Electric Properties of 1-Tetradecanol-Tetradecanoic Acid (Myristyl Alcohol-Myristic Acid) Mixed Bimolecular Films

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Stable bimolecular films (bilayers) were found to be formed in aqueous solutions from myristyl alcohol or from mixtures of myristyl alcohol and myristic acid. The electric capacitance and resistance of these bilayers were measured in solutions of pH 3—8. The capacitance of mixed bilayers was larger than that of myristyl alcohol bilayers (0.58 μF/cm²). When the bilayers were formed from sample solutions having mole ratios 1/1 and 2.3/1 (myristic acid to myristyl alcohol), the capacitance had minima at *ca.* pH 7. The resistance of mixed bilayers at *ca.* pH 5 was smaller than one tenth of that of the myristyl alcohol bilayer (*ca.* $7\times10^8\,\Omega\cdot\text{cm}^2$). The results are explained in terms of (i) van der Waals attraction between hydrocarbon chains, (ii) the tendency for myristic acid to form expanded layers, (iii) the head group attraction between the myristic acid ion and myristyl alcohol, and (iv) the tendency for myristic acid ions to dissolve in the aqueous phase.

Bimolecular films (bilayers) have been studied as a model of biological membranes.[1-5] Substances with simple structure were chosen for a systematic study on the relation between the properties and components of bilayers.

We were able to form stable bilayers of myristyl alcohol in water. At the air/water interface under normal conditions myristyl alcohol forms a condensed monolayer[6] while myristic acid forms an expanded monolayer.[7,8] Since mixed monolayers at water surface differ from those of the individual components, mixed bilayers in water are expected to differ in character from single component bilayers. In the present work the electric properties of single component bilayers (myristyl alcohol) have been compared with those of mixed bilayers (myristyl alcohol and myristic acid).

Experimental

Materials. Guaranteed Reagents (Nakarai Chemicals Ltd.) were used. Myristyl alcohol and myristic acid (purity >99%) were recrystallized. No impurities were detectable gas-chromatographically. Cyclohexane and *n*-decane were used as solvents without purification. The aqueous solution surrounding the bilayer was controlled by several buffer solutions, *i.e.* those of Sǿrensen (sodium chloride, sodium hydroxide, aminoacetic acid and hydrochloric acid), McIlvaine (sodium phosphate and citric acid), and Gomori (2,4,6-trimethylpyridine and hydrochloric acid). Chemicals of reagent grade (Wako Pure Chemical Industries Ltd.) were used for the buffer solutions.

Methods. The apparatus and procedures for bilayer-formation were similar to those employed by Hanai *et al.*[9] Sample mixtures of myristyl alcohol and myristic acid (total *ca.* 0.3 g) were dissolved in a *n*-decane–cyclohexane mixed solvent [(total 2 cm³), 1/1 (v/v)]. This sample solution was spread over a hole (diameter: 0.7 mm) of a Teflon beaker in a buffer solution through a Teflon tube (diameter: 1 mm) attached to a syringe. In order to hasten the bilayer-formation, small bubbles were ejected successively from the end of another tube to the front of the hole. When a black film was observed through a microscope, the electric capacitance (C_m) of the film was measured with an AC bridge (Ando Electric Co. Ltd., Model TR-1c). Measurements were carried out in the frequency range 1—3 MHz.

Resistances (R_m) of the bilayers were also obtained by the DC transient method. The applied polarizing potential was 25 mV. The potential across the bilayer was measured with an electrometer of high input impedance (Takeda Riken Industry Co. Ltd., Model TR-8651) through a pair of reversible Ag/AgCl electrodes. The area of the bilayer was measured by a photographic method. The temperature of the bilayer and its surrounding solution was controlled with an accuracy of ±0.2 °C by circulation of thermostated water.

Results

Over the pH range 1—12, bilayers of myristyl alcohol are stable with a lifetime of over one hour. However, with an increase in myristic acid content in mixed bilayers, the pH range for stability is greatly reduced. Only at pH—7, a stable bilayer can be obtained from a sample solution with a 4/1 myristic acid to myristyl alcohol mole ratio. Hereafter the various mixed bilayers will be referred to in terms of the molar ratios in the sample solutions from which the bilayers are formed. The mole ratio in bilayers is not always equal to that in the sample solution.

Conductance G_t and capacitance C_t of the whole system (*i.e.* of the bilayer and its surrounding aqueous solution) are dependent on frequency f. A plot of $G_t/2\pi f$ against capacitance gives a semicircle (Fig. 1). The capacitance at 1 kHz agrees with that obtained by the DC transient method (0.58 μF/cm²).

