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THE VESICULAR CLEAVAGE OF ACTIVATED PHOSPHATE ESTERS BY HYDROXIDE AND FLUORIDE IONS -- ARTIFACTUAL ENDOVESICULAR REACTIONS

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Summary: Previously reported endovesicular reactions in the cleavage of p-nitrophenyl diphenyl phosphate by hydroxide (or fluoride) ions in vesicles of dihexadecyldimethylammonium bromide appear to be artifacts.

A principal goal of membrane mimetic chemistry is the preparation of synthetic membranes that are chemically differentiated on "inner" and "outer" surfaces.[1] One approach is the treatment of surfactant vesicles with reagents designed to react (e.g.) only on the outer or exovesicular surface which could thus be differentiated from the inner or endovesicular surface.[2] In the course of studying the catalyzed basic cleavage of the activated phosphate ester p-nitrophenyl diphenyl phosphate (PNPDPP) in surfactant vesicles,[3] we noted the occurrence of kinetically distinct "fast" and "slow" processes, which were interpreted as exovesicular and endovesicular hydrolyses, respectively.[4] Because the surfactant catalyzed cleavage of activated phosphates, such as PNPDPP, with simple nucleophiles (OH^- or F^-) is of current interest,[5,6] we have continued our investigations in the area. We now report that new evidence requires revision of our previous analysis[4] of these reactions.

Vesicles of dihexadecycldimethylammonium bromide[4] (16_2) were created by sonication (Braun-sonic model 1510, immersion probe, 70-80 W, 30 min) in water at 60°C, slow cooling to 25°C, and filtration through a 0.8 Millex-PF filter. An aliquot of PNPDPP (in CH_3CN solution) was added to an appropriately diluted solution of 16_2, and this was reacted with 0.10 M aqueous solutions of KOH or KF at 25°C in the Durrum D-130 stopped-flow spectrometer. Final reaction conditions (after mixing) were $[16_2] = 0.25\text{-}15.0\times10^{-4}$ M, [PNPDPP] = 2.5×10^{-5} M, [KOH or KF] = 0.05 M. Reaction kinetics were followed by monitoring the appearance of p-nitrophenolate ion at 400 nM, and absorbance vs. time data were analyzed in the normal manner.[4,7]

In both KOH and KF vesicular cleavages of PNPDPP, "fast" and "slow" absorbance changes were observed that were similar to those previously reported.[4] Figure 1 correlates k_ψ^f, the

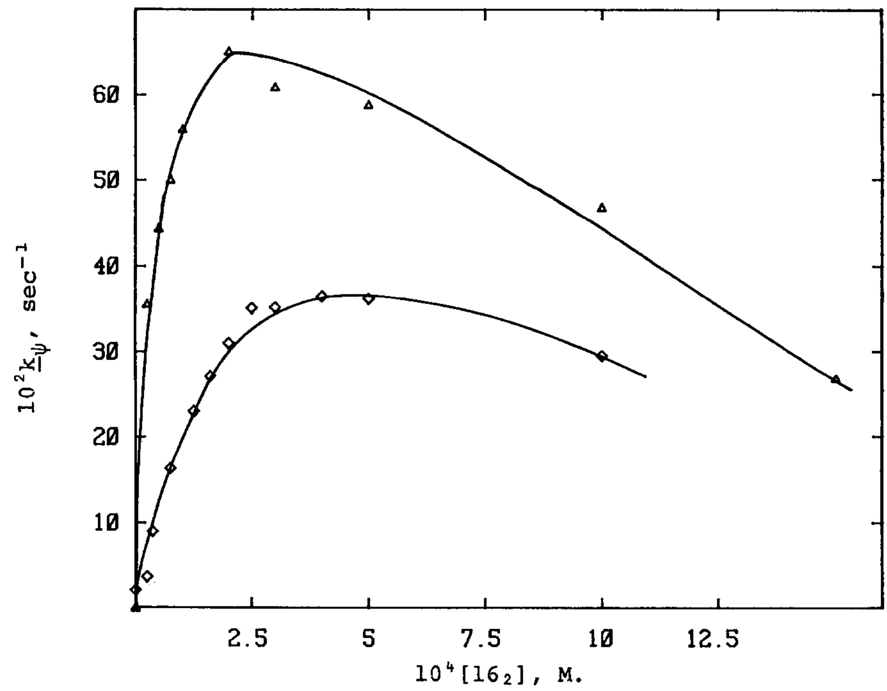

Figure 1. Pseudo-first-order rate constants for cleavages of PNPDPP in vesicular 16_2 by 0.05 M fluoride (△) or hydroxide (◇) ions vs. $[16_2]$. See text for conditions.

