it is clear that there is a solvent effect since values obtained in $CDCl_3$ differ slightly from those obtained in C_6D_6. Nevertheless, the overall agreement between simulated and observed values is quite satisfactory and would indicate that the proposed simulation does reflect the conformational flexibility of the molecule and can be a valid tool in investigating conformational behavior in solution.

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Registry No. β-Maltose octaacetate, 22352-19-8; amylose triacetate, 9040-62-4.

Separability of Endovesicular and Exovesicular Reactions

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Abstract: *p*-Nitrophenyl diphenyl phosphate (**3**) was cleaved in vesicles of *N,N*-dihexadecyl-*N,N*-dimethylammonium bromide (**1**) or *N,N*-dihexadecyl-*N*-(β-hydroxyethyl)-*N*-methylammonium bromide (**2**) in 0.01–0.1 M aqueous NaOH at 25 °C. When the substrate was bound to and entrapped by the vesicle before addition of the base, initiation of the reaction gave consecutive "fast" and "slow" pseudo-first-order processes, assigned to exovesicular and endovesicular reactions, respectively. When the substrate was added to preformed vesicles, only the exovesicular cleavage could be observed. It was shown by experiments with vesicle-entrapped indigo carmine that permeation of hydroxide ions was not rate-limiting in endovesicular cleavage reactions.

The vigorous investigation of synthetic surfactant vesicles[2] as chemically unique reaction media continues apace.[3] Vesicles possess exterior and interior membrane surfaces and interior volumes which can entrap reagents and substrates.[2,4] These architectural elements constitute specific reaction sites, so that attention now focuses on the separability of *exovesicular* and *endovesicular* reactions. Three approaches may be identified. (a) "Unsymmetrical" vesicles can be produced by chemical differentiation of the exterior and interior vesicle surfaces.[5-7] (b) A "complex" surfactant may generate two distinct, "insulated" binding sites upon vesicle formation.[8] (c) Exovesicular and endovesicular reactions may be directly observed in *native* vesicles; e.g., kinetically distinct exterior and interior esterolyses of *p*-nitrophenyl acetate were observed with fully functionalized thiol vesicles.[9]

In this report, we generalize approach (c), demonstrating that simple, unfunctionalized, synthetic vesicles support the kinetic separability of exovesicular and endovesicular reactions.

Results and Discussion

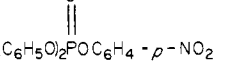
Vesicles of dihexadecyldimethylammonium bromide (**1**, 16_2)

$$(n\text{-}C_{16}H_{33})_2N^+Me_2,Br^-$$
1 (16_2)

or dihexadecyl-β-hydroxyethylmethylammonium bromide (**2**, 16_2OH)[9] were generated from the surfactants by injection[9] or

$$(n\text{-}C_{16}H_{33})_2N^+MeCH_2CH_2OH,Br^-$$
2 (16_2OH)

sonication[2] in water. Substrate *p*-nitrophenyl diphenyl phosphate (**3**) was introduced by *coinjection* or *cosonication* with the surfactant at pH 7 (method A), or by *subsequent* addition *after* the vesicles had been formed (method B). Initiation of the cleavage of **3** was accomplished by stopped-flow combination of (vesicles + substrate) at pH 7 with aqueous NaOH (final concentrations 0.01–0.1 M) (method A), or by stopped flow combination of substrate in pH 7 water with vesicles in NaOH solutions (method B).[10] Reactions were followed by monitoring the appearance of *p*-nitrophenolate ion at 392 nm, and rate constants were evaluated from photographs of absorbance vs. time as displayed on an oscilloscope.



3

As shown in Table I, the kinetics of method A phosphate cleavage in vesicular **1** or **2** were biphasic or triphasic. They featured a "fast" pseudo-first-order process (k_ψ^f), accounting for 71–95% of reaction, followed by a "slow" (k_ψ^s) pseudo-first-order

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(10) It is shown below that OH^- permeation of the vesicles under method A is rapidly driven by substantial (5–6 units) pH gradients, and is not rate-limiting in the observed cleavages of **3**.

