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# LATERAL DIFFUSION, ORDER PARAMETER AND PHASE TRANSITION IN PHOSPHOLIPID BILAYER MEMBRANES CONTAINING TOCOPHERYL ACETATE

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## SUMMARY

Lateral diffusion coefficient and order parameter measurements were made with pyrene excimer optical probes and fatty acid spin label probes respectively in pure dipalmitoyl phosphatidylcholine membranes and in membranes doped with tocopheryl acetate.

The investigation shows, that the lateral diffusion coefficient for pyrene in dipalmitoyl phosphatidylcholine membranes is decreased whereas the order parameter of the fatty acid chains is slightly increased in the inner part of the membranes by the addition of tocopheryl acetate.

The fluid-solid equilibrium phase diagram of dipalmitoyl phosphatidylcholine/tocopheryl acetate mixed membranes has been constructed from the measurements of the partition of (2,2,6,6-tetramethylpiperdine-1-oxyl) TEMPO spin labels between lipid and aqueous regions as function of temperature. In the membranes tocopheryl acetate induces a strong broadening of the temperature range of the phase transition. At low tocopheryl acetate concentrations dipalmitoyl phosphatidylcholine and tocopheryl acetate seem to be completely miscible in the solid and in the liquid crystalline state.

## INTRODUCTION

Little is known about the biochemistry of tocopherol and its derivatives in vivo. An antioxydative effect is postulated but the mechanism is not known in detail [1]. Lucy [2] observed inhibition of retinol hemolysis in erythrocytes by tocopherol. This effect might be due to a stabilising influence of tocopherol on the membrane structure. Because the molecules of tocopheryl compounds are amphiphilic they can be incorporated in an ordered manner into bilayers, and an influence on the structure and function of biological membranes can therefore be anticipated.

It seemed therefore of interest to investigate physical effects of tocopheryl acetate on bilayer model membranes.

## EXPERIMENTAL

Dipalmitoyl phosphatidylcholine/tocopheryl acetate dispersions were prepared in the usual way under oxygen free conditions. The label (zone refined pyrene I or stearic acid spin labels $II_{m,n}$) and tocopheryl acetate were dissolved in chloroform and a lipid film was formed by evaporation of the chloroform from the solution containing these components.

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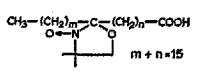


$II_{m,n}$

After addition of an aqueous phosphate buffer-solution (0.067 M $Na_2HPO_4$, pH = 7) the solution was shaken for about 30 min on a vortex mixer at a temperature of about 45 °C i.e. above the phase transition.

L-β,γ-Dipalmitoyl-α-phosphatidylcholine was purchased from Fluka, Buchs, tocopheryl acetate was D,L-α-tocopheryl acetate from Hoffmann-La Roche, Basel. Fluorescence measurements were made with a Perkin Elmer MPF-2A fluorescence spectrophotometer and EPR measurements with a Varian X-band E 9 EPR spectrometer.

## MEASUREMENTS

(1) *Pyrene diffusion*

The ratio of the diffusion coefficient ($D$) of dipalmitoyl phosphatidylcholine/tocopheryl acetate membranes to the diffusion coefficient ($D_0$) of pure dipalmitoyl phosphatidylcholine membranes for pyrene was obtained by measuring the relative excimer/monomer fluorescence quantum efficiency $\varphi$ according to the method introduced by Galla and Sackmann [3]:

$$\frac{D}{D_0} = \frac{\varphi \cdot \tau_0 \cdot c_0}{\varphi_0 \cdot \tau \cdot c} \tag{1}$$

with $\tau, \tau_0$, pyrene excimer lifetime in membrane with and without tocopheryl acetate $c$, $c_0$, pyrene concentration per unit area in membrane with and without tocopheryl acetate.

Fig. 1 shows the fluorescence spectrum of a membrane dispersion containing tocopheryl acetate. The relative quantum efficiency is taken as the spectral intensity ratio ($\varphi = (I'/I)$) at 480 nm and 373 nm respectively.

The linear dependence of $\varphi$ as a function of the pyrene concentration $c$ in the membrane above the phase transition (Fig. 2) shows that the excimer formation is diffusion limited [3].

The concentration $c$ ($Å^{-2}$) of pyrene per unit membrane area is given by: $c = (1/F)(R/(1+\alpha))$, with $\alpha$, molar ratio of tocopheryl acetate/dipalmitoyl phosphatidylcholine; $R$: molar ratio of pyrene/dipalmitoyl phosphatidylcholine and $F = F_0 - \Delta F$, average area per molecule in the bilayer.

