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Is there a 'cut-off' in the adsorption of long chain amphipathic molecules into lipid membranes?

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Electrokinetic mobilites of lipid vesicles have been measured in solutions of *n*-alkyltrimethylammonium ions. The electrokinetic (zeta) potentials calculated from the mobilities have been used to estimate the adsorption of the alkyl chain ions. No significant difference was found in the adsorption to egg phosphatidylcholine or egg phosphatidylcholine + cholesterol (2:1 mole ratio). For *n*-octyl- to *n*-octadecyltrimethylammonium, the standard free energy of adsorption increased on average by approx. 3 kJ · mol^{-1} from one homologue to the next, and there was no suggestion of a 'cut-off' in adsorption as the chain length increased. These results are considered in relation to recent reports of the action of alkyl chain molecules on biological membranes.

It was often been reported that long chain substances become biologically less active in membranes as their chain length increases [1–7]. For non-polar molecules, such as hydrocarbons, it seems fairly certain that this loss of activity arises because the larger homologues cannot be accommodated in the small hydrophobic regions of lipid bilayers or biological membranes without an energetically unfavourable distortion of the structure [2,3]. The polar substances, such as the *n*-alkanols and the *n*-alkyltrimethylammonium ions, which appear to become inactive at chain lengths beween C_{13} and C_{16} [4–7] are amphipathic molecules. Unlike the hydrocarbons these molecules tend to be located at interfaces and the explanation offered for the hydrocarbons does not apply, at least not in any simple way. The alternatives are broadly of two types; either the partitioning from the aqueous solution into the membrane increases less rapidly with increasing chain length than the solubility decreases, until a point is reached at which insufficient molecules are taken up by the membrane (i.e. a mechanism similar to that for the hydrocarbons but probably with different origins) or the uptake is maintained but the adsorbed molecules perturb the membrane in a way which no longer seriously affects its function. Were it feasible to measure the uptake of these molecules into the site of action in the membrane the viability of the two types of mechanism could immediately be assessed. Unfortunately the sites of action are neither known nor accessible to examination in this way. It is possible, however, that these sites are the lipid regions adjacent to the relevant membrane proteins. For this reason it is of interest to study the adsorption of long chain molecules into purely lipid membranes.

No clear demonstration exists that the adsorption of long chain molecules (C_{10}–C_{18}) (e.g. *n*-alkanols) into phospholipid bilayers follows the same rule as do the lower homologues. In fact there is evidence to the contrary [4]. Reliable data are,

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however, not easily obtained in these systems mainly owing to the very low aqueous concentrations involved and the consequent likelihood of depletion when surfaces of appreciable area are present. For the alcohols these problems are compounded by the difficulties of estimating the alcohol adsorption in very small quantities of lipid. For these reasons, the *n*-alkyltrimethylammonium ions have been selected for study. The advantages of this choice are two fold. First, the adsorption (or membranes/aqueous solution partition coefficient) is smaller than for the alkanols and hence depletion is less of a problem. Secondly, phosphatidylcholine vesicles (with or without added cholesterol) have practically zero electrokinetic potential and the ionic double layer produced by the adsorbed quaternary ammonium ions forms the basis of a simple measurement of adsorption (microelectrophoresis) which requires very little lipid. As a result, unambiguous data can be obtained for the adsorption of chain lengths up to and including C_{18}.

Vesicle suspensions were prepared by adding solutions of sodium chloride (25 mM) to known quantities of dried lipid in stoppered tubes. Dispersions were formed by manual shaking. As appropriate, *n*-alkyltrimethylammonium bromides were added to the sodium chloride solutions. The pH was not controlled since the electrophoretic mobility of phosphatidylcholine vesicles is constant in the range pH 2–11 [8]. The lipid suspensions were introduced into a microelectrophoresis apparatus and the electrophoretic mobilities (U) of the vesicles determined by visual observation of individual particles [9]. Electrophoretic (ζ) potentials were calculated from the mobilities by means of the Helmholtz-Smoluchowski equation [10]

$$\zeta = \frac{\eta}{\varepsilon_0 \varepsilon_r} U \qquad (1)$$

where η is the viscosity and ε_r the relative permittivity of the aqueous solution, and ε_0 is the permittivity of free space.

Results for *n*-octyl- to *n*-octadecyltrimethylammonium bromides in two lipid systems (egg phosphatidylcholine and egg phosphatidylcholine : cholesterol (2 : 1 mol ratio)) are shown in Fig. 1. The ζ vs. log(concentration) plots are approximately linear in each instance. This is probably fortuitous as it has no obvious theoretical basis. It does, however, make possible the use of the (dashed) regression lines for purposes of interpolation. The quantities of lipid used had to be drastically reduced in the experiments with the longer chain homologues in order to avoid depletion of the aqueous phase. This point is illustrated in Fig. 2 where the zeta