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Table I. Rate Constants for Cleavage of *p*-Nitrophenyl Diphenyl Phosphate (3)[a]

case	vesicle	method, [NaOH], M[b]	k_ψ^f, s^{-1} [c]	k_ψ^s, s^{-1} [d]	k_ψ^s, %[e]	$10^5 k_o$, M^{-1} s^{-1} (%)[f]
1	16_2	A, 0.10	$0.65 \pm 0.03_6$	0.022	5.0	*g*
2	16_2	A', 0.10	$0.84 \pm 0.04_3$	$0.032 \pm 0.003_2$	11	1.0 (5)
3	16_2	B, 0.10	$0.45 \pm 0.02_2$	*h*		*g*
4	16_2	A, 0.05	$0.53 \pm 0.03_4$	*i*		*i*
5	16_2OH	A, 0.10	$3.24 \pm 0.08_8$	$0.026 \pm 0.002_6$	14	$5 \pm 2_3$ (8)
6	16_2OH	A', 0.10	$1.73 \pm 0.10_3$	$0.075 \pm 0.003_2$	17	$10 \pm 3_2$ (12)
7	16_2OH	A', 0.10[j]	$0.75 \pm 0.04_3$	$0.052 \pm 0.007_2$	16	$2 \pm 1_2$ (5)
8	16_2OH	A, 0.10[k]	$1.65 \pm 0.09_3$	$0.061 \pm 0.003_3$	6.1	*g*
9	16_2OH	B, 0.10	$0.94 \pm 0.06_4$	*h*		*g*
10	16_2OH	B', 0.10	$1.13 \pm 0.10_3$	*h*		*g*
11	16_2OH	A, 0.05	$1.82 \pm 0.02_7$	$0.042 \pm 0.007_6$	4.4	$3 \pm 1_3$ (2)
12	16_2OH	A, 0.05[j]	$0.95 \pm 0.02_8$	$0.051 \pm 0.007_6$	10	$4 \pm 1_3$ (5)
13	16_2OH	A, 0.05[k]	$1.27 \pm 0.05_4$	$0.033 \pm 0.006_3$	8.6	*g*
14	16_2OH	A, 0.05[j,k]	$0.89 \pm 0.02_4$	0.021 1 run	13	*g*
15	16_2OH	B, 0.05	$0.86 \pm 0.02_4$	*h*		*g*
16	16_2OH	B, 0.05[j]	$0.60 \pm 0.01_4$	*h*		*g*
17	16_2OH	A, 0.01	$1.13 \pm 0.03_4$	*i*		*i*
18	16-OH[l]	A, 0.10	$2.29 \pm 0.05_4$	*h*		*g*
19	16-OH[l]	B, 0.10	$2.32 \pm 0.04_4$	*h*		*g*

[a] Conditions (*after* stopped-flow mixing): [vesicle] = 5.0×10^{-4} M, [3] = 1.0×10^{-5} M, 4% (v/v) EtOH, 0.2% (v/v) CH_3CN, 25 °C. [b] Methods of vesicle preparation: A, coinjection; A', cosonication; B, subsequent injection; B', sonication of surfactant followed by subsequent injection of substrate. See text for more detail. [NaOH] are final concentrations after mixing. [c] Rate constant of faster reaction; errors refer to average deviations of *n* (subscript) runs. [d] Rate constant of slower reaction; errors as in *c*. [e] Percent of total reaction occurring at slower rate. [f] Rate constant of apparent zero-order reaction linking fast and slow processes; percent of zero-order reaction in ( ). [g] Process not observed. [h] No slow reaction was observed. [i] Not studied in this case. [j] Ionic strength maintained constant during mixing with KCl. [k] 20 (wt) % of cholesterol incorporated into vesicle. [l] *Micellar* conditions with n-$C_{16}H_{33}N^+Me_2CH_2CH_2OH,Br^-$ (16-OH); [surfactant] = 2.0×10^{-3} M.