The change of the average area per lipid molecule ($\Delta F$) of dipalmitoyl phos-

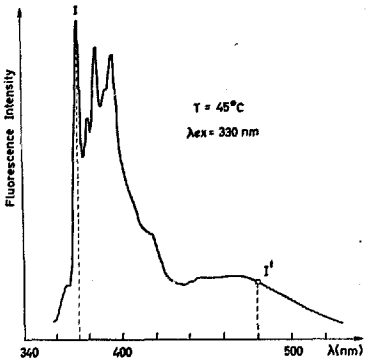


Fig. 1. Fluorescence spectrum of dipalmitoyl phosphatidylcholine membranes containing 1 mol % pyrene and 20 mol % tocopheryl acetate. Excitation and fluorescence bandwidths 6 and 2 nm respectively.

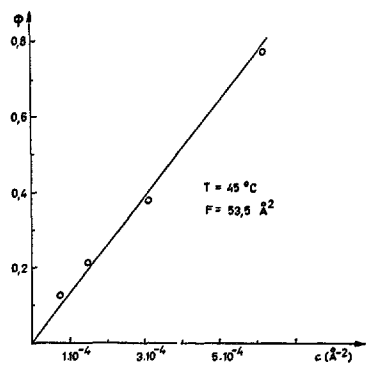


Fig. 2. Pyrene concentration dependence of relative excimer/monomer quantum efficiency of pyrene fluorescence in dipalmitoyl phosphatidylcholine membranes containing 12 mol % tocopheryl acetate.

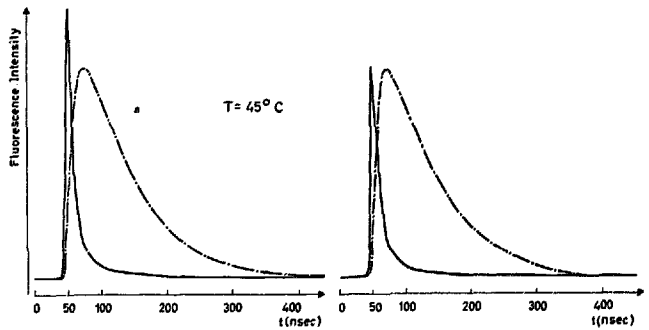


Fig. 3. Excimer fluorescence response (– · –) to an $N_2$-discharge light flash (—). (a) of dipalmitoyl phosphatidylcholine membranes containing 10 mol % pyrene and 12.6 mol % tocopheryl acetate. (b) of dipalmitoyl phosphatidylcholine membranes containing 10 mol % pyrene and no tocopheryl acetate. In the excitation and fluorescence beam an interference filter (Schott) with peak transmission at 334 nm and an edge filter (KV 408, Schott) respectively were used. The decay curves were measured by sampling 500 points per pulse and by averaging 512 pulses with a HP 5480 A CAT computer.

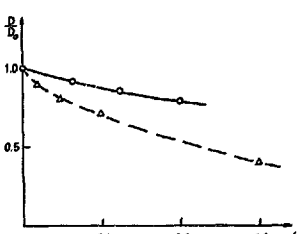


Fig. 4. Tocopheryl acetate concentration dependence of relative lateral diffusion coefficient of pyrene in dipalmitoyl phosphatidylcholine/tocopheryl acetate membranes containing 1 mol % pyrene (–○–). Cholesterol concentration dependence of relative lateral diffusion coefficient of pyrene in dipalmitoyl phosphatidylcholine/cholesterol membranes as measured by Galla and Sackmann [3] (–△–). $D_0 = 1.00 \cdot 10^{-7} \cdot cm^2 \cdot s^{-1}$ [3]. Temperature, 45 °C.

phatidylcholine monolayers spread on water by the incorporation of tocopheryl acetate has been measured in a Langmuir trough and was found to be approximately $\Delta F = 0.65 \cdot \alpha \cdot F_0$ at 45 °C and 35 dyn cm$^{-1}$. $F_0 = 58$ Å$^2$ being the area per molecule in pure dipalmitoyl phosphatidylcholine membranes at 45 °C measured by X-ray diffraction [4]. At 35 dyne · cm$^{-1}$ the dipalmitoyl phosphatidylcholine monolayer yields the same area per molecule. The excimer lifetime measurements were performed with light flashes of a pulsed nitrogen discharge of a few ns duration in bilayers of high pyrene concentration (Fig. 3).

There is no measurable difference in the lifetime of the excimer state in the pure dipalmitoyl phosphatidylcholine membranes and the tocopheryl acetate containing membranes at 45 °C. The excimer lifetime was evaluated from the flash response curve according to [5] and was found to be 73 ns*. From these measurements the ratio $D/D_0$ is obtained according to Eqn. 1 and plotted as a function of $\alpha$ the molar ratio of tocopheryl acetate to dipalmitoyl phosphatidylcholine in Fig. 4 at 45 °C.

