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THE INFLUENCE OF *n*-ALKANOLS ON THE CAPACITY PER UNIT AREA OF PLANAR LIPID BILAYERS

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The electrical capacities per unit area of planar lipid bilayers formed from monoolein/*n*-hexadecane, monoolein/squalane (or squalene) and monoolein/triolein have been measured in the presence of a range of *n*-alkanols. For monoolein/*n*-hexadecane bilayers, the effects of the *n*-alkanols are complicated but can be rationalized in terms of the likely changes in lipid chain order and the influence of the *n*-alkanol in the Plateau-Gibbs border. Monoolein/squalane (or squalene) and monoolein/triolein bilayers exhibit behaviour quite different from the *n*-hexadecane membranes. For both the squalane and triolein bilayers the shorter chain alkanols increase the capacity per unit area while the longer homologues have little effect. These results help to account for the influence of the *n*-alkanols on gramicidin single-channel lifetimes.

We have previously shown [1] that the influence of *n*-octanol on the capacity per unit area of planar lipid bilayers depends on the nature of the hydrocarbon solvent used to disperse the lipid. Thus *n*-octanol increased the capacity per unit area of membranes formed from monoolein/squalene (or squalane) but decreased that of monoolein/*n*-hexadecane bilayers. It was suggested that in the case of monoolein/*n*-hexadecane membranes, which contain approx. 20% (v/v) solvent in the control system [2], *n*-octanol increased the partitioning of *n*-hexadecane into the bilayer, thereby increasing its thickness and reducing its capacity. Bilayers formed from monoolein/squalene (or squalane), however, contain relatively little solvent [3] and the effect of *n*-octanol on these membranes was suggested to be a more reliable indication of its interaction with liposomes and cell membranes.

We have now extended this study and report the effects of a wide range of *n*-alkanols on the capacity per unit area of three planar bilayer systems. The lipid used was monoolein and the solvents investigated were *n*-hexadecane, squalane and squalene (which behave very similarly) and triolein. The use of triacylglycerols as solvents, thus giving a purely lipid membrane containing no hydrocarbon, was suggested by Waldbillig and Szabo [4]. To compare the effects of different members of the *n*-alkanol series, which cover a wide range of aqueous solubility, concentrations were referred to the film-forming solution. Published values of the partition coefficients of *n*-alkanols between aqueous solution and *n*-hexadecane [5] were used to estimate, for a given *n*-alkanol, the relevant aqueous concentration. Oil and aqueous phases were then made up and left in contact to equilibrate before use. Using appropriate quantities of oil and aqueous phases the resultant uncertainty in oil phase concentration is negligible. This procedure was not used with monoolein/triolein membranes, which formed bilayers with very little residual border (and therefore little bulk lipid is present in the experimental chamber) and with which only high concentrations of relatively water-soluble alkanols were used. Here

the *n*-alkanol concentrations are referred to the aqueous phase and solutions were not pre-equilibrated. No significant variation of capacity per unit area with time was detected in any bilayer system.

The techniques of membrane formation and electrical capacity measurement were as described previously [6]. Capacities were measured at 500 Hz and were assumed to be effectively the low frequency limiting values. The thickness, h, of the chain region can then be calculated from the simple expression

$$h = \frac{\epsilon_0 \epsilon}{C}$$

where ϵ_0 is the permittivity of free space, ϵ is the dielectric constant of the chain region (2.20 for pure monoolein [7]) and C is the capacity per unit area of the bilayer. In absolute terms this expression may give a small error in the thickness since it is thought that at approx. 500 Hz the polar groups of a bilayer may contribute $\lesssim 5\%$ to the capacity [8]. However, the introduction of hydroxyl groups from the adsorbed alkanols is likely to produce only a small change in this 5% and the differential effects of different alkanol homologues would produce even smaller changes. Thus the variations in h of current interest should be quite accurately reflected by the above expression.

Fig. 1 shows the changes in capacity per unit area of monoolein/hexadecane bilayers caused by various *n*-alkanols. The data are complex at first sight but may be accounted for in terms of the following considerations. First, the bilayer, and the *n*-hexadecane solvent in it, are in equilibrium with the Plateau-Gibbs border. Thus the amount of *n*-hexadecane in the bilayer can be affected by altering the physico-chemical properties of either the bilayer or the border, e.g. increasing the lipid concentration in the membrane-forming solution tends to thin the bilayer [9]. Second, short chain *n*-alkanols (*n*-nonanol and below) increase the fluidity of lipid membranes [10,11] and lower their phase transition temperature [12,13]. These effects are related to an alkanol induced decrease in membrane lipid chain order. Third, long chain alkanols (*n*-undecanol and above) have the opposite effect on bilayer fluidity [11] and transition temperature [11,14] and hence act to increase lipid chain order.