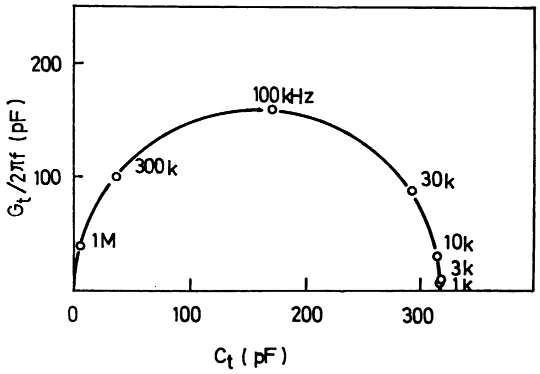

Fig. 1. Complex capacitance diagram of a whole system (a bilayer and its surrounding solution).
C_t, capacitance of a whole system; G_t, condnctance of a whole system; f, frequency; pH 5; The mole ratio of myristic acid to myristyl alcohol in sample solution is 1/1.

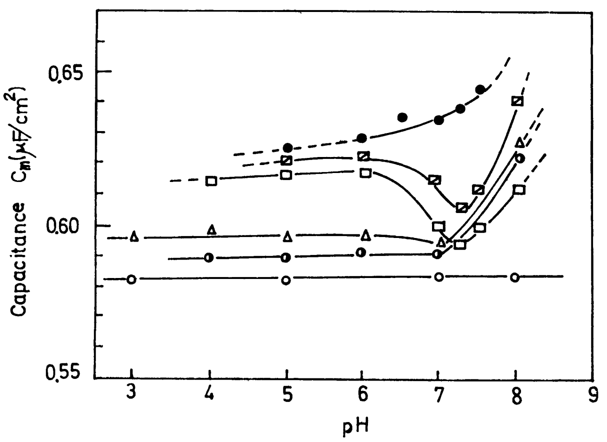

Fig. 2. The specific capacitances of bilayers as a function of pH (at 25 °C and 1 KHz). The mole ratio of myristic acid to myristyl alcohol in sample solution, ○, 0/1; ◑, 0.042/1; △, 0.25/1; □, 1/1; ▨, 2.3/1 and ●, 4/1. ----, unstable bilayer.

The influence of pH on the capacitance C_m of bilayers (equal to C_t) is shown in Fig. 2. The capacitance is independent of the nature of the buffer solution. The capacitance of the myristyl alcohol bilayer remains constant (0.58 μF/cm²) regardless of pH, while that of all mixed bilayers increases with an increase in pH from 7 to 8. The capacitance of 1/1 and 2.3/1 mixed bilayers shows minima at pH 7 while that of the 4/1 bilayer shows no minimum over the pH range 4—8. The capacitance of mixed bilayers increases rapidly up to a mole ratio of 1/1 (Fig. 3).

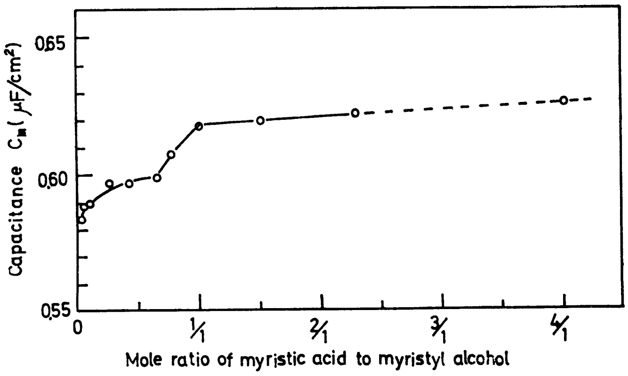

Fig. 3. Relation between the capacitance of mixed bilayer and the composition of sample solution from which the bilayer was formed (at 25 °C, 1 kHz, and pH 5). ----, unstable bilayer.

With temperature rise from 15 to 30 °C the capacitance of mixed bilayers increases gradually (Fig. 4). With increasing myristic acid content the temperature dependence of capacitance becomes more marked.

The DC resistance R_m of the myristyl alcohol bilayer has a constant value (7×10^8 Ω·cm²) regardless of pH (Fig. 5). The resistance R_m increases with pH for a 1/1 mixed bilayer. At pH 5 it is 4×10^7 Ω·cm², about one tenth of R_m for the myristyl alcohol bilayer.

