Tetrahedron Letters, Vol.31, No.52, pp 7559-7562, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

DYNAMICS OF LIPOSOMES CONSTRUCTED FROM PHYTANYL LIPIDS

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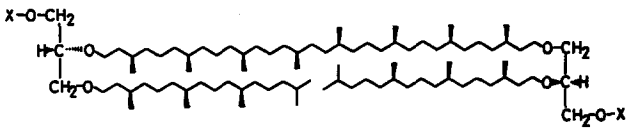
Summary. Liposomes constructed from phytanyl lipids 3-F/3-NF exhibit very facile trans-bilayer ("flip-flop") lipid migration.

Unusual lipids have been isolated from the thermophilic archaebacteria that thrive at high temperatures (50-80°C).[1] The example shown below has isoprenoid residues bound through ether linkages to phosphoglycerol residues bearing polar head groups.[1] This bifunctional lipid is long enough to form a single layer liposomal membrane.[1] Because the unusual

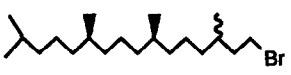


thermal resistance of the archaebacteria may reflect unique thermal properties of their uncommon membrane lipids, there is much current interest in the synthesis and behavior of these lipids and their analogues.[1-3]

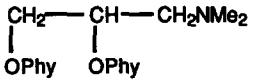
Recently, we developed methods for the chemical differentiation of the outer and inner surfaces of synthetic bilayer liposomes or vesicles,[4] and could then follow the decay of the imposed chemical asymmetry due to lipid trans-bilayer migration ("flip-flop").[4,5] The dynamics of the lipid molecules within the liposome could be related to lipid molecular structure.[5,6] Now we have synthesized novel phytanyl lipids, examined their flip-flop dynamics within a liposomal assembly, and correlated the observed behavior with key elements of molecular structure. These new results are reported here.

Phytol (Aldrich) was reduced to phytanyl alcohol (hydrazine hydrate and propionic acid, EtOH, 50-60°, 13 days, 89%),[7] which was converted to phytanyl bromide (1) by reaction with 47% aqueous HBr (cat. H_2SO_4, 140°/5.5 h, 81%). Reaction of 1 (refluxing THF, 64 h) with the dialkoxide derived (NaH, THF) from rac-3-dimethylamino-1,2-propanediol gave the dimethylaminodiphytanyl ether, 2, in 39% yield after purification by chromatography on silica gel (CH_2Cl_2/MeOH). Tertiary amine 2 was then quaternized with either MeBr (Et_2O, 25°, 72 h) to afford surfactant 3-NF (75%, chromatography as above), or with 3-(bromomethyl)-4-nitrophenyl benzoate[4] (THF, 25°, 48 h) to give surfactant 3-F (38% after chromatography on silica with $CHCl_3$/MeOH). Both 3-NF and 3-F gave structurally consistent nmr spectra and acceptable elemental analyses. We also made use of the related n-hexadecyl ether nonfunctional (4-NF) and functional (4-F), surfactants, that were available from a previous study.[6]

Covesicles of 3-F/3-NF, 4-F/3-NF, 3-F/4-NF, and 4-F/4-NF were created by sonication of $CHCl_3$-cast films of 1:10 F/NF surfactant blends in pH 3.9 aqueous HCl, μ = 0.01 (KCl).[8] The



1 (Phy-Br)



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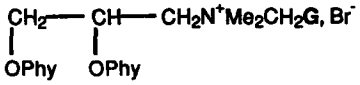
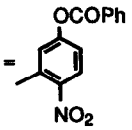
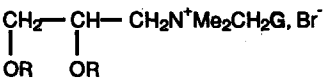


3-NF, G = H

3-F, G = 



4-NF, G = H, R = n-$C_{16}H_{33}$

4-F, G as in 3-F, R = n-$C_{16}H_{33}$

gel to liquid crystal phase 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;[4,9] results appear in Table 1. The phytanyl covesicle, 3-F/3-NF had T_c < 10°C, much lower than the dihexadecyl covesicle, 4-F/4-NF, where T_c = 37°C.[6] We assume that the T_c of the 1:10 4-F/3-NF covesicle is similar to that of the all phytanyl covesicle (<10°C). The T_c of the 1:10 3-F/4-NF covesicle was measured as 39°C.

To study lipid dynamics within the covesicles, we first surface-differentiated covesicles (prepared at pH 3.9) by brief exposure to 1 x 10^{-4} M glutathione in 0.01 M, pH 8 Tris buffer, μ = 0.01 (KCl).[4-6] Exovesicular p-nitrophenyl benzoate residues (G) of the functional lipids (3-F or 4-F) 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 1). Subsequent, slower endovesicular cleavages of G were rate limited by H^+/OH^- permeation across the liposomal membranes, driven by the imposed pH 8/3.9 gradient, yielding rate constants k_s[4,6,10] (Table 1).

The data indicate that the 3-F/3-NF and 4-F/3-NF covesicles (i.e., covesicles with >90% phytanyl residues) are simply too fluid and permeable at 25° (T_c < 10°) to maintain the pH gradient long enough to permit exovesicular/endovesicular chemical differentiation.[11] They can, however, be differentiated at ~5°C. In contrast, the covesicles with >90% dihexadecyl ether residues (3-F/4-NF and 4-F/4-NF) can be readily differentiated at 25°C, below their Tc's.

