Evidence from E.S.R. Studies for Virtual Immobility in Niosomes derived from Steroidal Lariat Ethers

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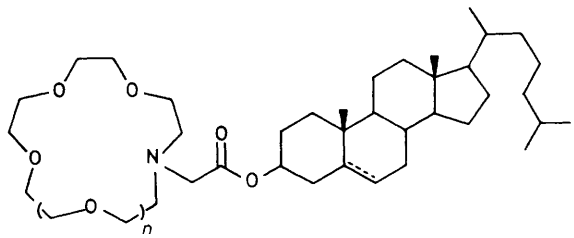
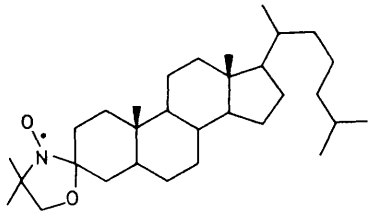
Nitroxide radical probes exhibit correlation times which are 300 times longer in steroidal lariat ether-derived niosomes than are observed in egg lecithin vesicles and the membranes are so rigid that the spectra are almost identical to spectra observed for frozen systems.

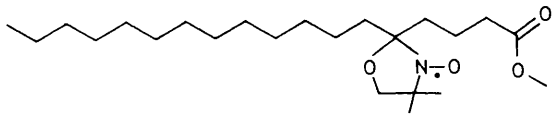
We have recently reported the first example of niosome formation from steroidal lariat ethers.[1] While striking in itself, the more remarkable thing about these aggregates is that they formed in the absence of cations, *i.e.*, the aggregates were (neutral) niosomes. To our knowledge, there is only one other report in the literature of niosomes formed from synthetic monomers in the absence of cholesterol.[2] It is, of course, well known that membrane rigidity is affected by cholesterol content. It was thus of interest to probe the rigidity of these systems which have, by definition, a very high steroid content.



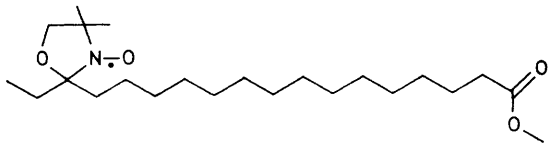
(1) n = 1, cholestanyl
(2) n = 1, cholesteryl
(3) n = 2, cholesteryl



(4)



(5)



(6)

Figure 1

E.s.r. of spin labelled probes is a well-established technique which was introduced by McConnell in 1965.[3] The use of nitroxide radicals has proved useful for the study of smectic crystals[4] as well as for the analysis of molecular interactions and motions in biological membranes.[5] The spin label may be intercalated or attached directly into the regions of interest. The anisotropy of the magnetic interaction of the label with the applied magnetic field reflects the local structure and serves as an indicator of conformational changes in the membrane.[6]

Compound (**1**) was synthesized and niosomes derived therefrom were prepared as recently reported.[1] The unsaturated analogue (**2**), and the 18-membered ring analogue of the latter (**3**) were synthesized and characterized as previously described.[7] Structures of the steroidal lariat ethers and of the commercially available nitroxide probes (**4**)—(**6**) are illustrated in Figure 1. The concentration of steroidal lariat ethers (5 mM) was typically 100-fold that of the nitroxide probes (0.05 mM). After water had been added to the mixture of lariat ether and probe, the combination was sonicated to obtain the probe-containing membrane system. E.s.r. spectra were measured using an IBM Bruker (ER-200 SRC) spectrometer equipped with an IBM 9000 computer. Samples were measured using borosilicate capillary tubes of 1 mm internal diameter. The order parameters, S, were calculated from sufficiently resolved spectra so that $A_{\parallel}$ and $A_{\perp}$ could be measured, as described by McConnell and co-workers.[8]