process, accounting for 5–17% of reaction. Occasionally, an apparently zero-order segment (2–12%) connected the fast and slow phenomena. Examination of the table permits the following observations. (a) Multiphasic kinetics are observed with both nonfunctional vesicular 16_2 (cases 1, 2) and functional vesicular 16_2OH (cases 5, 6). With 16_2, the nucleophile responsible for phosphate cleavage must be OH^-, whereas, with 16_2OH, the intervention of the surfactant's conjugate base, 16_2O^-, produces a 5-fold enhancement in k_ψ^f (cases 5 vs. 1).[11] (b) k_ψ^f for vesicular 16_2OH is sensitive to $[OH^-]$ or $[16_2O^-]$; cf. cases 5, 11, and 17. (c) Importantly, multiphasic kinetics *persist* in method A experiments whether the vesicles are prepared by injection or sonication[12] (cases 1 vs. 2, 5 vs. 6), whether or not osmotic shock is avoided by control of ionic strength (cases 6 vs. 7, 11 vs. 12), or when the vesicles are doped with cholesterol (cases 5 vs. 8, 11 vs. 13, 14).

We assign k_ψ^f to exovesicular cleavage of **3** bound to exterior vesicular surfaces, and k_ψ^s to endovesicular cleavage of entrapped[2a] **3**, or of **3** bound to interior vesicular surfaces.[13] We are uncertain of the origin of the apparently zero-order "connecting" phase often observed between k_ψ^f and k_ψ^s. In support of our assignments, note that *micellar* cleavage of **3** by 16-OH (cases 18, 19) proceeds with a rate constant similar to k_ψ^f for vesicular cleavage by 16_2OH (cases 5, 6). The former reaction occurs in the Stern layer of micellar 16-OH,[11] which should provide a microenvironment similar to the exterior surface of 16-OH vesicles. More convincingly, biphasic kinetics vanish when **3** is added *after* the vesicles have been formed (Table I, all method B experiments). In these cases, substrate does not survive transit of the outer vesicular surface; it is cleaved by exovesicularly approximated OH^- and only k_ψ^f is observed. Only when substrate has been prepositioned *inside* the vesicles (method A or A') can endovesicular (k_ψ^s) reactions be seen.[14]

It was important to show that the "slow" endovesicular cleavages observed in method A experiments were not rate-limited by OH^- permeation across exterior vesicle membranes. Accordingly, 16_2 or 16_2OH vesicles were created by injection of surfactant into a 0.01 M, pH 7 aqueous solution of indigo (carmine) disulfonate (pK_a = 11.4–13, Aldrich). Sephadex G-25-80 chromatography afforded vesicle-entrapped[4a] dye (eluted with the void volume), which was then combined with NaOH solution (final $[OH^-]$ = 0.01 or 0.1 M) in the stopped flow spectrometer.[15] Monitoring the disappearance of "acidic" dye at 610 nm and $[OH^-]$ = 0.1 M, we found $k = 95 \pm 3$ s^{-1} for free aqueous dye vs. $k = 108 \pm 3$ s^{-1} for 16_2OH-entrapped dye[16] (128 ± 3 s^{-1} for 16_2-entrapped dye). With $[OH^-]$ = 0.01 M, comparable k's were 55 ± 8 s^{-1} (H_2O) and 66 ± 2 s^{-1} in 16_2OH (51 ± 9 s^{-1} in 16_2) vesicles. Although deprotonation of the indigo disulfonate is rather slow, hence readily measurable,[17] the rate constants are essentially identical for deprotonation of free or vesicle-entrapped dye, and they indicate that permeation of OH^- (here driven by pH gradients of 5–6 units) is considerably faster than the cleavage reactions of **3** observed under comparable conditions.[18] Permeation of OH^- is therefore not rate-limiting in endovesicular cleavage of **3**. Presumably, it is the lower activity of endovesicular OH^- (or 16_2O^-) which is responsible for the substantially slower cleavage of endovesicular **3**, relative to exovesicular substrate.

