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EXAMPLES OF TRANSVESICULAR REACTIONS[1]

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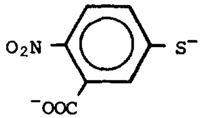
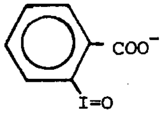
Summary. The oxidation of vesicle-entrapped Ellman's anion (1) by exovesicular o-iodosobenzoate ion (2) is a permeation-limited, transvesicular reaction; transvesicular esterolyses reactions are also illustrated.

A surfactant vesicle[2] affords at least three distinguishable loci for chemical reactions. Two of these are well documented: the exterior vesicular surface (exovesicular reaction) and the interior of the vesicle or an interior surface (endovesicular reaction). We have reported kinetically differentiated exo- and endovesicular reactions in functional and nonfunctional synthetic surfactant vesicles.[3] The third reaction locus is the outer vesicle bilayer. We define transvesicular reactions as those which are rate-limited by reagent diffusion across this barrier. There are several examples of transvesicular, photochemically driven electron transfer reactions,[4] but we present here what we believe to be the first well-characterized examples of bimolecular reactions which are rate-limited by permeation of one of the reactants through the outer bilayer of a synthetic surfactant vesicle.[5]

The clearest example is provided by the oxidation of Ellman's anion 1 (Ell^-) by o-iodosobenzoate 2 (ArIO) in vesicles of 3 (16_2). Protonated 1 was obtained from its dimer (Ellman's reagent[6]) by dithiothreitol reduction followed by acidification with HCl. The reactions between Ell^- and ArIO were followed under various conditions at pH 8, monitoring the bleaching of Ell^- at 450 nm. Vesicles of 16_2 were created by injection techniques,[3] and were presumably multilamellar.[2,3] Table I summarizes reaction conditions and rate constants. The latter were generally obtained under pseudo-first-order conditions and converted to second order rate constants[7,8] by dividing by the concentration of excess reactant. In two cases, we also determined k_2 for oxidation of Ell^- by iodosobenzene (PhIO). Due to the low solubility of PhIO, these studies were subject to larger uncertainties (~11%) in k_2.



1 (Ell^-)



2 (ArIO)

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

3 (16_2)

In water, oxidative quenching of Ell^- by iodosobenzoate proceeded rapidly, with k_2 = 675 L/mol-sec. The reaction between these anions was catalyzed about 10-fold (k_2 = 6400) in cationic

Table I. Oxidation of Ellman's Anion (1) by o-Iodosobenzoate (2)

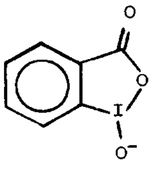
Conditions[a]	Reagent Sequence	k_2^{ArIO} [b]	k_2^{PhIO} [b]
No surfactant	Ell⁻, then ArIO	675 ± 7[c]	-
CTABr micelles	Micelles, then Ell⁻, then ArIO	$6400 \pm 400_3$	-
16_2,Br vesicles	Vesicles, then Ell⁻, then ArIO	$616 \pm 4_2$[d]	$9000 \pm 1000_2$[d,e]
16_2,Br vesicles	(16_2 + Ell⁻ coinjected), then Sephadex, then ArIO	$1.4 \pm 0.2_3$[f]	$6900 \pm 800_2$[f,g]

[a] [Surfactant] = 1×10^{-3} M; [Ell⁻] = $2\text{-}4 \times 10^{-5}$ M; [ArIO] = 2.5×10^{-4} M (unless otherwise indicated); pH 8.0; 0.01 M Tris, μ = 0.01 (KCl), 25°C. [b] L/mol-sec. Errors are average deviations of n runs (n = subscript). [c] k_2 is the slope of k_ψ^{obs} vs. [ArIO] at 5 concentrations of [ArIO], $2.5\text{-}25 \times 10^{-4}$ M. [d] Exovesicular reaction; no slow phase observed. [e] [PhIO] = 9×10^{-6} M; [Ell⁻] = 4.3×10^{-5} M. [f] Endovesicular reaction of compartmentalized substrate. [g] [PhIO] = 4.3×10^{-5} M; [Ell⁻] = 2.5×10^{-5} M; rate constant from second order rate law.

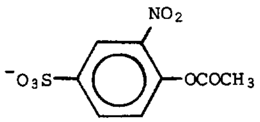
Table II. Cleavage of ANBS (4) or ANB (5) by o-Iodosobenzoate (2')

Case	Conditions[a]	Reagent Sequence	k_2^{ANBS}, L/M-s	k_2^{ANB}, L/M-s
1	No surfactant	Substrate, then ArIO	$53 \pm 2_2$	$38 \pm 0.3_2$
2	CTABr micelles	Micelles, then substrate, then ArIO	$1180 \pm 80_2$	$335 \pm 5_2$
3	16_2,Br⁻ vesicles	Vesicles, then substrate, then ArIO	$720 \pm 40_2$	$260 \pm 30_2$
4	16_2,Br⁻ vesicles	(16_2 + substrate coinjected), then ArIO	(k_2^f) $550 \pm 50_2$ (k_2^s) $10 \pm 2_2$[b]	$222 \pm 1_2$ 5.2[c]
5	16_2,Br⁻ vesicles	(16_2 + substrate + ArIO coinjected at pH 2), then pH 7.5	$278 \pm 5_3$[d]	$42.5 \pm 0.3_2$[d]

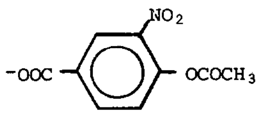
[a] [Surfactant] = 1×10^{-3} M; [ANBS] = $2\text{-}4 \times 10^{-5}$ M; [ANB] = 4×10^{-5} M; [ArIO] = $5\text{-}10 \times 10^{-4}$ M; pH 8.0 (unless otherwise indicated), 0.01 M Tris buffer, μ = 0.01 (KCl), 25°C. Errors in rate constants are average deviations of n runs (n = subscript). [b] Biphasic kinetics, % slow phase = 20%. [c] Biphasic kinetics, % slow phase = 12%, single run. [d] "Monophasic" kinetics.



