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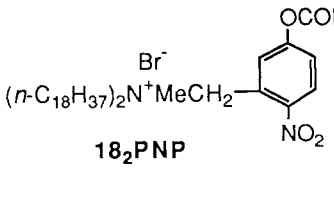
Some Kinetic Properties of Mixed Chain Alkylammonium Ion Vesicles

Tsunehisa FUJITA and Robert A. MOSS*
Department of Chemistry, Rutgers, The State University of New Jersey,
New Brunswick, New Jersey 08903, U.S.A.

The relationship between lipid structure and lipid dynamics of bilayer covesicles derived from mixed chain dialkylammonium ion lipids was investigated.

The properties of molecular aggregates have attracted much attention, and bilayer lipid membranes have been closely studied for applications in the pharmaceutical field.[1] Most of the membranes used for these purposes were constructed of the phosphatidylcholine type natural lipids that have glycerol "backbones". In contrast, are the synthetic, dialkylammonium ion lipid bilayer membranes whose properties were first investigated by Kunitake and Fendler.[2] Other types of synthetic lipids for membrane formation include alkylpyridinium,[3] dialkylsulfosuccinate,[4] and dialkylphosphate.[5] Less is known about the relation of structure to properties of the synthetic lipid membranes as compared to the natural lipid membranes.[6] We reported that H^+/OH^- permeation across the membrane[7] and lipid trans-bilayer migration ("flip-flop")[8] become more difficult with increasing chain length of the dialkylammonium ions in bilayer membranes. Here, we describe the effects of *dissimilar* alkyl chains on the permeation and dynamic properties of dialkylammonium ion vesicles.

We studied two types of "mixed chain" lipid systems: (1) covesicles of 2 different, but equal chain length dialkylammonium ions $[(C_m)_2N^+Me_2 \cdot Br^- + (C_n)_2N^+Me_2 \cdot Br^-]$, and (2) vesicles of mixed chain dialkylammonium ion lipids $[C_mC_nN^+Me_2 \cdot Br^-]$.[2c] These vesicular systems largely consisted of the indicated nonfunctional dialkylammonium ion lipids (91%), but also contained 9 mol-% of the probe functional lipid, *N,N*-dioctadecyl-*N*-methyl-*N*-(2-nitro-5-benzoyloxy)benzylammonium bromide (**18_2PNP**); see Scheme 1.



18_2PNP

$(n\text{-}C_nH_{2n+1})_2N^+Me_2$, Br^-

16_2: n = 16
18_2: n = 18
20_2: n = 20
22_2: n = 22

$(n\text{-}C_mH_{2m+1})(n\text{-}C_nH_{2n+1})N^+Me_2$, Br^-

16-18: m = 16, n = 18
16-20: m = 16, n = 20
18-20: m = 18, n = 20
18-22: m = 18, n = 22

Scheme 1

The nonfunctional lipids were prepared by quaternization of the appropriate dimethylalkylamine with the appropriate alkyl bromide.[9] The functional lipid, **18_2PNP**[10] was prepared by quaternization ($CHCl_3$, 25 °C, 48 h) of *N,N*-dioctadecyl-*N*-methylamine with 4-nitro-3-(bromomethyl)phenyl benzoate; it had mp 72.5-73.5 °C. All lipids were purified by chromatography, recrystallized from EtOAc or acetone, and characterized by NMR and microanalysis. Lipid structures are shown in Scheme 1.

Covesicles of functional (**F**) and nonfunctional (**NF**) lipids were generated by the sonication of $CHCl_3$-evaporated films of 1:10 **F/NF** lipid blends in pH 3.9 aqueous HCl, μ =0.01 (KCl).[11] Dynamic light

scattering measurements afforded hydrodynamic diameters for the covesicles in the range 300 to 400 Å. The gel to liquid crystal transition temperatures (T_c) of the covesicles were determined from temperature-dependent discontinuities in the fluorescence polarization of covesicallized 1,6-diphenyl-1,3,5-hexatriene,[12] and are recorded in Table 1.

