Tetrahedron Letters,Vol.25,No.37,pp 4063-4066,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

TRANS-MEMBRANE TRANSPORT OF 1-ANILINO-8-NAPHTHALENESULFONATE IN SIMPLE CATIONIC SURFACTANT VESICLES

Robert A. Moss,* Thomas F. Hendrickson, Shanti Swarup, Yongzheng Hui, Luis Marky, and Kenneth J. Breslauer
Department of Chemistry, Rutgers, The State University of New Jersey
New Brunswick, New Jersey 08903

Summary. The title fluorescent probe crosses dihexadecyldimethylammonium bromide vesicular membranes 1-2 orders of magnitude faster than it crosses the liposomal membranes of dipalmitoylphosphatidylcholine.

The membrane mimetic properties of simple surfactant vesicules continue to attract much attention.[1] Fundamental to the problem of chemically differentiating exovesicular and endovesicular loci of fully-functionalized, synthetic surfactant vesicles,[2] is the competition between reaction of an added substrate at the exovesicular surface and its permeation to endovesicular reactive sites.[3] Studies have been made of the permeation of simple ions in synthetic surfactant vesicles,[4] but kinetic investigations of more complicated permeants are few.[5] The useful fluorescent probe of trans-membrane transport, 1-anilino-8-naphthalenesulfonate (ANS), although extensively studied in liposomal assemblies such as dipalmitoylphosphatidylcholine (DPPC),[6] does not appear to have been examined with cationic vesicles, e.g., dihexadecyldimethylammonium bromide (16_2). We now provide initial ANS permeation results for 3 different types of 16_2 vesicles, and comparative data for small and large DPPC liposomes. Additionally, we describe other important properties of these molecular assemblies.

Vesicle preparation. Vesicles of 16_2 and DPPC were prepared in pH 7.4, 0.01 M aq. imidazole buffer by (a) sonication [Braun-Sonic model 1510, immersion probe, 55-60°C, 100 W, 10 min]; (b) rapid injection (250 µl Hamilton syringe) of ethanolic surfactant into buffer at 55-60°C;[7] or (c) slow injection (0.15 ml/min) of 1 ml of surfactant in $CHCl_3$ into 4 ml of buffer at 70°C,[8] with $CHCl_3$ removed by continuous N_2 sparging during injection. Methods (a)[1] and (c)[8] afford unilamellar vesicles or liposomes, whereas (b)[7] yields multilamellar assemblies. Our own electron microscopic investigations are in accord with these reports. All vesicle preparations were cooled to 23°C and filtered through 0.8 µ Millex-PF filters before use.

Dynamic light scattering. Apparent hydrodynamic diameters were determined by dynamic light scattering and are collected in Table I. Sonication affords "small" vesicles,[1] rapid injection gives "medium" sized assemblies,[7] and slow injection affords large, unilamellar vesicles.[8] All types of vesicles are larger at 45°C, above their phase transition temperatures (T_c), than they are at 23°C, below T_c. In each size class, the DPPC liposomes appear larger than the corresponding 16_2 vesicles. The large vesicles are size-stable (light scattering) for > 7 days, but the small and medium vesicles become visibly inhomogeneous after 12 or 24 hrs, respectively.

Table I. Physical Properties of Vesicles and Liposomes[a]

Vesicle	Type	Diameter (Å)[b] 23°C	(Var.)	45°C	(Var.)	Phase Transitions[c] T_{c_1} (°C)	ΔH_1[d]	T_{c_2} (°C)	ΔH_2[d]
16_2	small	330	(0.45)	560	(0.72)	25.1 26.5	5.46	40.2	2.04
16_2	medium	1100	(1.02)	1250	(0.90)	24	<0.3	35.2	2.76
16_2	large	2740	(1.13)	3460	(1.19)	25	8.10	36.8	0.50
DPPC	small	480	(0.52)	830	(0.69)	37[e]	7.54[f]	41.1	7.54[f]
DPPC	large	3600	(1.10)	4300	(1.20)	35[g]	1.60[g]	41.2[g]	8.20[g]

[a]Methods of vesicle preparation and details of aqueous medium are given in the text. [b]Apparent hydrodynamic diameter, Nicomp TC-100 computing autocorrelator; Ar laser (488 nm), 90° scattering angle, Hazeltine microcomputer, cumulant program. The variant (var.) indicates marked polydispersity when >1.0. [c]Microcal-1 differential scanning calorimeter [W.M. Jackson and J.F. Brandts, Biochem., 9, 2294 (1970)]. T_c values are rounded to nearest deg. [d]Enthalpy of transition in kcal/mol. [e]Very similar values appear in ref. 9. [f]Total for both transitions. [g]Values for "large multilamellar" liposomes given in ref. 9.