We conclude that vesicles of nonfunctional 16_2, as well as functional 16_2OH, bind phosphate **3** in both endo- and exovesicular

(11) Bunton, C. A.; Ionescu, L. G. *J. Am. Chem. Soc.* **1973**, *95*, 2912, report a 26-fold enhancement in the cleavage of **3** by *micellar* 16-OH, relative to CTABr, in 0.01–0.02 M aqueous NaOH. See also Begunov, A. V.; Rutkovskii, G. V.; Kuznetsov, S.-G. *J. Org. Chem. USSR* **1981**, *17*, 1668.

(12) Sonication was carried out at 60–65 °C with a Bransonic Model 221 bath-type sonifier, operated at maximum power (225 W) for 30 min.

(13) Electron microscopy supports the formation of versicles from $R_2N^+Me_2,Br^-$ surfactants such as 16_2. Cf. Kunitake, T.; Okahata, Y.; Tamaki, T.; Kumamaru, F.; Takayanagi, M. *Chem. Lett.* **1977**, 387, and ref 2a. Electron microscopy of vesicular 16_2OH, prepared by injection into 2% uranyl acetate, revealed vesicles of 1500-Å diameter with a 270-Å wall, corresponding to ~6 bilayers. Fine structure was not visible due to lack of contrast. A large circular object of 3700-Å diameter and no fine structure was also observed, but it may have been an artifact.

(14) We do not believe that the biphasic kinetics reflect separate reactions catalyzed by morphologically distinct vesicle populations. Extensive sonication[12] of vesicle/substrate preparations (which should degrade multilamellar to smaller, bilayer vesicles[2a,4a]) does not obviate biphaseic kinetics. Moreover, only monophasic kinetics are observed when substrate is added after vesicle formation. This is understandable if k_ψ^s represents endovesicular chemistry, but not if it reflects reactions occurring on a second kind of vesicle.

(15) Spectroscopic experiments give the following data for indigo disulfonate disodium salt: acidic form, λ_{max} 610 nm, $\epsilon \sim 2 \times 10^4$ (free) and 609 nm (entrapped); basic form, λ_{max} 448 nm (free) and 447 nm (entrapped).

(16) A similar rate constant was measured with 16_2OH entrapped dye by monitoring the appearance of the basic form at 448 nm and $[OH^-]$ = 0.1 M.

(17) Cf. Rose, M. C.; Stuehr, J. *J. Am. Chem. Soc.* **1968**, *90*, 7205.

(18) These results are consistent with a report of Kunitake, T.; Okahata, Y.; Yasunami, S. *Chem. Lett.* **1981**, 1397.

microenvironments and that hydroxide-mediated cleavages of the differentially bound substrate molecules are kinetically separable. This kinetic separability appears to be a general property of functional or nonfunctional vesicles, at least when the rate of the observed reaction is comparable to or faster than the rate of *substrate* permeation through vesicular membranes,[2a,4a,19] so that differentially bound substrate molecules kinetically reflect their specific microenvironments.

Experimental Section

Materials. Substrate **3** was prepared by literature methods.[20] Surfactant **2** (16_2OH) was prepared from *N*-methyl-2-aminoethanol by successive cetylations with cetyl bromide.[9] The experimental details of this preparation appear in another publication.[21] Surfactant **3** (16_2) was a gift from Professor T. Kunitake. Indigo (carmine) disulfonate was obtained from Aldrich Chemical Co.

Kinetic Studies. Vesicular solutions were prepared by injection or sonication.[12] In the injection technique,[22] usually 0.25 mL of $\sim 10^{-3}$ M ethanolic solutions of surfactant (or surfactant + substrate) were injected into 3 mL of various aqueous solutions held at ~60 °C during injection. The injection syringe was fitted with a capillary tapering to a 100–200-nm exit aperture. Specific details of reactant concentrations, experimental method, and additives appear in Table I.

Reactions were followed with a Durrum Model D-130 stopped-flow spectrophotometer equipped with a Beckman DU-2 monochromator and a Tektronix Model 5103N/D15 storage oscilloscope. The oscilloscope trace was photographed for permanent recording. A constant-temperature circulating bath maintained the reaction temperature at 25 ± 0.2 °C. All buffers or other aqueous solutions were prepared from nitrogen-purged steam-distilled water and were purged again immediately before use.