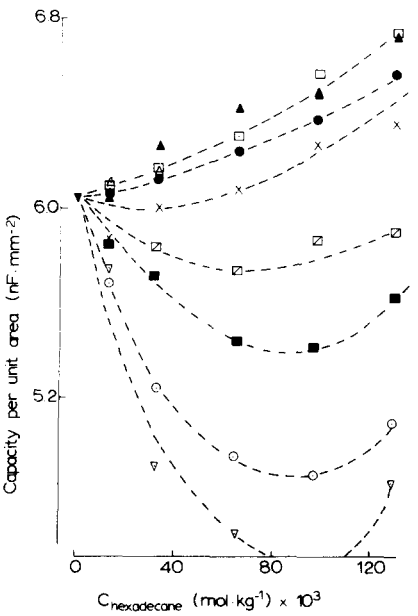

Fig. 1. The influence of *n*-alkanols on the capacity per unit area of monoolein/hexadecane membranes. ▼, control; ▽, *n*-heptanol; ⊙, *n*-octanol; ■, *n*-nonanol; ▨, *n*-decanol; ×, *n*-undecanol; ●, *n*-dodecanol; ⊡, *n*-tridecanol; ▲, *n*-tetradecanol; △, *n*-hexadecanol. $C_{\text{hexadecane}}$ is the concentration of *n*-alkanol in the film-forming solution. The aqueous phase, which was 0.1 M NaCl, contained in each case a concentration of alkanol calculated using the data of Aveyard and Mitchell [5]. The aqueous and oil phases were pre-equilibrated. The monoolein concentration was 8.4 mM and $t = 21 \pm 2$°C. Each point represents the mean of 30–40 measurements. Error bars are less than the size of the points. The dashed lines have no theoretical significance and are purely to assist in discerning trends in the data.

Finally, the order of the lipid chains in a planar bilayer affects the distribution of hydrocarbon between a bulk phase (such as the Plateau-Gibbs border) and the bilayer [15].

It can be seen from Fig. 1 that increasing the concentration of long chain *n*-alkanols produced an increase in bilayer capacity per unit area. This must be the result of either an increase in bilayer dielectric constant or a decrease in bilayer thickness. A decrease in h would be expected to follow a reduction in the amount of hexadecane in the bilayer, and this could occur both by an alkanol induced increase in lipid chain order and, as the concentration of *n*-alkanol in the border increases, by a reduction in the overall chemical potential of solvent. It will be noticed that the capacity per

unit area does not rise above the value for monoolein/squalane membranes (shown in Fig. 2b). The short chain length alkanols, however, cause a concentration dependent fall and then a rise in bilayer capacity per unit area. Because the alkanol dielectric constant is higher than that of the bilayer, a fall in capacity per unit area can only be the result of a membrane thickness increase. The initial fall in capacity could therefore derive from an alkanol-induced decrease in lipid chain order, which in turn would increase the partitioning of hexadecane from the bulk border to the bilayer interior and increase the bilayer thickness. The later rise in capacity could be a consequence of the increasing concentration of *n*-alkanol in the border hydrocarbon, which would lead to thinning in the same way as for the longer chain homologues discussed above.

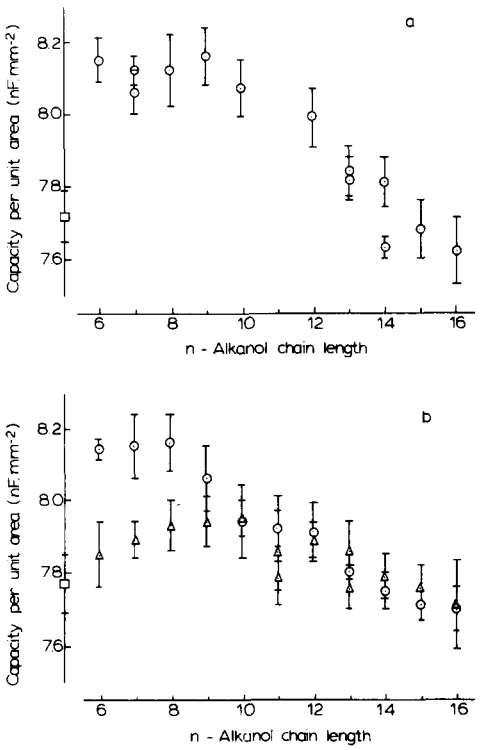

Fig. 2. The influence of *n*-alkanols on the capacity per unit area of bilayers formed from (a) monoolein/squalene, and (b) monoolein/squalane. In (a) the *n*-alkanol concentration in the film-forming solution was 0.058 mol/kg squalene; in (b) the concentrations are △, 0.031 and ⊙, 0.062 mol/kg squalane. The oil and aqueous phases were pre-equilibrated. The monoolein concentration was 28 mM and the aqueous solution was 0.1 M NaCl. $t = 21 \pm 2\,^{\circ}C$. The error bars show the standard deviation of 30–40 measurements. Where two complete experiments were performed with a given *n*-alkanol and concentration, both results are shown. The control values, ⊡, are shown on the ordinates.