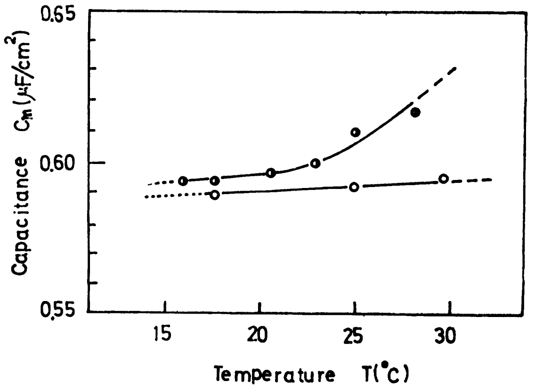

Fig. 4. The specific capacitance of bilayers as a function of temperature (at pH 5 and 1 kHz). The mole ratio of myristic acid, ○, 0.25/1; ◑, 1/1. ----, unstable bilayer, ····, stable black film, at the periphery of which some crystal parts are observed.

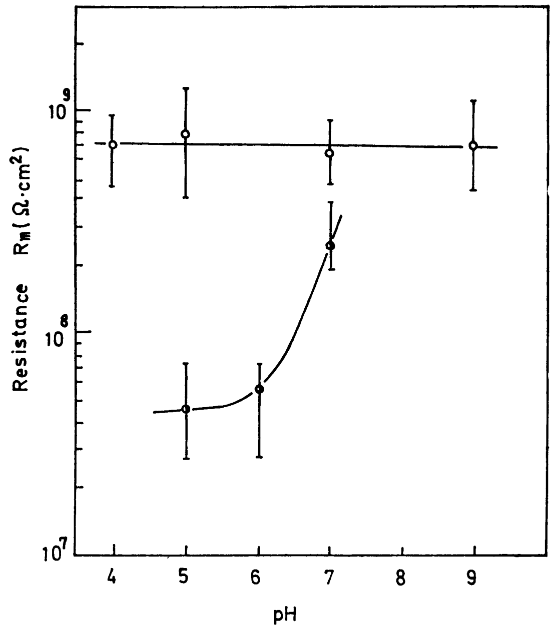

Fig. 5. The DC specific resistance of bilayers as a function of pH at 25 °C. The mole ratio of myristic acid to myristyl alcohol in sample solution. ○, 0/1; ◑, 1/1. Both ends of vertical line indicate maximum and minimum of the observed values, respectively. The mark ○ or ◑ shows the mean value.

Discussion

The semi-circular relationship between the capacitance of the whole system and its conductance divided by 2π times the frequency (Fig. 1) is similar to that obtained by Hanai *et al.*[9] for egg lecithin bilayers. They concluded from the shape of the complex plane plots that the static capacitance of the whole system was equal to the capacitance of the bilayer itself, $C_t(0\text{ Hz})=C_m(0\text{ Hz})$. For several systems we studied the static capacitance and the capacitance at 1 kHz agree with each other regradless of temperature and pH, $C_t(0\text{ Hz})=C_t(1\text{ kHz})$. Thus the static capacitance of the bilayer itself in various environments can be approximated by the observed capacitance of the whole system at 1 kHz, $C_m = C_m(0\text{ Hz}) \doteqdot C_t(1\text{ kHz})$.

Assuming the myristyl aclohol film to be a parallel plate condenser whose dielectric constant is 2.0, we find from the observed value of the static capacitance (0.58 μF/cm^2) that film thickenss is 31 Å, approximately twice the myristyl alcohol chain length. It seems reasonable to conclude that the film is bimolecular.

At pH 5, the capacitance of mixed bilayers increases with increasing myristic acid content (Figs. 2 and 3), *i.e.* the thickness of bilayers decreases. This is considered to be due to an increased expansion of the myristic acid parts in the mixed bilayers. The monolayer of myristic acid on water at pH 5 is not condensed, but expanded.[7,8] This property of myristic acid may appear in its mixed bilayers.

The occurrence of change of slope at a mole ratio 1/1 is noteworthy (Fig. 3), since peculiar phenomena at a mole ratio of 1/1 have been frequently reported in mixed monolayer of ionic and non-ionic surface active substances (though not as yet in mixed bilayers) *e.g.* Bernard and Dervichian.[10]

It is suggested that in mixed bilayers the myristic acid molecules oscillate chaotically and intertwine randomly although not to the degree pictured by Gaines.[11] The molecular motion, which we shall call "vermiculation", may be regarded as a type of micro Brownian motion which does not include the motion of the center of mass of the long chain molecule. Besides the myristic acid chains, even some chains of myristyl alcohol situated between myristic acid chains can vermiculate. Moreover, hydrocarbon chains in the bilayer may be more mobile than those in the monolayer, since a small amount of solvent (decane and cyclohexane) still remains among the chains in the bilayer (which will then comprise long chain molecules, solvent molecules and voids). Owing to vermiculation, the effective chain lengh (end-to-end distance) becomes shorter than the chain length of their fully extended forms. Consequently, the mixed bilayer is thinner than the single component bilayer of myristyl alcohol, the capacitance decreasing thereby.