Rate constants were obtained from computer-generated correlations of log $(A^\infty - A^t)$ with time in the standard manner. For studies of biphasic kinetics, reactions were monitored with oscilloscope time bases of 0.1 or 0.2 s/div (k_ψ^f) or 2.0 or 5.0 s/div (k_ψ^s). Rate constants were calculated from the appropriate traces. First-order kinetic behavior was observed for fast and slow reactions, with correlation coefficients >0.999 and >0.99, respectively. "Crossover", or contributions of the k_ψ^f process to the absorbance belonging to the k_ψ^s process (and vice versa) was usually <0.5% of the total absorbance change, and never more than 3%, i.e., of the order of the experimental error in reading the oscilloscope photographs.

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Mechanism of the Apparent Electron-Transfer Reaction between *tert*-Butoxide Ion and Nitrobenzene

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Abstract: Potassium *tert*-butoxide reacts with nitrobenzene in tetrahydrofuran to produce *tert*-butoxynitrobenzenes. During the reaction at least 2 equiv of the potassium salt of nitrobenzene radical anion precipitates per equivalent of substitution products. The reaction is first order in nitrobenzene and second order in *tert*-butoxide. When a mixture of deuterated and undeuterated nitrobenzene is used, the substitution reaction is selective for the undeuterated reagent. In the presence of molecular oxygen, the nitrobenzene radical anion does not form but the substitution reaction proceeds at the same rate. The isotopic selectivity is undiminshed by the presence of molecular oxygen, demanding that the hydrogen-transfer step occur prior to the electron-transfer steps. These experimental observations demonstrate that substitution occurs by dianion formation followed by electron transfer.

Replacement of hydrogen by nucleophilic substitution in aromatic nitro compounds is not a frequently encountered reaction despite being known since 1899.[1] Nitrobenzene, $PhNO_2$, has been shown to react with both oxygen and nitrogen nucleophiles to produce substitution products although usually in low yield.[2]

Several of our publications[3] have dealt with processes requiring both alkoxide and $PhNO_2$ to be present for the production and oxidation of carbanions. In addition we have studied the potassium *tert*-butoxide, *t*-BuOK, catalyzed hydrogen–deuterium exchange reaction of $PhNO_2$ in *tert*-butyl alcohol-*d*.[4] In connection with running controls for these reactions, we became interested in the sometimes-observed background process in which $PhNO_2$ is consumed through direct reaction with alkoxide.

Results and Discussion

Reaction Products. At temperatures above 50 °C, $PhNO_2$ is slowly destroyed by *t*-BuOK in *t*-BuOH.[4] The products include 4-*tert*-butoxynitrobenzene (4-*t*-BuO$PhNO_2$), azoxybenzene, azobenzene, an intractable black resin, and a large balance of unisolated material. We also detected the formation of nitrobenzene radical anion ($PhNO_2^{-}\cdot$). This was demonstrated by the observation of exchange broadening of $PhNO_2$ peaks in the NMR spectrum of the reaction and confirmed by direct ESR measurement. At room temperature, $PhNO_2^{-}\cdot$ reaches a constant concentration after a few hours and remains at this level indefinitely. It may be destroyed by O_2 but reforms on degassing of the solution. This cycle can be repeated indefinitely. Even at 50 °C, however, O_2 consumption is immeasurably small over a period of several days.

The conditions used by Russell and Bemis[5] for the preparation of $PhNO_2^{-}\cdot K^+$ suggested that tetrahydrofuran (THF) would be a good choice of solvent in which to examine the details of this reaction. If $PhNO_2^{-}\cdot K^+$ is a major product of the reaction between *t*-BuOK and $PhNO_2$, it should precipitate from this solvent. In a typical experiment, 2 mmol of $PhNO_2$ was allowed to react with 2 mmol of *t*-BuOK in 11 mL of THF at 50 °C for 72 h under argon gas. A copious brown precipitate was found to contain 0.93 mmol of $PhNO_2^{-}\cdot K^+$ as evidenced by its ESR spectrum in dimethyl sulfoxide (Me_2SO) and determined quantitatively by GC analysis

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