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THE INTERACTION OF *n*-OCTANOL WITH BLACK LIPID BILAYER MEMBRANES

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Summary

The electrical capacities of black lipid films formed from monoolein + *n*-hexadecane and monoolein + squalane (or squalene) solutions have been measured in the presence of various concentrations of *n*-octanol. In addition, partition coefficients for *n*-octanol between *n*-hexadecane and 0.1 M NaCl, dielectric constants for octanol-hexadecane mixtures and the interfacial tension of films and film-forming lipid solutions against the aqueous phases have been determined.

It is concluded that in "solvent-free" bilayers the octanol is unlikely to have changed the bilayer thickness by more than about 1 Å. The bilayer tension, on the other hand, increases appreciably in the presence of octanol.

It has recently been argued that the adsorption of *n*-alkanes into lipid bilayers inhibits ion conduction by the pore-forming polypeptide gramicidin by increasing both the thickness and tension of the membrane [1]. Evidence has also been presented that a similar type of mechanism may be responsible for the blockage by the alkanes of the sodium and potassium currents in the nerve axon [2,3]. The *n*-alkanols, like the *n*-alkanes, also block the sodium and potassium currents in excitable membranes [4,5] and it is of interest to enquire whether these substances also affect lipid bilayer thickness and tension. Ashcroft, Coster and Smith [6] showed that benzyl alcohol reduced the electrical capacity per unit area of black films formed from lecithin and tetradecane and, from this, they argued that the alcohol thickened the bilayer. The same type of result was obtained in unpublished work in this laboratory using ethanol and monoolein-hexadecane membranes.

The difficulty with this type of experiment is, however, that in a black film the adsorption of the alcohol could well lead to an increase in the amount of tetradecane or hexadecane in the interior of the membrane and hence produce a result quite different from that for a solventless bilayer. This possibility has been underlined by a recent study by Turner and Oldfield [7] where it was concluded that benzyl alcohol did not affect the thickness of multibilayers of dimyristoyl phosphatidylcholine.

The present paper is concerned with the interaction of octanol with black lipid films. It will be shown that the choice of hydrocarbon solvent greatly affects the results, but that conclusions relevant to solvent-free bilayers may nevertheless be reached. The black films were formed from monoolein, using as the non-polar solvent *n*-hexadecane, squalane or squalene. The techniques of film formation and of electrical capacity measurement were as described previously [8]. The *n*-octanol, to which the films were exposed, was allowed to reach its equilibrium distribution between the monoolein-hydrocarbon and aqueous solutions before films were formed. The final concentration of the octanol in the aqueous solution was determined by measuring the surface tension against air of a sample of the solution (using the drop-volume method [9]) and comparing the result with a calibration curve for known octanol concentrations. By equilibrating suitably large volumes of lipid-hydrocarbon solution against suitably small volumes of aqueous solution, the amount of octanol added initially to the oil phase could be assumed to remain effectively the same.

In Fig. 1 are shown the changes in capacity per unit area produced by the *n*-octanol in the various monoolein bilayers. The capacities were mea-

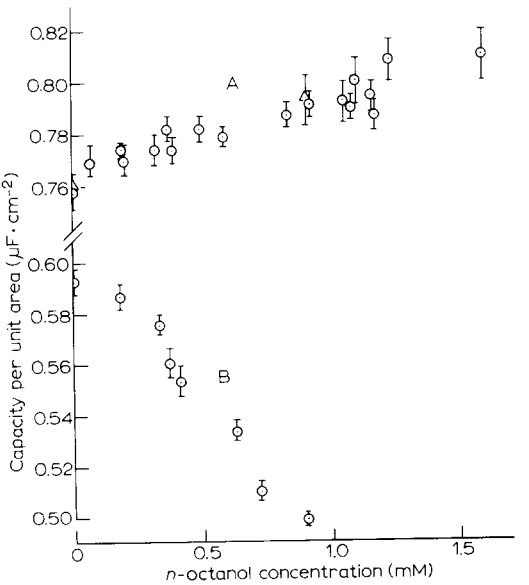

Fig. 1. Capacities per unit area as a function of aqueous *n*-octanol concentration for monoolein-squalane (or squalene) membranes (A) and for monoolein-hexadecane membranes (B). In A the monoolein concentration was 28 mM (○) or 400 mM (△) (the latter being in squalene solution); in B the monoolein concentration was 8.4 mM. The aqueous solution was 0.1 M NaCl; $T = 20 \pm 1$ °C. The error bars represent standard deviations for 20—30 readings.

sured at 500 Hz and showed no significant frequency dependence. It was assumed, as in previous studies, that these capacities were effectively the low frequency limiting values and that they arose only from the chain region of films [8]. Thus, the thickness, h, of this chain region (of dielectric constant ϵ) should be given by [8]

$$C = \frac{\epsilon}{4\pi h} \tag{1}$$

where C is the capacity per unit area of the bilayer.

The capacities for zero octanol concentration (with the exception of those for squalane, which gives results very similar to squalene) have been discussed elsewhere [8,10—12]. Bilayers formed from squalane and squalene solutions are thought to be effectively solvent-free [12]. Those formed from *n*-hexadecane solutions contain approximately 20% (v/v) alkane and are thicker than the solvent-free structures by about this same percentage [10,11]. As shown in Fig. 1, octanol decreases the capacity per unit area of the membranes formed using hexadecane. Since the dielectric constant of *n*-octanol is 10.3, as compared with about 2.1 for the alkanes, ϵ in Eqn. 1 could increase owing to the partitioning of alcohol into the interior of the leaflet. For this reason, the capacity decrease observed in this system can only reflect a thickening of the membrane. The capacity increases in the other systems could arise either from an enhanced dielectric constant or from a decreased thickness or, perhaps, from a combination of these effects. No clear distinction can be made using the present methods, but some useful conclusions can be reached.

