^{-5} M PNPDPP increases to 1.43×10^{-2} s^{-1}, equivalent to a second order rate constant (based on 3) of 75 $M^{-1}s^{-1}$, and representing a kinetic enhancement of ~180 for covesicular 3/2 vs. native vesicular 3.[9] Covesicular 3/2 is also a true catalyst, capable of cleaving excess substrate. With $5.0 - 7.0 \times 10^{-5}$ M 3 (and twice as much 2), $1.8 - 2.5 \times 10^{-4}$ M PNPDPP cleaves with "burst" kinetics[2c] in pH 8 buffer. The "turnover" rate constant for (rate-limiting) basic cleavage of the putative phosphorylated-3 intermediate[2c] is $1.1 - 1.2 \times 10^{-3}$ s^{-1}, whereas k_ψ for the initial PNPDPP cleavage is $8 - 9 \times 10^{-3}$ s^{-1}.

We believe that similar factors govern the reactivities of both vesicular 3 or its covesicles with 2. Their intrinsic reactivities are mitigated in the native vesicles because of extensive electrostatic interactions between adjacent zwitterionic head groups[10] that largely confine the functional groups to the vesicular surfaces. Moreover, the diester backbones of 1 and 3 are relatively rigid and impermeable,[1,8,11] further restricting access to endovesicular functional groups. However, the zwitterionic head groups behave as "molecular electrometers"[12a] or sensors[12b] of electric charge, changing their conformations and orientations with respect to the membrane in response to dipole-charge or dipole-dipole interactions with membrane-bound or intercalated addends such as 2.

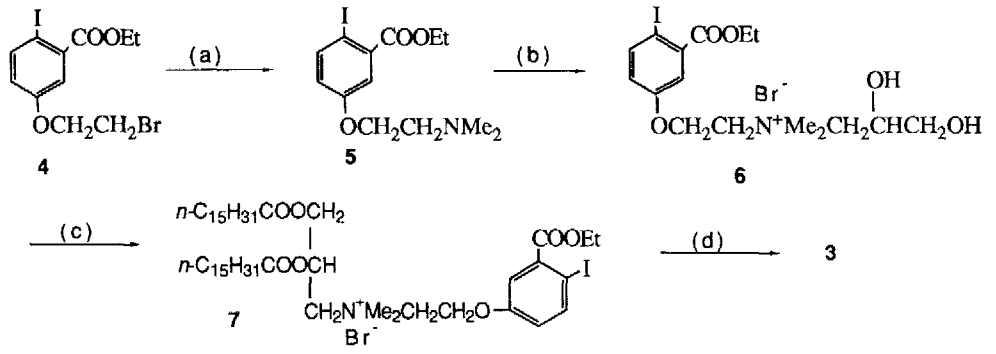
Native vesicular 3 is in the "rigid" gel phase at 25°C (T_c = 36°C), with a microviscosity of ~92 cP.[13] Stopped-flow spectroscopy with the 1,8-anilinonaphthalene sulfonate (ANS) probe, where the half-time for the development of ANS fluorescence is inversely related to vesicle permeability,[8,14] reveals no ANS permeation. Presumably the head groups of 3, involved in $N^+/^-O$-I interactions, lie parallel to the membrane surface, while the bilayer region itself is relatively impermeable. Covesicular (1:2) 3/2, however, is much more fluid and permeable. T_c is reduced to 28°C, microviscosity[13] drops to 16 cP, and ANS permeation now occurs with $\tau_{1/2}$ = 0.11 sec at 25°C.[15] At the same time, electrostatic interactions between the cationic head groups of 2 and the zwitterionic head groups of 3 must alter the orientations of the latter



1 3

$(n\text{-}C_{16}H_{33})_2N^+Me_2$, Br^- (2) $(PhO)_2POC_6H_4NO_2$ (PNPDPP)

Scheme I



(a) Me_2NH, EtOH, 90°, 30 h, 64%; (b) $BrCH_2CHOHCH_2OH$, MeOH, reflux, 24 h, 74%; (c) $(C_{15}H_{31}CO)_2O$, DMAP, CH_2Cl_2, 25°, 48 h, 73%; (d) Cl_2, $CHCl_3$, 0-5°, dark, 2 h; then satd aqueous Na_2CO_3, MeOH, 5°, 30 min, 66%.