Table 1. Gel to Liquid Crystal Transition Tempertures (T_c) of Vesicles[a]

No.	Matrix	Observed T_c /°C	Calculated T_c /°C[b]	ΔT_c /°C
1	16_2	21	--	--
2	18_2	36	--	--
3	20_2	45	--	--
4	22_2	66 [c]	--	--
5	16 - 18	26	29	-3
6	16 - 20	26	33	-7
7	18 - 20	40	41	-1
8	$18_2 + 20_2$	42	41	+1
9	18 - 22	40	51	-11
10	$18_2 + 22_2$	44.5	51	-6.5

a) Nos. 1-3 were observed in the presence of the corresponding *N*,*N*-dialkyl-*N*-methyl-*N*-(2-nitro-5-hydroxy)benzylammonium bromide (**n_2PNPOH**); **n_2** : **n_2PNPOH** = 10 :1; Nos. 5-10 were observed in the presence of **18_2PNP**. b) The "calculated" T_c is the simple average of the T_c's of the symmetrical lipid vesicles of the appropriate lipid chain lengths. For example, the calculated T_c for Nos. 9 or 10 is the average of the T_c's of Nos. 2 and 4. c) T_c of the *holovesicle* **22_2** was reported as 70 °C.[13]

The kinetics and dynamic properties of the covesicles were investigated as follows. We first surface-differentiated the covesicles by brief exposure to 1 x 10^{-4} mol/L glutathione in 0.005 mol/L, pH 8 Tris buffer, μ =0.01 (KCl).[6,8,10] The *exovesicular* *p*-nitrophenyl benzoate residues of the functional lipid (**18_2PNP**) were thus rapidly cleaved to the corresponding *p*-nitrophenoxide moieties, a process that was followed spectrophotometrically at 400 nm, affording the rate constants k_f (Table 2). Subsequent, slower *endovesicular* cleavages of *p*-nitrophenyl benzoate residues were rate limited by H^+/OH^- permeation across the vesicle membranes, driven by the imposed pH 8/3.9 gradient, yielding the rate constants k_s[6,7,10] (Table 2). After the end of the endovesicular cleavage, reacidification with HCl to pH 2.6 brought about an immediate protonation of the exovesicular *p*-nitrophenoxide ions (instantaneous loss of proportionate absorbance at 400 nm), followed by a time-dependent loss of the residual 400 nm absorbance, affording the permeation rate constant, k_{per}.

For studies of lipid flip-flop, the following protocol was adopted. Immediately after the end of exovesicular ester cleavage, the external pH was lowered to 3.9 (HCl), preventing further ester cleavage. The differentiated vesicles were then "incubated" at a specific temperature for a given time, permitting lipid flip-flop. The pH was then readjusted to 8 (NaOH), initiating a new "fast" ester cleavage that was due to the formerly endovesicular functional lipid molecules that had "flipped" to exovesicular sites during the incubation.[6,10] The final, subsequently observed k_s reaction represented the cleavage of residual endovesicular **18_2PNP**. The extent of flip-flop equilibration induced by a particular incubation regimen could be estimated from the partition between the post-incubation k_f and k_s reactions. Approximate flip-flop equilibration half-times were derived from series of incubation experiments carried out with appropriate time increments. Results appear in Table 2.

We note at once that k_f, corresponding to the initial exovesicular ester cleavage reaction, is rather independent of the vesicles' chain length composition, with k_f varying from 0.1-0.25 s^{-1}. On the other hand, both k_s (which is rate limited by H^+/OH^- permeation[6,8,10]) and k_{per} depend on chain length, becoming smaller as the lipid chain length increases; *cf.*, Nos. 1-3.[14] This behavior is in keeping with expectations.[15]

Systems 4-11 are constructed of heterogeneous chain length lipids ranging from C_{16}-C_{22}. Several interesting observations can be made from the data in Table 2. Firstly, *covesicles* constructed of 2 different homogeneous lipids (*e.g.*, No. 6, **18_2 + 20_2**) are similar in k_s and k_{per} to the vesicles constructed of the corresponding mixed chain lipids (*e.g.*, No. 10, **18-20**). Pertinent comparisons are Nos. 4 with 8, 5 with 9, 6 with 10, and 7 with 11. These results suggest that the **M_2/N_2** covesicles and the corresponding **M/N** vesicles are similarly packed, and that individual lipid domains are not generally formed in the covesicles.