Table II. Fluorescence of Pyrene and Permeation of ANS in Vesicles and Liposomes

Vesicle	Type	Pyrene Fluorescence[a] I_{390}/I_{370}	I_{475}/I_{392}	$\tau_1/2$ (s^{-1}) of ANS Permeation[b,c] 15°C	25°C	35°C	40°C	45°C
16_2	small	0.78	0.14	0.40[d]	0.20	0.08	0.07	
16_2	medium	0.78	0.18	0.60[e]	0.19[f]			
16_2	large	0.78	0.32	0.08	0.07	0.03		
DPPC	small	0.88	0.23	g	g	g	4.5	9.0[h]
DPPC	large	0.93	0.45	i	i	2.5	14.[j]	8.0

[a]$[16_2]=[DPPC]=1.0\times10^{-3}$M; SLM 4800 spectrometer, Xe lamp; 23±1°C; $[Pyrene]=2\times10^{-6}$M (390/370) or 1×10^{-5}M (475/392); λ_{ex}=337 nm. [b]Durrum D-130 stopped-flow spectrometer modified for fluorescence (90°), 150 W Xe lamp (368 nm), 420 nm cut-off filter. Final concentrations: $[vesicles]=5.0\times10^{-4}$M, $[ANS]=5.0\times10^{-5}$M. See text for vesicle preparation and buffer. [c]$\tau_1/2$ is half-life time for development of ANS fluorescence at indicated temperatures. [d]Maximum, 0.70 sec at 12°C. [e]Maximum value at 14-15°C. [f]23°C. [g]No fluorescence below ∿38°C. [h]Maximum, 14 sec at ∿43°C. [i]No fluorescence below ∿30°C. [j]Maximum, 24 sec at ∿42°C.

Phase transitions. Differential scanning calorimetry of $2x10^{-3}$ M vesicle solutions afforded the T_c values and associated enthalpies in Table I. Generally, T_c values for the major transitions of the DPPC liposomes are significantly higher than those of the corresponding 16_2 residues. The two widely-separated, high-enthalpy T_c's of the small 16_2 vesicles may signify the presence of different vesicle populations.[9] The T_c data define appropriate temperature ranges for the permeation experiments.

Fluoroescence studies. Table II collects fluorescence data for pyrene (Py) solubilized in vesicles and liposomes. The intensity ratio of Py fluorescence peaks III and I (∿390 and ∿370 nm) reflects the polarity of the pyrene's microenvironment.[10a] Py solubilized in micellar CTABr (I ratio=0.80) and all types of 16_2 vesicles experiences a polarity comparable to that of CH_3OH (I ratio=0.75).[10a] Py microenvironments seem less polar in DPPC liposomes, resembling that of ethanol (I ratio=0.91).[10a] The intensity ratio of Py excimer fluorescence (475 nm) to monomer fluorescence (392 nm) is indicative of the medium's microviscosity.[10b] The data in Table II suggest that DPPC liposomes offer less resistance to Py translation than corresponding 16_2 vesicles, and that translation is easier in the larger vesicles.

Trans-membrane transport. The time-dependent development of ANS fluorescence on stopped-flow mixing of aq. solutions of ANS and DPPC liposomes has been taken as a kinetic measure of the transport or permeation of the ANS across the exoliposomal surface and into the liposome.[6a] We measured half-lives ($\tau_{1/2}$) for the development of ANS fluorescence with each type of vesicle, over temperatures from ∿10°-45°C. Selected data appear in Table II. With 16_2 vesicles, permeation occurred rapidly (<1 sec) at all temperatures sampled, both below and above T_c. With DPPC liposomes, no permeation was evident below ∿30°C, and $\tau_{1/2}$ values were 1-2 orders of magnitude greater than in 16_2 vesicles at comparable temperatures.[11] There is no direct correlation between "membrane fluidity" (Table II) as reported by Py translation and excimer formation, and ANS transport; the former phenomenon is greater in DPPC liposomes than in 16_2 vesicles, but ANS transport is slower in the liposomes. Possibly, the charge type of the surfactant assemblies plays a role in determining the rate of trans-membrane transport of ANS and other large ionic probes (at least above the T_c). Anionic ANS may experience more difficulty crossing the zwitterionic surface of DPPC, where it is strongly bound,[6b] than the cationic surface of 16_2.[12] This is not true of simple ions, however.[13]

Our results are potentially important for the design of exovesicular reactions.[2,3] Taking $\tau_{1/2}$ ∿0.1-0.2 sec for the permeation of 16_2 vesicles at 25°C by ANS-like probes, leads to k_{perm} ∿3-7 sec^{-1}. If 16_2 vesicles can serve as models for fully-functionalized cationic surfactant vesicles, then exovesicular reactions of the latter with ANS-like substrates will require $k_{\psi} >> 10$ sec^{-1} to ensure exclusive exovesicular reaction. We intend to extend the present studies to other kinds of vesicles and probes in order to better define those factors essential to locus-specific vesicular reactions.

Acknowledgments. We thank the U.S. Army Research Office and the Petroleum Research Fund for financial support. Y. Hui thanks the Shanghai Institute of Organic Chemistry, Academia Sinica, China, for an Academic Leave. R.A. Moss is grateful for an Erna and Jacob Michael Visiting Professorship and to The Weizmann Institute of Science for its hospitality during the composition of this manuscript. We are also grateful to Prof. D. Haynes for helpful discussions.

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11. Our 40°C (small) DPPC result resembles that of Haynes[6a] obtained with dimyristoylphosphatidylcholine liposomes ($\tau_1/_2$=5.7±1.3 sec, 35°C, pH 7.4, 0.01 M imidazole buffer).
12. For example, ANS does not permeate (no fluorescence) anionic dicetyl phosphate vesicles (below 45°C), nor does it permeate net anionic, 10% dicetyl phosphate - 90% DPPC sonicated covesicles.However, cosonicated vesicles of 90% DPPC - 10% 16_2 (which are net cationic) gave lower $\tau_1/_2$ permeation values at all temperatures, relative to pure DPPC vesicles.
13. Reference 4, papers of Okahata et al.

(Received in USA 5 June 1984)