(2) *Order parameter*

The fatty acid spin labels $II_{m,n}$ perform a rapid anisotropic motion in fluid phospholipid membranes [6, 7]. The averaged components of the hyperfine splitting tensor $T_{\parallel}$ and $T_{\perp}$ in a membrane bound reference frame with the z'-axis forming the axis of fast molecular rotation are taken from the spectra [6, 7].

Fig. 5 shows the EPR spectrum of a $II_{11,4}$ spin label in a dipalmitoyl phosphatidylcholine/tocopheryl acetate membrane. From $T_{\parallel}$ and $T_{\perp}$ the local order parameter $S$ of the fatty acid chain, which is dependent on the depth of the location of the spin label in the hydrophobic part of the membrane, can be evaluated approximately:

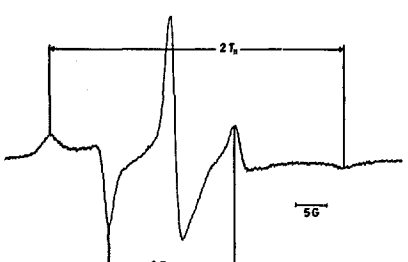


Fig. 5. ESR spectrum of $II_{11,4}$ spin label in dipalmitoyl phosphatidylcholine membranes containing 28 mol % tocopheryl acetate.

* Galla and Sackmann found 100 ns for the excimer lifetime in dipalmitoyl phosphatidylcholine membranes at 45 °C in 2 mM CsCl aqueous solution [3]. The difference may be due to the different ionic solutions used. Because the absolute value of $\tau$ does not influence our results we did not investigate this difference further.

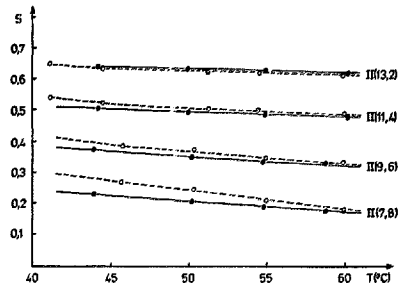


*Fig. 6.* Temperature dependence of order parameter of various spin labels $II_{m,n}$ in dipalmitoyl phosphatidylcholine membranes containing 28 mol % tocopheryl acetate (–○–) and pure dipalmitoyl phosphatidylcholine membranes (●).

$$S = \frac{T_{\parallel} - T_{\perp}}{T_{zz} - T_{xx}} \cdot \left(\frac{a_N}{a'_N}\right)$$

where $T_{zz} = 30.8$ G and $T_{xx} = T_{yy} = 5.8$ G [6, 7] are the components of the hyperfine splitting tensor in a molecular reference frame having a z-axis parallel to the 2 p$\pi$ orbital of the unpaired electron as determined by single crystal experiments. $a_N$ and $a'_N$ are the isotropic hyperfine splitting constants in single crystals and in the model membranes respectively.

In Fig. 6 the order parameter is plotted against temperature for various spin labels in dipalmitoyl phosphatidylcholine/tocopheryl acetate membranes. The absolute values of $S$ obtained in this way should be taken with care since it has been shown [8] that spin label measurements do not necessarily give the correct values for the order parameter in membranes. But the relative values of order parameters of dipalmitoyl phosphatidylcholine membranes with and without tocopheryl acetate are significant. From Fig. 6 it can be seen that an increase of the order parameter is induced by tocopheryl acetate only in the inner part of the membrane.

(3) *Phase transition*

In order to measure the fluid (liquid crystalline)- solid equilibrium phase diagram the spin label TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) was dissolved in the aqueous dipalmitoyl phosphatidylcholine/tocopheryl acetate dispersions in a concentration of $5 \cdot 10^{-4}$ M.

This spin label distributes between the aqueous and lipid phases and is excluded from the lipid phase when it becomes solid. From the relative intensity of the signal of the spin label in the membranes to the signal of the total spin label, the $f$-parameter can be calculated as function of temperature (Fig. 7) and the phase diagram of dipalmitoyl phosphatidylcholine/tocopheryl acetate membranes can be constructed [9].

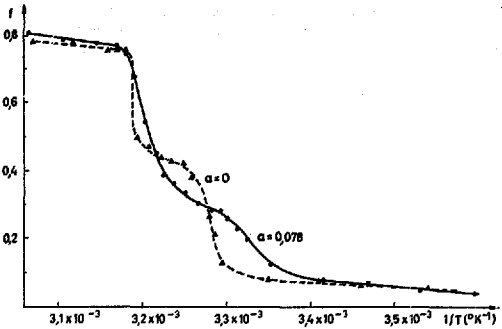


*Fig. 7.* Temperature dependence of *f*-parameter for dipalmitoyl phosphatidylcholine membranes containing 7,8 mol % tocopheryl acetate and pure dipalmitoyl phosphatidylcholine membranes.