With temperature rise the capacitance of the mixed bilayer increases, while that of the bilayer of myristyl alcohol does not change (Fig. 4). This could be also explained by the increasing tendency to the expanded state caused by temperature rise.

On the other hand some myristic acid ions in a one component monolayer dissolve in water at pH>5.[7] It is possible that myristic acid ions in the mixed bilayer may also dissolve at pH>5. Since the dissolution decreases the number density of the long chain compounds remaining in the bilayer, it should decrease the degree of molecular orientation in the bilayer, and decrease the thickness. This should give rise to an increase in capacitance. In fact, for the 4/1 bilayer the capacitance increases with increasing pH (Fig. 2). At pH>7.5, the bilayer is unstable, since too many long chain ions escape from bilayer into the aqueous phase. But for the 0.042/1 and 0.25/1 mixed bilayers, the increase of capacitance begins from pH 7. At pH<7, capacitance of these mixed bilayers is constant, regardless of pH values. Therefore, no myristic acid molecule in the two mixed bilayers seems to dissolve in the aqueous phase at pH<7. This might be explained in terms of the "ion-dipole attraction" discussed by Goodrich[12] and Spink.[7]

"Ion-dipole attraction" does not mean an attraction between the myristic acid ion and the dipole of water molecule, but the head group attraction between myristic acid ion and myristyl alcohol. The former attraction pulls the long chain ion into the aqueous phase out of the bilayer, while the latter induces the long ion to remain within the bilayer, the dissociation of myristic acid thus bringing about two opposing effects.

The van der Waals' attraction between the long chains and the ion-dipole head attraction causes the long chain molecules and ions to approach each other as closely as possible and to be fully extended normal to the bilayer surface. Under such conditions it is difficult for the myristic acid ions to escape from the mixed bilayer into the aqueous phase.

The results of our monolayer experiments might support the above conclusions. At pH 2—7 and 24 °C the surface pressure-molecular area (π-A) curve of 0.25/1 mixed monolayers was found to be independent of pH. Thus no myristic acid in the mixed monolayer is dissolved in the aqueous phase at pH<7.

With regard to interfacial forces, the bilayer/water interface more closely resembles the oil/water interface than the air/water interface. In general it is more difficult for long chain molecules to escape from an oil/water interface into water than from an air/water interface.[13] Therefore our monolayer experiments suggest that the dissolution of myristic acid in water from mixed bilayers is reduced by ion-dipole attraction with adjacent myristyl alcohol molecules.

Although the 1/1 mixed bilayer is rich in myristic acid content, it is difficult for such an intertwined structure as pictured by Gaines[11] to exist between pH 6 and 7. Since some myristic acid molecules ionize within the bilayer, ion-dipole attraction is added to the van der Waals attraction between the long chains so that some molecules and ions may be fully extended. Such an increase in bilayer thickness leads to a decrease in its capacitance. At pH 7—8, however, there is an increase in capacitance (Fig. 2) because of dissolution of myristic acid ions. Since myristic acid makes up a considerable proportion of the bilayer, the marked effect of its dissociation and hence dissolution more than compensates the effects due to ion-dipole interaction, such as a decrease in vermiculation.

In the 2.3/1 mixed bilayer, there are insufficient myristyl alcohol molecules for ion-dipole attraction to play a significant role. Thus the effect is much weaker than in the 1/1 bilayer. As a consequence dissolution of myristic acid in the aqueous phase is more pronounced and the minimum in capacitance at pH 7 is shallower (Fig. 2).

No minimum in the pH-capacitance curve is observed for the 4/1 bilayer. Since myristic acid makes up a very large proportion of the bilayer the marked effect of its dissociation and dissolution outweighs competing effects. Figures 2 and 3 show that the 4/1 bilayer is the only, unstable one at pH 5. This may be attributed not only to a strong tendency towards expanded layers (because of the high myristic acid content) but also to the absence of ion-dipole attraction at pH 5.

The DC resistance of a 1/1 mixed bilayer is lower than that of a myristyl alcohol bilayer (Fig. 5). However, the former increases and approaches the latter when the pH increases from 6 to 7. The resultant increase in ion-dipole attraction together with the van der Waals attraction between the long chains causes the long chain molecules and ions in the bilayer to pack together as closely as in the crystalline state. Therefore it becomes more difficult for small ions (*e.g.* H_3O^+, OH^-, *etc.*) to cross the bilayer, or to pass between the long molecules along the direction of their orientation.

The results show that bilayers having a very low resistance typical of biological membranes may be produced by incorporating into the sample solutions substances which can form expanded monolayers. The structures of such mixed bilayers may be loosened by increases in temperature or changes in pH.

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