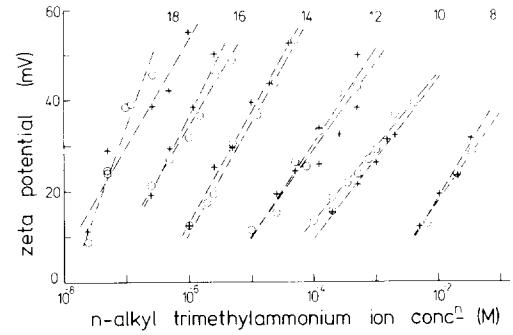

Fig. 1. Electrophoretic (zeta) potentials of lipid vesicles in solutions of *n*-alkyltrimethylammonium ions in 25 mM NaCl. ⊙, egg phosphatidylcholine; +, egg phosphatidylcholine + cholesterol (2 : 1, mol ratio). The numbers against each set of results indicate the number of carbon atoms in the long alkyl chain of the quaternary ammonium derivative. The dashed lines represent regression lines and are for interpolation only. At zero quaternary ammonium ion concentration the zeta potential was 0 ± 2 mV for both lipid preparations. The phospholipid concentrations were for the *n*-octyl and *n*-decyl homologues 130 μM; for the *n*-dodecyl, *n*-tetradecyl and *n*-hexadecyl homologues, 13 μM and, for the *n*-octadecyl, 1.3–3.9 μM. Vesicle mobilities were measured across a 79 μm graticule in fields of 3–6 V/cm. At last six different vesicles (diam. -10 μm) were timed in each direction. This yielded an S.E. for each point of ≤ 1 mV. $T = 25°C$.

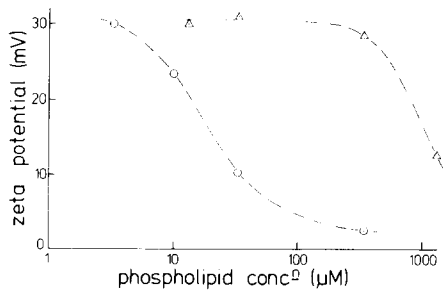

Fig. 2. The dependence of the zeta potential of egg phosphatidylcholine vesicles in quaternary ammonium ion solutions on phospholipid concentration. ⊙, *n*-octadecylammonium bromide (0.06 μM); ▵, *n*-hexadecylammonium bromide (0.6 μM).

potential for a given quaternary ammonium ion concentration is plotted against the concentration of phospholipid in the solution. It is clear that while, for the *n*-hexadecyl derivative, 130 μM was acceptable, rather less than 13 μM was desirable for the *n*-octadecyl homologue.

Since the head group was the same for each of the substances tested it is reasonable to suppose that, at low adsorptions at least, equal zeta potentials represent equal number densities of ions at the surfaces of the lipid vesicles. Further, if it is assumed that the zeta potential may be equated to the diffuse layer potential (ϕ_0) then, for potentials $\lesssim 25$ mV, the number N of quaternary ammonium ions adsorbed per cm^2 of lipid surface is given by the Gouy-Chapman equation [10] as

$$N \simeq 2.3 \cdot 10^{11}\, \zeta \qquad (2)$$

where ζ is in millivolts and the temperature has been taken as 25°C. At 20 mV (a value selected arbitrarily as being both fairly low and also fairly accurate), there should be approximately one ion per 2200 Å^2. Since $\zeta \leq \phi_0$ [11], this charge density is likely to be an underestimate, not that this affects the present arguments. The logarithm of the aqueous phase concentration of quaternary ammonium derivative ncessary to give $\zeta = 20$ mV is plotted against chain length in Fig. 3. The plot is

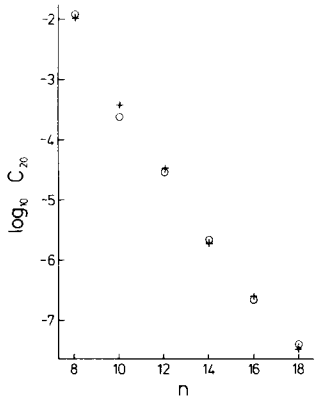

Fig. 3. The effect of chain length on the adsorption of the *n*-alkyl trimethylammonium ions onto egg phosphatidylcholine (⊙) and egg phosphatidylcholine + cholesterol (2:1 mol ratio) (+) vesicles. C_{20} is the aqueous concentration in mmol/l required to give a zeta potential of 20 mV; n is the number of carbon atoms in the *n*-alkyl chain. Points were obtained by interpolation from Fig. 1.

effectively linear over the whole range of chain length both for the phosphatidylcholine-cholesterol vesicle and for phosphatidylcholine alone. A routine thermodynamic analysis based on regression lines for the points in Fig. 3 (see for example Ref. 12 or 13) gives the standard free energy of adsorption per CH_2 from the aqueous phase to the lipid as -2.92 kJ · mol^{-1} and -3.10 kJ · mol^{-1} for the phospholipid and phospholipid-cholesterol systems, respectively.

The results show no suggestion of a decline or cut-off in adsorption for the longer chain homologues. It cannot, of course, be concluded from this that the *n*-alkanols also exhibit no cut-off. However, in view of the physico-chemical similarities between the *n*-alkanols and the quaternary ammonium ions it would be surprising if the adsorption of the two types of substance was not also very similar, at least in its chain length dependence. It seems therefore that any disappearance of biological activity, certainly in the quaternary ammonium series, cannot be attributed to the absence of these ions in the lipid bilayer regions of the membrane. An obvious remaining possibility is that interactions directly with membrane proteins are involved. It still cannot be ruled out, however, that the perturbation of lipid bilayer structure by the longer chain homologous differs in important respects from that caused by the shorter homologues and that this, rather than a decline in adsorption, underlies the reported biological effects.

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