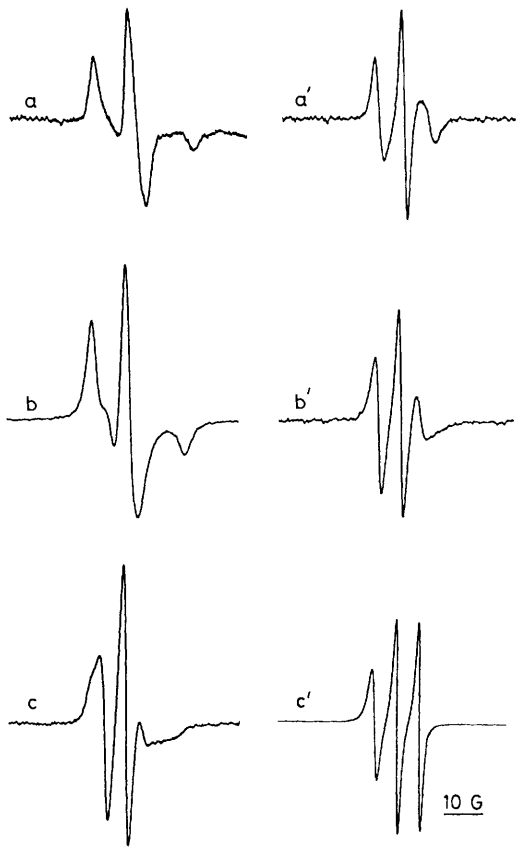


Figure 2. E.s.r. spectra of probes (**4**), (**5**), and (**6**) in niosomes of (**1**) (a—c) and lecithin vesicles (a'—c') (1 G = 10^{-4} T).

Spectra recorded for dispersions of compounds (**1**)—(**3**) containing probes (**4**)—(**6**) exhibit substantial anisotropy and, therefore, diminished mobility of the nitroxide probe. In Figure 2, spectra for probes (**4**), (**5**), and (**6**), in steroidal lariat ether niosomes [derived from (**1**), unprimed] and egg lecithin vesicles (primed) are shown as a, b, and c, respectively. The spectra of these probes in lecithin vesicles suggest, as expected, a more mobile nitroxide environment than observed in the corresponding steroidal systems. Spin labelled niosomes of (**1**) and (**2**) and micelles of (**3**) containing (**4**) exhibit spectra approaching the rigid limit of frozen solutions. The measured values of $2A_{max.}$ for dispersions of (**1**), (**2**), and (**3**) in the presence of probes (**4**), (**5**), and (**6**), are shown in Table 1. Notice that the values corresponding to probe (**4**) in all three dispersions are very near the rigid limit of 66.8 G obtained for the frozen solutions. This is excellent evidence for extreme membrane rigidity. Estimation of the correlation time[9] for probe (**4**) in niosomes of (**1**) (slow-tumbling region) give a value of 500 ns while the corresponding value for the lecithin system is 1 ns. This probe in the lariat ether niosomes is therefore about 300-fold less mobile than in the lecithin system. Similar behaviour was observed for (**4**) in niosomes formed from (**2**) and micelles of (**3**).

It is interesting to compare our systems with a literature value for a phospholipid having a similar phase transition temperature (*ca.* 40 °C). The rigidity of our systems near the crown head-group is similar to that of dipalmitoylphosphatidylcholine (DPPC) near its highly polar head-group [$2A_{max.}$ value for spin label (**4**) is *ca.* 64 G at room temperature].[5a] However, for a probe located *ca.* five carbons from the polar head-group, the values of $2A_{max.}$ are 43.6 and 60 G for DPPC[5c] and our systems, respectively.

Table 1. Experimental values of $A_{max.}$ at room temperature for the different dispersions of (**1**), (**2**), and (**3**) in the presence of probes (**4**), (**5**), and (**6**).

Compound	Probe	$2A_{max.}$/G
(**1**)	(**4**)	64.0
(**1**)	(**5**)	60.0
(**1**)	(**6**)	46.5
(**2**)	(**4**)	65.2
(**2**)	(**5**)	60.5
(**2**)	(**6**)	43.6
(**3**)	(**4**)	64.2
(**3**)	(**5**)	59.7
(**3**)	(**6**)	43.4

Calculation of the order parameters, S,[6b,8] for (**6**) in dispersions of (**1**), (**2**), and (**3**) gave 0.50, 0.47, and 0.46, respectively. This corresponds to an angular amplitude of motion of about 53°. This angle is smaller than those calculated for lecithin liposomes containing (**6**) but agrees with values obtained for phosphatidylcholine liposomes containing (**5**) (>75 and 63°, respectively). We interpret this to mean that the probes align themselves in the steroidal niosomes with the polar head groups in the same direction as the crown ring. For (**4**), the head group is the nitroxide; for (**5**) and (**6**), the ester group is the head group. The nitroxide is thus expected to be in a more rigid environment in compounds (**4**) and (**5**) than when it is at the terminus of a long alkyl chain that probably extends into the more flexible junction of steroid tails in the bilayer.

Simulations of the spectra are underway to quantitate these observations of remarkable rigidity.

We warmly thank the National Institutes of Health for a grant (GM-33940) that supported this work.

Received, 19th July 1988; Com. 8/02933D

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