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PERMEATION OF ELLMAN'S ANION IN CATIONIC SURFACTANT VESICLES

Robert A. Moss,* Shanti Swarup, and Ronald P. Schreck

Department of Chemistry, Rutgers, The State University of
New Jersey, New Brunswick, New Jersey 08903

Summary. The redistribution (permeation) of Ellman's anion (2-nitro-5-thiolatobenzoate) from surface to interior sites of cationic vesicular dihexadecyldimethylammonium bromide is characterized by $\underline{k}_{apparent}$ = 0.8-1.7 sec^{-1}.

Fluorescence stopped-flow spectroscopy has been employed to study the permeation of (*e.g.*) 1-anilino-8-naphthalenesulfonate (ANS) into liposomes and vesicles.[1] Absorption spectroscopy is much less commonly applied to permeation problems, although Murakami has recently described the dynamics of binding of *p*-*n*-hexyloxynitrobenzene to cationic vesicles derived from alanine.[2] In the course of our continuing studies of the dithionite cleavage of Ellman's reagent in dihexadecyldimethylammonium bromide (16_2) vesicles,[3] we have observed time-dependent uv spectral behavior of Ellman's *anion* (Ell^{-2}, *cf.*, Figure 1)[4] in 16_2 vesicles. These apparent permeation phenomena are described here and shown to afford kinetic parameters similar to those derived from the ANS fluorescence[1c] and dithionite[3] chemical studies.

As shown in Fig. 1, Ell^{-2} is very sensitive to its microenvironment. The uv spectra afford λ_{max} (nm) and (log ε) values of 407 (4.14) in water, 438 (4.15) in $5x10^{-3}$ M cetyltrimethylammonium bromide (CTABr) micelles, and 450 (4.16) in $2x10^{-3}$ M 16_2 vesicles.[5] Time dependent phenomena are observed when aqueous Ell^{-2} is mixed (< 1 msec) with 16_2 vesicles[6] and then, after a variable aging time (50 msec - 10 sec), admitted to the observation cuvet of a Dionex D-132 multimixing unit.[3] Representative data, monitored at 450 nm, are shown in Figure 2. These time dependent absorption increases each yield apparent first order rate constants, $\underline{k}_{app}$ ~0.9 ± 0.1 sec^{-1}. With increasing aging of the Ell^{-2}/16_2 sample, decreasing ΔA_{450} is observed, and, after 8-10 sec aging only a maximum, time independent absorbance could be detected. Combination of Ell^{-2} with CTABr *micelles* gives a similarly intense but *time independent* absorbance at our shortest aging time, 1.5 msec.

We interpret these observations as very fast, non-measurable (< 1 msec)[1a] binding of Ell^{-2} to the 16_2 vesicles or CTABr micelles followed, in the vesicular case, by measurable time-dependent redistribution of the Ell^{-2} between surface (exovesicular) and unspecified endo-

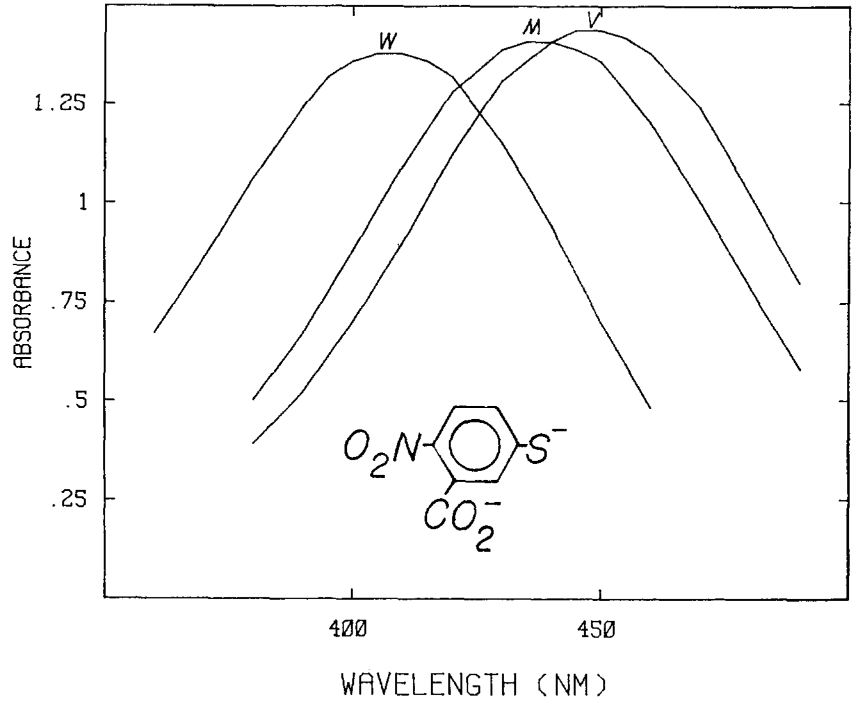

Figure 1. Uv spectra of 1.0×10^{-4} M Ellman's anion in water (W), 5×10^{-3} M micellar CTABr (M), and 2×10^{-3} M 16_2 vesicles (V); all solutions were prepared in T-8 buffer.[5]

vesicular binding sites. This process leads to a net bathochromic spectral shift which appears as an increasing absorbance at 450 nm.[7]

