BBA Report

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A comparative study of cholesterol and a cholesterol-like ESR probe in pure and mixed monomolecular films

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SUMMARY

Pure and mixed monomolecular films of the *N*-oxyl-4′,4′-dimethyloxazolidine derivatives of 5α-androstan-3-one and 5α-cholestan-3-one have been studied with myristic acid. The results show that the film behavior of these ESR probes differ considerably from that of the "parent" cholesterol molecule and indicate that quantitative extrapolation of information from such probes to the parent molecule must be made with considerable caution.

One of the most interesting developments in cell membrane studies over the past five years has been the use of spin-labeled probes to provide information on both structure and function[1,2]. Such work has shown, for example, that the freedom of motion or fluidity of the lipid hydrocarbon chains appear to increase, for both lipid bilayers and membranes, as the terminal methyl group is approached[2]. Other observations are of a semi-quantitative or quantitative nature and particularly where these are concerned objections have been raised that such probes constitute impurities in the membrane and that the behavior observed is not representative of typical membrane lipid components. McConnell and McFarland[1] have not been insensitive to such criticisms but conclude that provided such materials retain "essential" structural features the spin labels constitute "tolerable" structural perturbations. Certainly they have taken increasing care to synthesize and utilize membrane probes of very similar molecular structure to the molecules they are intended to substitute. Nevertheless the suspicion remains that the attachment of a "tumor"-like probe to an alkane chain or a polar group of a membrane lipid may significantly change its behavior, and such a possibility should be checked in each individual instance. One possible approach, and the one described here, would be to carry out a comparative study of both the probe and the "parent" molecule in pure and mixed lipid films. Since a monolayer may be reasonably taken as half a bilayer[3], and at least representative of the lipid portion of a membrane[4], the information thus made available on molecular packing, orientation and interaction should be highly relevant.

Samples of two ESR probes resembling cholesterol were generously provided by Dr. A. Keith of the Genetics Department of the University of California at Berkeley. The samples were the *N*-oxyl-4',4'-dimethyloxazolidine derivatives of 5α-androstan-3-one (3-nitroxide androstane) and 5α-cholestan-3-one (3-nitroxide cholestane). Both samples had been purified using thin-layer chromatography and gave a single spot on a silica-gel plate. Regretably both samples were too small to attempt any further purification.

Films of both probes were spread as pure components and as mixed films with myristic acid (99.5% pure, Applied Science Laboratories) on an automated film balance. The film balance and general techniques[5], as well as modifications of these techniques for mixed films[6] have been adequately described elsewhere. Comparative isotherms for pure cholesterol[7] and mean molecular area plots for mixed films for the cholesterol–myristic acid system[6] have also been published elsewhere.

Both the substituted androstane and cholestane showed isotherms which were significantly more expanded than those for cholesterol[7]. That 3-nitroxide androstane should show an expanded film is only to be expected since the molecule has two hydrophilic groups at the 3- and 17-positions and is capable of assuming a horizontal orientation at the air–water interface, (*cf.* β-estradioldiacetate[7]). In view of this observation it was decided to restrict further work to the 3-nitroxide cholestane since this differed from cholesterol only in the saturation of the 5–6 double bond and the substitution of a nitroxide group for the hydroxyl group at the 3-position. Moreover L.L.M. van Deenen (personal communication) has shown that hydrogenating the cholesterol has little or no effect on its behavior in liposomes. The more expanded isotherm exhibited by the 3-nitroxide cholesterol (see Fig. 1)

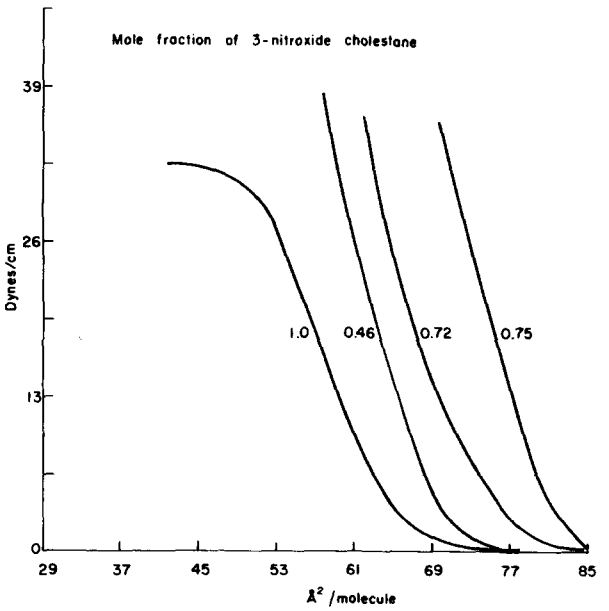

Fig. 1. Isotherms of pure and mixed films of 3-nitroxide cholestane with myristic acid. The numbers at the solid lines refer to the mole fraction of 3-nitroxide cholestane. All isotherms were determined on pH 2, 2 M NaCl aqueous substrates. Temp., 14.5°. Ordinate: surface pressure (π) in dynes/cm. Abscissa: area/molecule in $Å^2$/molecule.

would seem, therefore, to be due to the substituted polar group. Such an observation would be in accord with the earlier observation of Adam *et al.*[8] that a ketone rather than a hydroxyl group at the 3-position would produce an increase in the area/molecule.