2'



4 (ANBS)



5 (ANB)

CTABr micelles. When Ell$^-$ was added to preformed ("empty") cationic 16_2 vesicles, followed by the addition of ArIO, we observed quenching with k_2 similar to that observed in water. We believe, however, that this was an exovesicular reaction occurring on the 16_2 vesicular surface. The rate retardation (relative to the micellar case) is anticipated because "two negatively charged reactants...are less likely to encounter when bound to a surface at close proximity than when they are free to move about."[9]

Most importantly, Ell$^-$ was coinjected with 16_2, and the resulting vesicles were chromatographed on Sephadex G-25 to give vesicle-entrapped Ell$^-$ eluted in the column void volume. Subsequent reaction with iodosobenzoate gave a very slow (k^s = 1.4 L/mol-sec) oxidation, inhibited by a factor of ~440, relative to the exovesicular process. We suggest that this represents a *permeation-limited*, *transvesicular* reaction of ArIO and Ell$^-$, in which the anionic ArIO must "slowly" diffuse across the cationic bilayer in order to react with the trapped, dianionic Ell$^-$.[10] In agreement with this idea, we note in Table I that oxidation of Ell$^-$ with *neutral* iodosobenzene (PhIO) was very fast, whether the Ell$^-$ was situated on the surface or within the 16_2 vesicles; permeation was no barrier to the neutral PhIO.[11]

In a second group of experiments, excess ArIO (presumably reacting in its nucleophilic, valence tautomeric form, *2'*)[12] was used to cleave the anionic activated esters *4* (ANBS)[13] and *5* (ANB).[14] These reactions were spectrophotometrically monitored by following the pseudo-first-order appearance of the corresponding nitrophenoxide ion at 407 nm (ANBS) or 401 nm (ANB). Rate constants obtained under various reaction conditions were converted to second order (division by [ArIO]) and appear in Table II.

Consider first the ANBS data. Cases 1, 2, and 3 record the rate constants for esterolyses in water, in micellar CTABr (22-fold catalysis), and (exovesicularly) on 16_2 vesicles (13.6-fold catalysis). In case 4, ANBS was coinjected with 16_2, so that it was present both within and outside of the 16_2 vesicles. Subsequent addition of ArIO afforded the anticipated *biphasic* kinetics, represented by the rate constants k_2^f and k_2^s. However, when ANBS and ArIO were *both* coinjected with 16_2 at pH 2 (so that substrate and reactant were present in both vesicular locations), and esterolysis was subsequently initiated by raising the pH to 8, we observed (case 5) only *monophasic* kinetics with an intermediate rate constant.

We interpret this surprising result as follows. To detect biphasic kinetic behavior from sequential reactions, the rate constant ratio, $k_2^f//k_2^s$, must be comparable to or exceed the "phase ratio", $\%k_2^f/\%k_2^s$. In the present case, we suspect that this condition was not fulfilled. Moreover, taking $\%k_2^f/\%k_2^s$ ~4 (similar to the observation in case 4), then k_2^f/k_2^s cannot exceed ~4 if monophasic kinetics are to be observed. With k_2^f ~550 (case 4), k_2^s for the ("invisible") slow reaction would be at least 137 L/mol-sec. Since the observed slow reaction in case 4 has k_2^s = 10 L/mol-sec, we suggest that it corresponds to a permeation-limited transvesicular reaction between ANBS and ArIO. The "monophasic" reaction observed in case 5 corresponds to an inseparable sum of slow and fast reactions with k_2^f/k_2^s < 4.

A very similar pattern emerged upon study of the ester cleavage of the closely related anionic carboxylate substrate *5* (ANB). The experiments, results, and interpretation parallel those of the ANBS experiments. Again, we suggest that the biphasic reaction of ArIO with coinjected vesicular ANB/16_2 (Table II, case 4) includes a permeation-limited "slow" transvesicular component.

There is an ambiguity in the interpretation of the ANBS or ANB + ArIO reactions which is absent in the Ell^- + ArIO reaction. In the latter case, the substrate (Ell^-) is a dianion at pH 8.[10] It is prepositioned inside of the 16_2 vesicles, and we feel reasonably certain that the slow oxidation reaction observed upon subsequent addition of monoanionic ArIO represents the permeation-limited diffusion of ArIO into the vesicle. However, in the reactions between ArIO and ANB or ANBS, both reagent and substrates are *mono*anionic. It is not presently clear whether the observed permeation-limited transvesicular reactions represent ArIO diffusing into the vesicle to react with entrapped substrate, substrate diffusing out of the vesicle[15] to react with excess, surface-adsorbed ArIO, or a blend of both processes.

We have here delineated three examples of permeation-limited transvesicular reactions. Taken in concert with previous results,[3] it is clear that judicious choice of reagent, substrate, and surfactant can lead to endovesicular, transvesicular, or exovesicular reactions. It should now be possible to study the effects of reagent or substrate structural modification on the occurrence of these reaction types in synthetic or natural surfactant vesicles.

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References and Notes

(1) This work formed part of a presentation at The Second International Kyoto Conference on New Aspects of Organic Chemistry, Kyoto, Japan, August 18, 1982.

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(15) In contrast to Ell^-, ANBS and ANB are not completely retained within 16_2 vesicles during the time scale of Sephadex chromatography and subsequent preparations for kinetic studies (~20 min.).

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