Immediately after surface differentiation of the covesicles, the external pH is lowered to 3.9 (HCl), preventing further benzoate cleavage. The differentiated vesicles are then "incubated" at 12° or 25°C for a given time, permitting lipid flip-flop, and then readjusted to pH 8 (NaOH). The new, fast (k_f) appearance of p-nitrophenoxide absorbance, initiated by the pH readjustment, represents cleavage of formerly endovesicular G residues that have "flipped" to exovesicular sites during the incubation.[4,6] The subsequently observed k_s

Table 1. Dynamics of Phytanyl Liposomes

Covesicle[a]	T_{rx} (°C)[b]	T_c (°C)[c]	k_f, s^{-1}	k_s, s^{-1}	%f : %s[d]	$t_{1/2}$ flip[e]
3-F/3-NF	25	<10	0.098	f	f	
	5-12[g]	<10	0.096	0.011	66:34	<1 min at 12°C
4-F/3-NF	25	<10[h]	0.12	f	f	
	5-12[g]	<10[h]	0.11	0.034	68:32	<1 min at 12°C
3-F/4-NF	25	39	0.18	0.010	59:41	1 min at 25°C
	5-12[g]	39	0.086	0.0050	51:49	2-3 min at 12°C
4-F/4-NF	25	37[i]	0.20	0.00016	64:36	>10 min at 25°C[j]
						2-4 min at 40°C[k]

[a]See text for structures; F/NF = 1:10. [b]Reaction temperature. [c]Temperature of gel to liquid crystal transition. [d]Ratio of fast to slow kinetic phases. [e]Approximate half-time for decay of surface differentiation; see refs. 4 and 5. [f]The slow kinetic phase was <12%. [g]Surface differentiation at 5 - 12°; flip-flop studies at 12°C. [h]Estimated; see text. [i]Ref. 6; 4-F/4-NF = 1:7. [j]No flip-flop was observed after 20 min incubation of surface differentiated vesicles at 25°C. [k]$t_{1/2}$ for 1:7 covesicles was determined as 5 min at 40°C in ref. 6.

reaction represents the cleavage of residual endovesicular G groups.[12] The extent of flip-flop equilibration induced during a particular incubation follows from the partition between the post-incubation k_f and k_s reactions. Approximate flip-flop half-times can be derived from series of incubation experiments with appropriate time increments; results appear in Table 1.

We have shown that hydrophobic chain length, and especially the way in which the chains are linked to the head group of a lipid molecule, strongly influence the lipid's dynamics within a liposome.[5,6] In the present case, it is clear that chain branching is also a critical determinant of the lipid's resistance to thermally-induced trans-bilayer migration within the liposomal membrane. Thus, branched chain phytanyl liposomes of 3-NF provide fluid, liquid crystalline membranes in which either phytanyl (3-F) or hexadecyl (4-F) functional lipid probes rapidly equilibrate between endovesicular and exovesicular loci; even at 12°C, $t_{1/2}$ < 1 min. In contrast, the straight chain hexadecyl lipid covesicles (4-F/4-NF) are quite resistant to flip-flop at 25°C (>20 min.). This enhanced stability parallels the higher T_c of these liposomes (37°C), which are in their more ordered and rigid gel phase at 25°C, where lipid flip-flop is suppressed. At 40°C, above the T_c, the 4-F/4-NF covesicles exhibit significant flip-flop,[6] with $t_{1/2}$ ~ 2-4 min. The covesicles with 1:10 phytanyl probe (3-F) in a hexadecyl ether lipid matrix of 4-NF exhibit intermediate behavior; they can be differentiated at 25°C, but flip-flop equilibration of probe 3-F is much more rapid than that of probe 4-F in the 4-NF covesicles.

Clearly, the methyl substituents of phytanyl residues interact strongly with neighboring lipids so as to prevent optimal chain packing and facilitate trans-bilayer lipid migration. These effects are most apparent in the phytanyl matrix (3-NF) covesicles, but even 9% of phytanyl chains, as in 3-F/4-NF covesicles, is sufficient to markedly affect the ease of lipid flip-flop.

The disruptive effects of phytanyl residues on lipid chain packing and motional behavior have been demonstrated by calorimetric[2] and nmr studies.[3] Menger et al. have elegantly explored the ability of methyl substituents to disrupt phosphatidylcholine chain packing in both monolayer films and liposomes.[13] Our results make it clear that, whatever special properties may be associated with the isoprenoid lipid membranes of the archaebacteria, the particular arrangement of phytanyl residues designed into the simple model lipids 3-F or 3-NF afford bilayer membranes with substantial lipid mobility.

Acknowledgments. We thank Dr. Kiyoshi Yamauchi of Osaka City University for experimental details concerning the preparation of Phy-Br, and Mr. Shovan Ganguli, of this laboratory, for samples of 4-F and 4-NF. We are grateful to the U.S. Army Research Office for financial support.

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(7) The reduction afforded a mixture of alcohols that were diastereomeric at C_3 (TLC, NMR). Only the major (60%) diastereomer, separated by chromatography on silica gel (6:1 hexane/EtOAc), but of unknown configuration at C_3, was carried through the remaining synthetic steps.
(8) The general procedure is described in ref. 4. Final surfactant concentrations were F = 5×10^{-5} M, NF = 5×10^{-4} M. The sonication methods employed here generally produce unilamellar vesicles.[4] Dynamic light scattering measurements give the hydrodynamic diameters of the 3-F/3-NF and 4-F/3-NF covesicles as 600-700 Å.
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(11) The apparent rate constants for pH gradient decay could be measured[10] using the corresponding p-nitrophenol functionalized covesicles; these rate constants were >0.2 s^{-1}.
(12) In all cases, the sum of the p-nitrophenoxide absorbance corresponding to the initial exovesicular esterolysis (k_f), and the post-incubation k_f and k_s absorbances, was equal to the expected, stoichiometric absorbance for the esterolysis of all G groups.
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(Received in USA 10 October 1990)