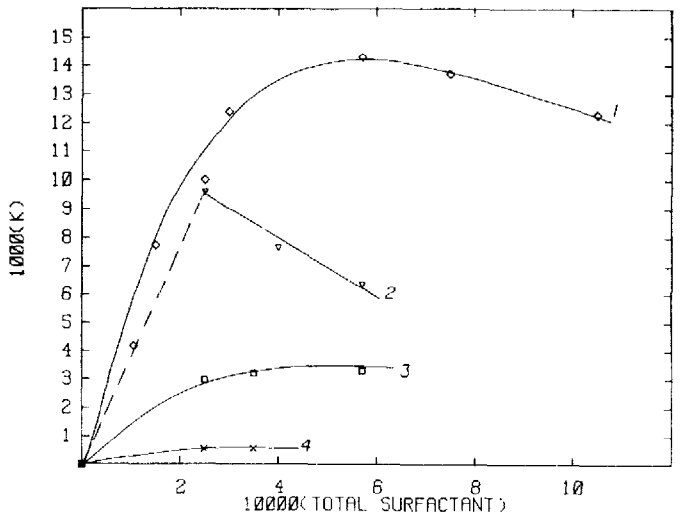


Figure 1. Pseudo-first-order rate constants (k, s^{-1}) vs. total surfactant concentration (M) for the cleavage of PNPDPP by (1) 1:2 covesicular **3/2**; (2) 1:3 covesicular **3/2**; (3) 1:1 covesicular **3/2**; (4) native vesicular **3**. Rate constants for curve 4 have been multiplied by 5 to bring them on scale. Reaction conditions are given in the text.

and their pendant iodosobenzoate moieties. Together, these effects greatly enhance iodosobenzoate accessibility and reactivitiy toward PNPDPP in the covesicular 3/2. We are continuing our studies of novel vesicular surfactants with adjustable reactivities.

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(3) Satisfactory ^{1}H nmr spectra and elemental analyses were obtained for all stable new compounds.

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(5) Titrimetric iodoso activity varied between 88-135% over 4 batches of 3. We believe that "overoxidation" to the iodoxy analogue of 3 is responsible for values >100%; cf., A.R. Katritzky, B.L. Duell, H.D. Durst, and B.L. Knier, Tetrahedron Lett., **28**, 3899 (1987). Kinetic properties of 3 agreed within 17% from batch to batch.

(6) Covesicles of 3/2 were prepared by direct sonication for 30 min. of their suspension in aqueous buffer.

(7) T_c was determined from sharp discontinuities in the temperature dependent fluorescence polarization of covesicallized 1,6-diphenylhexatriene: (a) M.P. Adrich and J.M. Vanderkooi, Biochem., **15**, 1257 (1976); (b) R.A. Moss and S. Swarup, J. Org. Chem., **53** 5860 (1988).

(8) See R.A. Moss, T.F. Hendrickson, S. Swarup, Y. Hui, L. Marky, and K.J. Breslauer, Tetrahedron Lett., **25**, 4063 (1984).

(9) The pK_a of 3 in 1:2 3/2 covesicles is 7.2, as determined from the discontinuity in a pH vs. k_ψ profile for cleavages of PNPDPP in 8 buffers with $5.5 < pH < 8.0$. For an illustration of this method, see ref. 2b. pK_a ~7.2 is typical of iodosobenzoates in cationic surroundings,[2a,b] and indicates ~80% ionization to I-O$^-$. Thus, 3 is largely a zwitterion in 3/2 covesicles. Reactions of native vesicular 3 with PNPDPP are too slow at pH < 8 to permit the determination of a pH/k_ψ profile.

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(13) Microviscosities were determined from the fluorescence polarization of 1,6-diphenylhexatriene in vesicular 3 or covesicular 3/2 at 25°C. For references and a description of the method, see ref. 7b.

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(15) Interestingly, the corresponding microviscosity of 1:1 3/2 is 55 cP, and ANS permeation is not observed, indicating that the 1:1 covesicles are less fluid and permeable than the 1:2 covesicles. Figure 1 reveals parallel differences in their reactivities toward PNPDPP.

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