Note, however, that $\underline{k}_{app}$ for approach to the equilibrium $Ell^{-2}/16_2$ distribution is not independent of $[Ell^{-2}]$ or $[16_2]$. As $[Ell^{-2}]$ decreases from 5×10^{-5} M to 2.5×10^{-5} M, with $[16_2] = 5\times10^{-4}$ M, $\underline{k}_{app}$ increases from 0.9 ± 0.1 sec^{-1} to 1.7 ± 0.2 sec^{-1}. As $[16_2]$ decreases from 5×10^{-4} M to 2.5×10^{-4} M, with $[Ell^{-2}] = 2.5\times10^{-5}$ M, $\underline{k}_{app}$ decreases from 1.7 sec^{-1} to 0.8 sec^{-1}. If we take 5,000 - 10,000 as the aggregation number of the 16_2 vesicles,[8] then the ratio of Ell^{-2} anions to 16_2 vesicles varies from ~250 - 1000 under our conditions. We might then expect modification of the cationic vesicular surface by the excess anionic Ell^{-2}. Binding and redistribution of Ell^{-2} in the 16_2 vesicles would be increasingly electrostatically hindered as the excess of Ell^{-2} rises, and more time would be required to achieve the equilibrium distribution at higher anion/vesicle ratios.

Comparison of the present $Ell^{-2}/16_2$ results with those obtained for the exovesicular to endovesicular redistribution of Ellman's reagent (the disulfide dimer of Ell^{-2})[3] or ANS[1c] in 16_2 vesicles affords $\underline{k}_{app}$ (sec^{-1}) values of 0.8-1.7 (Ell^{-2}), 3.8 (Ellman's reagent), and 3.5 (ANS). Although determined with three different probes using three different methods, these

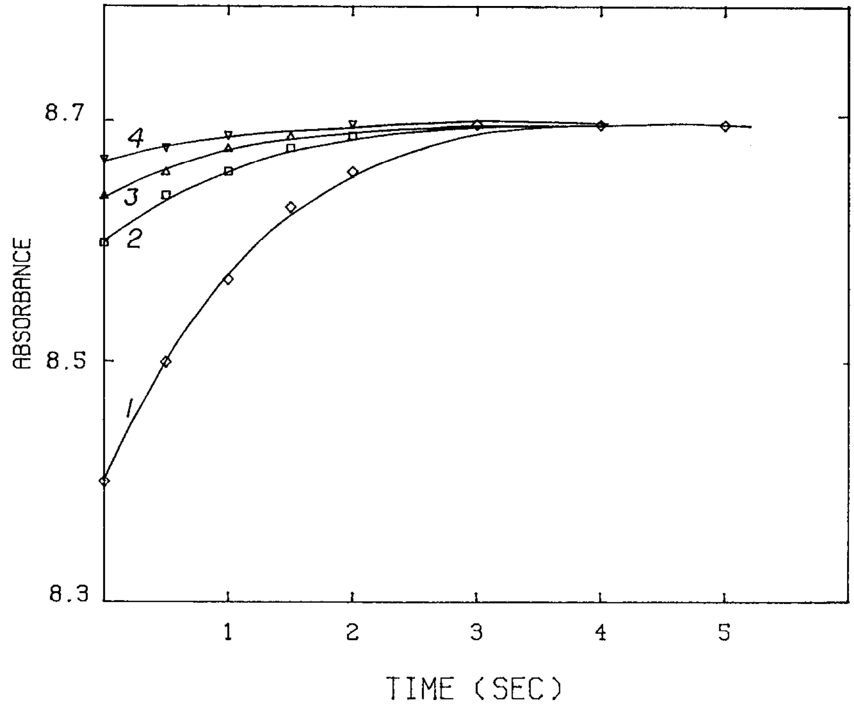

Figure 2. Time dependent changes in the absorbance (arbitrary units) at 450 nm of 5×10^{-5} M $E11^{-2}$ following addition to 5×10^{-4} M 16_2 vesicles in T-8 buffer.[5] The $E11^{-2}/16_2$ solutions were aged for (1) 0.046, (2) 2.05, (3) 5.05, or (4) 7.05 sec before recording the data.

$\underline{k}_{app}$ values are of similar magnitude, indicative of related redistribution processes for each of these hydrophobic anions in cationic 16_2 vesicles. We note too that the binding of *p*-hexyloxynitrobenzene to cationic vesicles is reported[2] to exhibit biphasic dynamic behavior characterized by relaxation times of 0.71 and 2.17 sec. The latter parameter, taken to represent penetration of the probe from the vesicular surface to its hydrophobic interior,[2] is of an order similar to the time dependent permeation phenomena observed in our laboratory.

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References and Notes

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(4) Ell^{-2} was obtained by dithiothreitol reduction (aq. Na_2CO_3) of Ellman's reagent, followed by acidification to afford the (solid) diprotonated form of Ell^{-2}. The acidic form was dissolved as needed in pH 8 buffer to afford Ell^{-2}.

(5) Experiments were carried out in N_2-purged, 0.01 M aqueous Tris buffer, pH 8.0, $\mu = 0.01$ (KCl) ("T-8 buffer").

(6) 16_2 surfactant was sonicated in T-8 buffer using a Braun-Sonic model 1510 probe type sonifier (70 W, 30 min, 60°C). Vesicles were filtered before use through 0.8 μ Millipore "Millex-PF" filters. These conditons afford 16_2 vesicles with 400-500 nm hydrodynamic diameters by dynamic light scattering measurements.

(7) The observed ΔA_{450} is a small (~4%) portion of the overall A_{450}. Most of the Ell^{-2} absorbance is immediately established upon mixing of the Ell^{-2} and vesicle solutions; only ΔA_{450} due to redistribution is measurable.

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