Waldbillig and Szabo [13] showed that in monoolein-decane black films which normally contain ca. 49% (v/v) decane, and have the maximum observed thickness [11], increasing the lipid concentration to exceptionally high levels caused considerable reductions in the solvent retention and film thickness. The use of high (0.4 M) monoolein concentrations in the squalene systems with or without octanol present did not, however, affect the membrane capacity. It is concluded, therefore, that the squalene and squalane-monoolein membranes did not absorb solvent in the presence of the octanol and consequently are unlikely to have thickened. They could, however, have thinned and the maximum amount that they could have done so, as calculated from Eqn. 1 assuming ϵ to have remained constant, is 1.6 Å. From partitioning experiments it was found that at 20°C 1.6 mM octanol in 0.1 M NaCl was in equilibrium with 330 mM octanol in hexadecane. The dielectric constant of this octanol-hexadecane mixture was 0.10 larger than for pure hexadecane. If this bulk phase result is assumed to apply to the black film the thickness decrease is calculated to be 0.5 Å. The bulk phase partition coefficient is unlikely to be a very precise indication of partitioning into a bilayer (for *n*-octane the bulk coefficient is some 30% higher than for the bilayer [10,11]) but the effects are sufficiently small that whatever assumption is made, within reason, the conclusion remains that little or no change occures in the membrane thickness.

Although this conclusion is in contrast to that for e.g. *n*-octane [1,2,10, 11], both the alkane and the alkanol increase the bilayer tension. In Fig. 2

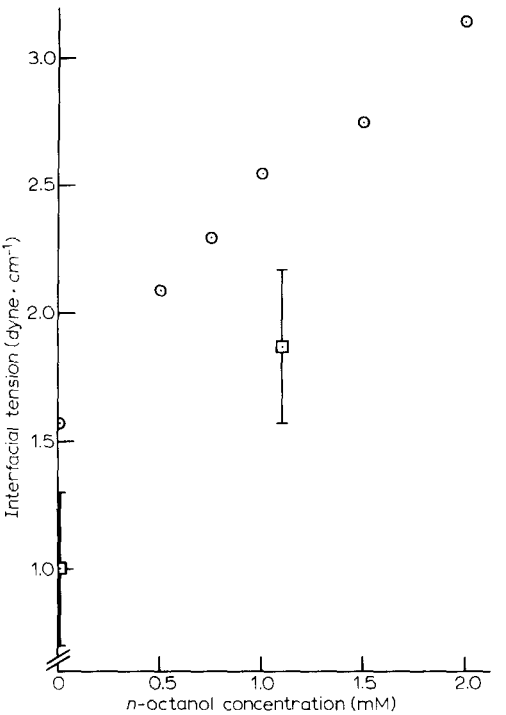

Fig. 2. Interfacial tensions between membrane-forming solutions of monoolein (28 mM) in squalane and 0.1 M NaCl containing *n*-octanol (○) and the interfacial tension (film tension/2) of black films formed from similar solutions (□). The error bars for the latter points represent the standard deviations for seven determinations. The aqueous and non-aqueous phases were brought to equilibrium prior to the measurements being made. $T = 20 \pm 0.2^{\circ}$ C.

is shown the variation in interfacial tension of the film-forming monoolein-squalane solution against 0.1 M NaCl as the *n*-octanol concentration is increased. At 1.6 mM octanol the tension is approx. 1 dyne/cm above the value for the alcohol-free system. A similar result is obtained for the hexadecane system. Arguments given elsewhere [14] suggest that the tension changes in the membranes will be very similar. Direct measurements of film tension have been attempted, using the bulging technique [14], which confirm the above conclusion (Fig. 2)*.

Since *n*-octanol and benzyl alcohol [7] do not apparently thicken bilayers it may seem that they cannot act on nerve channels in the way proposed for the alkanes [2,3]. But this is to overlook the tension increase. If, for example, there were a natural mis-match in the lengths of the lipophilic exterior of the channel proteins and the chains of the lipid (especially if the latter were the greater) the observed increase in membrane tension would still have the effect of pulling the channels apart, or away from the interfaces. It is also of interest that if the bilayer thickness does not change, purely geometrical considerations require that alcohol goes into the centre of the bilayer as well as into the surfaces (in about equal quantities for

*These measurements are inherently much less accurate than the bulk interface results and, while the film tensions must be less than those of the adjacent single interfaces, part of the discrepancy apparent in Fig. 2 could have arisen from systematic errors.

n-octanol in monoolein bilayers). The presence of such molecules could weaken hydrogen bonded protein structures in the non-polar regions of the membranes.

The results of the present studies of the adsorption of an alcohol into a single planar bilayer seem to be consistent with those obtained using multi-bilayers [7]. However, from thermodynamic considerations there is no reason to suppose that in general the adsorption in the two types of system should be the same. This is particularly so for non-polar substances like the alkanes which tend to adsorb into the centre of the bilayer and to increase the tension. The swelling of the multi-layered structure which is necessary to accommodate the leaflet thickening involves work against surface and capillary forces and hence acts to inhibit adsorption. Rough calculations suggest that, owing to this effect, adsorption in multi-layered systems could be well below that for planar bilayers or cell membranes [15].

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