Table 2. Dynamics of Vesicles[a]

No.	Matrix	k_f /s^{-1}	10^3 k_s /s^{-1}	f : s[b]	k_{per} /s^{-1}	$t_{1/2}$, flip[c]
1	16_2	0.18	> 30.0	94 : 6	0.94	< 1 min at 25 °C
2	18_2	0.17	1.84	50 : 50	0.023	2 min at 40 °C[d]
3	20_2	0.16	18.64, 0.13	57 : 14 : 29	0.012	10 min at 40 °C[d]
4	16_2 + 18_2	0.11	7.25	58 : 42	0.11	2 min at 25 °C
5	16_2 + 20_2	0.15	49.0	81 : 19	0.47	< 1 min at 25 °C
6	18_2 + 20_2	0.26	0.46	59 : 41	0.0067	2 min at 40 °C[d]
7	18_2 + 22_2	0.25	0.30	60 : 40	0.018	50 min at 25 °C[e]
8	16 - 18	0.15	3.34	52 : 48	0.076	< 1 min at 25 °C
9	16 - 20	0.20	23.0, 8.10	58 : 22 : 20	0.201	2 min at 25 °C
10	18 - 20	0.25	0.36	55 : 45	0.006	10 min at 25 °C
11	18 - 22	0.25	0.38	62 : 38	0.028	5 min at 25 °C

a) See Scheme 1 for structures. The reaction temperature was 25 °C. b) Ratio of fast to slow kinetic phases. c) Approximate half-time for the decay of surface differentiation; see Refs. 8 and 10. d) Little or no flip-flop was observed after 20 min of incubation at 25 °C. e) Fast flip-flop was observed at 40 °C ($t_{1/2}$ < 1 min).

There are indications that vesicular systems in which the chain length "mismatch" is 2 carbon atoms (Nos. 4, 6, 8, 10) are better packed than those systems in which the mismatch is 4 carbons (Nos. 5, 7, 9, 11). Thus, k_s or k_{per} are frequently larger for the latter systems than would be anticipated. For example, k_s and k_{per} of **16_2 + 20_2** (No. 5) are much larger than the corresponding parameters of **16_2 + 18_2** (No. 4). Similar phenomena are observed with **16-20** vs. **16-18** (Nos. 9 and 8). Analogous, less dramatic, enhancements in k_{per} are seen upon comparisons of the 18 and 22 carbon vesicular systems, Nos. 7 vs. 6, and 11 vs. 10.

Thus, the properties of the vesicular systems are very sensitive to lipid chain length. When there is a serious mismatch in chain lengths, the vesicles appear to be less well packed and more "leaky" to H^+/OH^-. Similar ideas follow from a consideration of the T_c data in Table 1. When the chain length mismatch is 2 carbon atoms, the "calculated" T_c is similar to the observed T_c (Nos. 5, 7, and 8). However, when the chain length mismatch is 4 carbon atoms (Nos. 6, 9, and 10), the observed T_c is considerably *lower* than the value calculated from the average of the appropriate holovesicular T_c's.

Flip-flop equilibrations of the surface differentiated vesicular systems reveal similar dependences on chain length matching. In constant chain length systems (Nos. 1-3), or in 2 carbon mismatched systems (Nos. 4 vs. 6

or 8 vs. 10), increasing chain length engenders greater resistance to flip-flop lipid redistribution. The 4 carbon mismatched systems, however, appear to equilibrate faster than anticipated; compare Nos. 5 with 4, 7 with 6, and 11 with 10.

In glycerol-based phospholipid bilayers, there is evidence that grossly mismatched chains that are longer than 16 carbon atoms can interdigitate among the chains of the opposing bilayer leaflet and lead to greater local ordering of chains, decreased fluidity, and, presumably, better packing.[16,17] Our present results with dialkylammonium ion bilayers suggest that interdigitation here, assuming that it does occur in mismatched chain length bilayers, does not necessarily lead to enhanced bilayer packing and stability.[17]

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