One notices in Fig. 7 that the pretransition of dipalmitoyl phosphatidylcholine at 36 °C is still clearly visible in membranes containing 7.8 mol % tocopheryl acetate. At higher tocopheryl acetate contents the transition broadens strongly and a pretransition can not be seen any more. Higher contents than 40 mol % of tocopheryl acetate in dipalmitoyl phosphatidylcholine membranes were excluded from the membranes and separated as small droplets in the dispersion. A possible explanation is that tocopheryl acetate does not form stable bilayers by itself, otherwise, one would probably have found phase separation within the membranes at higher tocopheryl acetate contents.

In Fig. 8 the incomplete solidus and liquidus curves of the dipalmitoyl phos-

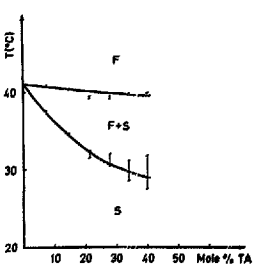


*Fig. 8.* Incomplete phase diagram of dipalmitoyl phosphatidylcholine membranes containing tocopheryl acetate. Above 40 mol % tocopheryl acetate is excluded from the membranes.

phatidylcholine/tocopheryl acetate dispersions for the main transition up to this critical tocopheryl acetate concentration are plotted. From the shape of these curves it can be seen that up to this critical tocopheryl acetate concentration the components are completely miscible in the solid and liquid crystalline phases.

## CONCLUSIONS

This investigation shows, that the lateral diffusion coefficient for pyrene in dipalmitoyl phosphatidylcholine membranes is decreased (Fig. 4) and the order parameter of the fatty acid chains increased (Fig. 6) in the hydrophobic inner part by tocopheryl acetate.

A higher order parameter can be interpreted as decreased fatty acid chain mobility (trans-gauche isomerisation) [10]. Therefore it seems that tocopheryl acetate reduces the probability for trans-gauche isomerisation in the inner part of the membrane. Molecular models show that in the tocopheryl acetate-molecule the methyl groups of the sidechain impede trans-gauche isomerisation by steric interaction. This induces increased order in the bilayer.

This increase of $S$ in the hydrophobic inner part of the membranes also explains the reduction of the diffusion coefficient of pyrene. It is in qualitative agreement with the picture of the diffusion of this molecule as being a hopping process determined by the rate of formation of free volume in the hydrophobic part of the membrane by thermally created rotational isomers (kinks) in the phospholipid molecules [11]. Cholesterol also increases the order parameter of dipalmitoyl phosphatidylcholine membranes above the phase transition but to a much higher degree than tocopheryl acetate and in contrast to tocopheryl acetate the effect is higher in the more hydrophilic outer part of the membrane [12]. Cholesterol reduces the pyrene lateral diffusion more than tocopheryl acetate in the same molar ratio (Fig. 4).

Tocopheryl acetate has a strong effect on the phase transition of dipalmitoyl phosphatidylcholine/tocopheryl acetate membranes (Fig. 8). It broadens the temperature range of the transition appreciably up to a critical tocopheryl acetate concentration from where on tocopheryl acetate is excluded from the membranes. Below this critical tocopheryl acetate concentration dipalmitoyl phosphatidylcholine and tocopheryl acetate seem to be completely miscible in the solid and liquid crystalline state of the membrane.

## ACKNOWLEDGMENT

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## REFERENCES

1 Green, J. and Bunyam, J. (1969) Nutr. Abstr. Rev. 39, 321–345
2 Lucy, J. A. (1972) Ann. N.Y. Acad. Sci. 203, 4–11
3 Galla, H. J. and Sackmann, E. (1974) Biochim. Biophys. Acta 339, 103–115
4 Träuble, H. and Sackmann, E. (1972) J. Am. Chem. Soc. 94, 4499–4510
5 Birks, J. B., Dyson, D. J. and Munro, I. H. (1963) Proc. Roy. Soc. Lond. A 275, 575–588
6 Hubbell, W. L. and McConnell, H. M., (1971) J. Am. Chem. Soc. 93, 314–326

7 Seelig, J. (1970) J. Am. Chem. Soc. 92, 3881–3887
8 Niederberger, W. and Seelig, J. (1974) Ber. Bunsenges. 78, 947–949
9 Shimshick, E. J. and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
10 Axel, F. and Seelig, J. (1973) J. Am. Chem. Soc. 95, 7972–7977
11 Galla, H. J. and Sackmann, E. (1974) Ber. Bunsenges. 78, 949–953
12 Schreier-Muccillo, S., Marsh, D., Dugas, H., Schneider, H. and Smith I. C. P. (1973) Chem. Phys. Lipids 10, 11–27