observed psuedo-first-order rate constants for the fast reactions, with $[16_2]$. Classic[8] rate constant vs. [surfactant] profiles were obtained. The maximum observed $\underline{k}_\psi^f$ values were $0.365 \pm 0.002_5$ sec^{-1} for OH^- at $[16_2] = 4.0 \times 10^{-4}$ M, and $0.65 \pm 0.01_5$ for F^- at $[16_2] = 2.0 \times 10^{-4}$ M. Relative to analogous cleavages of PNPDPP in the absence of surfactant,[9] these rate constants represent vesicular kinetic advantages of 17.4 and 360, respectively. The fluoride ion is clearly the more potent reagent, as it is in cetyltrimethylammonium bromide (CTABr) micellar cleavages of PNPDPP.[10,11] From the similarities in kinetic behavior of vesicular and micellar[11] OH^- and F^- cleavages of PNPDPP, it is reasonable to conclude that these fast processes occur in similar microenvironments; most likely the Stern layer of micellar CTABr and the exovesicular surface region of 16_2.

Accompanying the fast vesicular reactions of PNPDPP with both OH^- and F^-, were slower, secondary absorbance changes at 400 nm. These were most easily followed on a conventional spectrometer after manual combination of reagents, and they gave good pseudo-first-order rate constants. The latter were 50-100 times smaller than the $\underline{k}_\psi^f$ values depicted in Figure 1, and similar to the previously observed slow processes.[4] Crucially, the slow absorbance phenomena (but not the rapid reactions) persisted when 0.10 M OH^- or F^- solutions were combined with vesicular 16_2 solutions in the absence of PNPDPP, and afforded "rate constants" similar to those observed for the slow processes in the presence of substrate. It follows that the slow "reactions" cannot be attributed to PNPDPP cleavage; our previous[4] identification of these phenomena as endovesicular reactions must be retracted.

What causes the slow absorbance changes? Surfactant vesicles are known to be sensitive osmometers, and should respond to the sudden hyperosmolarity imposed by the rapid mixing of the 16_2 and 0.1 M salt solutions.[12] The osmotic response of the vesicles will be a complex blend of univesicular shrinkage and multivesicular aggregation and flocculation.[12] These lead to enhanced light scattering by the vesicles and thus to increased "absorption".[12d]

The principal experimental argument for our previous assignment of the slow processes to endovesicular cleavage of PNPDPP was their disappearance when aqueous PNPDPP solutions at pH 7 were added to 16_2 vesicles already equilibrated in concentrated (0.1-0.2 M) NaOH solutions. It was suggested[4] that, with this order of addition, the substrate did not survive transit of the outer vesicular surface and was, instead, rapidly cleaved by exovesicularly approximated OH^-. This interpretation must now be regarded as incorrect. Most likely, the hypoosmolarity imposed on the vesicular solutions in these experiments led to vesicular swelling; this would not result in enhanced turbidity and absorption.

Recognition of the previously reported 16_2 reactions as artifactual, naturally casts suspicion on other examples of complex (biphasic) kinetic behavior in vesicular reactions: (*e.g.*) the cleavage of *p*-nitrophenyl acetate (PNPA) by fully functionalized thiol vesicles,[2a] or the scission of Ellman's reagent by dithionite ion in 16_2 vesicles.[13] Appropriate control experiments have shown that the two-site vesicular processes observed in the latter case (under conditions of near constant ionic strength) are genuine, and occur much more rapidly than any dithionite-induced, spectroscopically-observable phenomena. On the other hand, preliminary experiments[14] indicate that endovesicular processes observed during the vesicular thiolysis of PNPA[2a] may be artifactual. This matter is under further investigation, and will be addressed in a full paper.

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9. $(\underline{k}_o)_{OH^-}=0.021$ sec^{-1} and $(\underline{k}_o)_{F^-}=0.0018$ sec^{-1}.

10. Reference 5, last citation.

11. We find CTABr micellar catalysis of the OH$^-$ and F$^-$ cleavages to be quite comparable to the 16$_2$ vesicular results given in the text. Thus $(\underline{k}_\psi)_{OH^-} = 0.31\pm0.02_5$ sec^{-1} at [CTABr] = 8.0×10^{-4} M, and $(\underline{k}_\psi)_{F^-} = 0.41\pm0.01_5$ sec^{-1} at [CTABr] = 5.0×10^{-4} M, under conditions identical to those employed in the vesicular reactions.

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