The observations on *n*-hexadecane-containing membranes are interesting in themselves, but are not helpful in deciding the effect of *n*-alkanols on membranes of biological relevance. Fig. 2 shows the influence of *n*-alkanols on the capacity per unit area of monoolein/squalane and monoolein/squalene bilayers, which contain at most approx. 4% (v/v) solvent [16]. *n*-Hexadecanol, *n*-pentadecanol and *n*-tetradecanol have little effect on membrane capacity per unit area in these systems. These *n*-alkanols do, however, affect the surface tension and contact angle of monoolein-squalane or squalene bilayers (Ref. 16, and Needham, D., personal communication) and reduce the lifetime of gramicidin channels in monoolein-squalane bilayers [17]. This indicates that their lack of effect on capacity does not arise from a failure to adsorb into the bilayer.

The lower chain length alkanols produce an increase in the capacity per unit area of monoolein/squalene or squalane membranes. As stated above, capacity increases in these systems cannot be attributed unequivocally to a thickness decrease because *n*-alkanol adsorption may increase the bilayer dielectric constant. To affect the dielectric constant of the bilayer portion of the membrane, the hydroxyl group of the alkanol would have to be buried in the hydrocarbon chain region. Measurements of the adsorption of *n*-alkanols from hydrocarbon to the hydrocarbon/water interface show a strong partition of the hydroxyl to that interface, such that molecules oriented with the hydroxyl to the surface outnumber those with buried hydroxyls by approx. 1000 : 1 [18]. It would therefore seem likely that capacity changes derive from effects on bilayer thickness. The mechanism of bilayer thinning by short chain alkanols is presumably quite simple. The insertion of a six- or eight-carbon chain molecule between two eighteen-carbon chains must create a transitory hole, which the lipid chains then fill by kinking inwards [19]. This decreases both the membrane thickness and lipid chain order. Benzyl alcohol also increases the capacity per unit area of planar lipid bilayers containing little hydrocarbon solvent

TABLE I

THE INFLUENCE OF VARIOUS *n*-ALKANOLS ON THE CAPACITY PER UNIT AREA OF MONOOLEIN/TRIOLEIN BILAYERS

The film-forming solution contained 0.43 mole fraction monoolein in triolein. The aqueous phase contained 0.1 M NaCl. The oil and aqueous phases were not pre-equilibrated. The ratio of oil to aqueous phase volumes was approx. $1:3\cdot10^4$. $t = 21 \pm 2\,^{\circ}C$.

n-Alkanol	Aqueous concentration	No. bilayers, no. measurements	Capacity per unit area ($\pm$S.D.) ($nF\cdot mm^{-2}$)
Control	0	7, 47	8.37 ± 0.04
n-Hexanol	15 mM	2, 38	9.00 ± 0.05
n-Hexanol	26 mM	4, 18	9.56 ± 0.10
n-Hexanol	30 mM	2, 16	9.77 ± 0.08
n-Heptanol	0.5 saturated	3, 18	9.16 ± 0.06
n-Octanol	approx. 0.95 saturated	1, 65	9.17 ± 0.09
n-Decanol	approx. 0.9 saturated	2, 10	8.58 ± 0.03

[20,21], presumably by the same mechanism.

Table I shows the influence of some short chain *n*-alkanols on the capacity per unit area of monoolein/triolein membranes, which contain no hydrocarbon solvent. As for the monoolein/squalane membranes, short chain alkanols cause a concentration- and chain-length-dependent increase in capacity per unit area. The effects, though significant, are not large. If the results for 30 mM *n*-hexanol are considered, then the maximum possible thickness decrease (assuming that the bilayer dielectric constant remains 2.20) is from 2.33 nm to 1.99 nm. Conversely, if the bilayer thickness had remained constant, the dielectric constant would have had to increase from 2.20 to 2.57. With the present techniques it is not possible to distinguish between the two possibilities.

A differential effect of long and short chain *n*-alkanols on bilayer thickness may explain the results of Pope et al. [17] concerning the influence of the *n*-alkanol series on gramicidin channel lifetime in monoolein/squalane membranes. In this work it was found that although all *n*-alkanols tested decreased channel lifetime, the long chain members of the series were the more effective. Elliott et al. [22] have shown that gramicidin lifetime is very sensitive to thickness changes in the region of the solventless membrane thickness. A 0.15 nm decrease in bilayer thickness (from 2.45 nm to 2.30 nm) increased channel lifetime by a factor of 3.4. It is therefore possible in the experiments of Pope et al. [17], that bilayer thickness decreases which would have been caused only by the short-chain alkanols, acted to partially offset the general *n*-alkanol induced lifetime reduction.

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