Clearly any comparison of the behavior of cholesterol and a cholesterol-like probe must be made in mixed monolayers having a wide compositional range. Fig. 1 shows the isotherms for three intermediate compositional mixed films. It is, however, difficult to evaluate molecular behavior from such a representation. When the same behavior is plotted as a mean molecular area (total area per mixed molecule) as a function of composition for a fixed temperature and pressure the behavior is much more readily interpreted. Thus if in Figs. 2 and 3 the data fall along the broken line either the components would be immiscible or the mixed film would exhibit ideal behavior. Positive deviations such are observed indicate that the two components are miscible and that the 3-nitroxide cholestanone induces even further expansion in the already partially expanded myristic acid film. This is clearly shown by the fact that the expansion is even greater at 8.9° than at 14.5°. At 8.9° (Fig. 2) myristic acid is just below the liquid condensed–liquid expanded transition point[9], and the introduction of a fully expanded second component appears to enhance the mobility of the alkane chains[10]. At 14.5° the myristic acid is already in the liquid expanded state and the extent to which it can be further expanded is reduced (Fig. 3).

In contrast to the above, cholesterol at similar temperatures and surface pressures exhibits the well known condensation effect on myristic acid[6,11], with negative deviations in the mean molecular area plot. The modification of the cholesterol molecule to form an

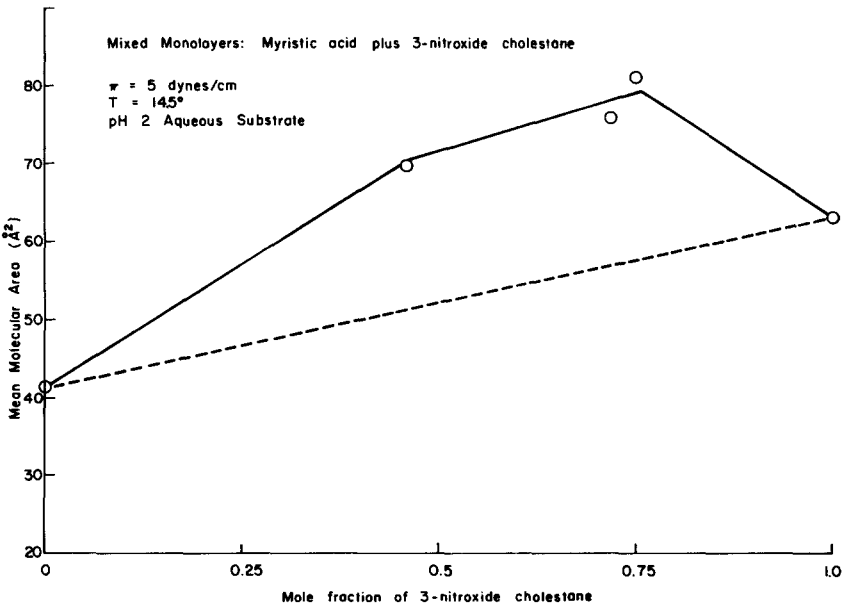

Fig. 2. Mean molecular areas of pure and mixed films of 3-nitroxide cholestane and myristic acid (pH 2, 2 M NaCl aqueous substrates at π = 5 dynes/cm and 8.9°) as a function of the mole fraction of 3-nitroxide cholestane.

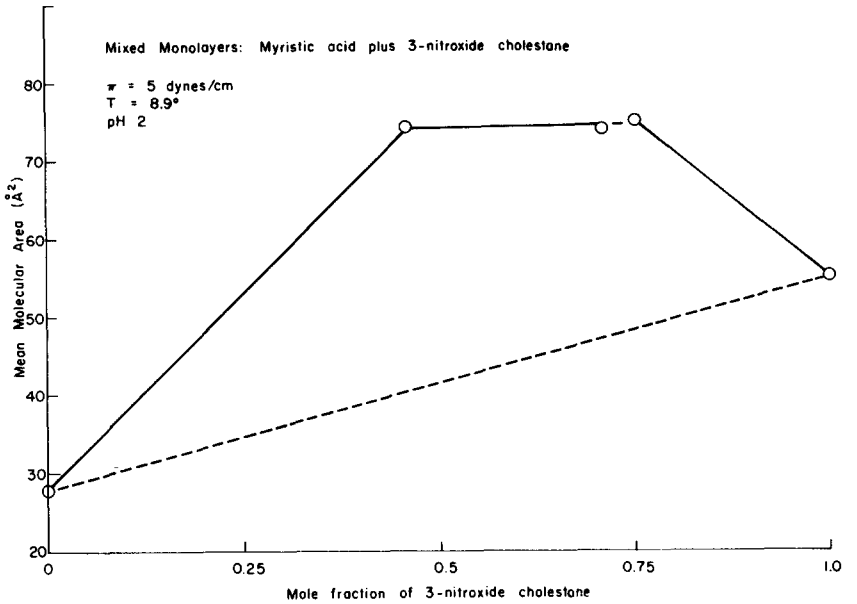

Fig. 3. Mean molecular areas of pure and mixed films of 3-nitroxide cholestane and myristic acid (pH 2, 2 M NaCl aqueous substrates at $\pi = 5$ dynes/cm and 14.5°) as a function of the mole fraction of 3-nitroxide cholestane.

ESR probe has therefore substantially affected its film behavior in both pure and mixed monomolecular films and it would appear likely that the same would be true concerning liposome or membrane behavior. The low probe concentrations used by McConnell and McFarland[1] should not negate this conclusion since the ESR signal will see only the immediate environment of the probe molecule itself.

In summary it would seem that quantitative observations of lipid mobility in membranes may be fully justified only for the lipid probe itself. Extrapolation to the "tumor-less" molecule must be considered at best semi-quantitative and may on occasion be totally misleading. Further studies to substantiate and expand on the above are presently underway.

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