VITAMIN E - PHOSPHOLIPID MEMBRANE INTERACTIONS: ESR AND STESR STUDY

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ABSTRACT. The effect of α-tocopherol on a phospholipid model membrane has been investigated by Electron Spin Resonance and Saturation Transfer ESR by using stearic acid and perdeutero- di-t-butyl nitroxide spin probes. It is observed that in the gel phase it induces a decrease of order and increase in fluidity; while in the liquid crystalline phase an indication of a slight increase in ordering and a clear decrease in fluidity are registered.

INTRODUCTION

Vitamin E, whose main constituent is α-Tocopherol (αT), is an indispensable lipid component of biological membranes. It is a chain-breaking antioxidant preventing peroxidation of the highly unsaturated fatty acids in membrane lipids [1]. It has also been suggested that it might stabilize biological membranes by restricting the molecular mobility of their components [2].

In the present work we report the effects of αT, in the gel and liquid crystalline phases, both on the rate of the stearic acid spin label motion and on the phospholipid chain order by using conventional ESR and STESR spectroscopy. These results, together with the dynamical information obtained by using PDDTBN, are critically analysed in order to obtain a clearer insight on the effect of αT on lipid membranes.

MATERIALS AND METHODS

D-α-tocopherol and Dipalmitoyl- L- α- phosphatidylcholine (DPPC) were obtained from Sigma Chem. Co. and used without any further purification. 5-keto and 16-keto stearic acid spin labels (5 SASL and 16 SASL, respectively) were purchased from SYVA, Palo Alto. C^{13}-depleted PDDTBN was kindly provided by Prof.W.Plachy of San Francisco State University.

Stearic acids labelled DPPC liposomes were prepared according to the procedure reported in ref [3], but in the present case, stearic acid spin probes, dissolved in ethanol, were mixed with αT and DPPC at the beginning of the sample preparation (probe to lipid molar ratio was 1:100). Dry films were hydrated with borate buffer at pH 9.4. This pH has been chosen to ensure that all spin probe carboxyl groups are ionized in the phosphatidylcholine membranes [4].Samples were contained in 1mm i.d. 100 μl glass capillaries within standart 4 mm diameter quartz tubes. The ESR apparatus and settings were reported in ref.[3].

For randomly oriented liposome samples, where the stearic spin labels undergo rapid anisotropic motion, the order parameter S was estimated as reported in ref. [5]. For samples in which the spin label motion was slower ($\tau_c > 10^{-8}$s), some sort of information about the relative order was obtained by taking into account the $2A_{||}$ splitting [6].

For the samples showing very slow motion ($\tau_c \sim 10^{-7} - 10^{-3}$ s) we used second harmonic absorption out-of-phase detection at high power (STESR). Effective correlation times were obtained by using the reference curves given in ref.[7].

RESULTS AND DISCUSSION

Fig 1.a and b shows the ESR spectra of 5 SASL and 16 SASL ,respectively in 20 mol % αT containing DPPC liposomes at different temperatures. 5 SASL inserted into the bilayer is able to monitor the region near to the polar interface of the membrane and 16 SASL inserted into the bilayer is able to monitor the deep interior of the

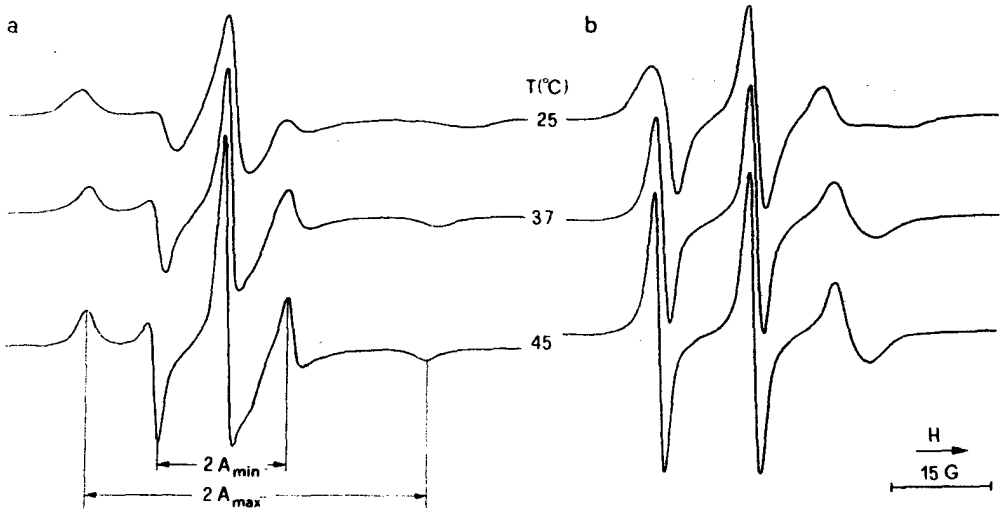

Fig.1. Conventional first harmonic in phase absorbtion ESR spectra of: 5 SASL (a) and 16 SASL (b) in DPPC liposomes containing 20 mol % αT at different temperatures.

bilayer far away from the polar interface of the membrane. When αT is added the ESR spectra obtained are similar to those of pure DPPC liposomes except that the sudden change in line-heights, qualitatively monitoring the main phase transition, is not now observed.

Fig.2 shows the variation of A_{max} for 5 SASL and 16 SASL as a function of temperature for DPPC liposomes with and without addition of αT. S order parameter values were also calculated. It is found that for 41° C, by using 5SASL, S=0.52 for pure DPPC liposomes and S=0.55 for 20 mol % αT containing DPPC liposomes. At 39° C, by using 16 SASL, S is found to be 0.31 for pure DPPC and 0.10 for 20 mol % αT containing DPPC liposomes. Examination of Fig.2 and order parameter values reveals that, below the phase transition temperature, addition of αT slightly decreases order in the region near the phospholipid polar heads; while above the phase transition an almost negligible increase is registered. Concerning the interior of the bilayer, deeply inside the acyl chains, 16 SASL monitors a significant decrease of the phospholipid chain order in the presence of αT below the main phase transition. The effect of αT on ordering of the biomembrane is usually mixed up throughout the related literature [8]., Our results agree with those of Wassall et al.[9].

Fig. 3 shows the second harmonic 90° out of phase ESR spectra (V_2'), recorded at different temperatures, for DPPC multilamellar dispersions labelled with 5 SASL in the absence (Fig.

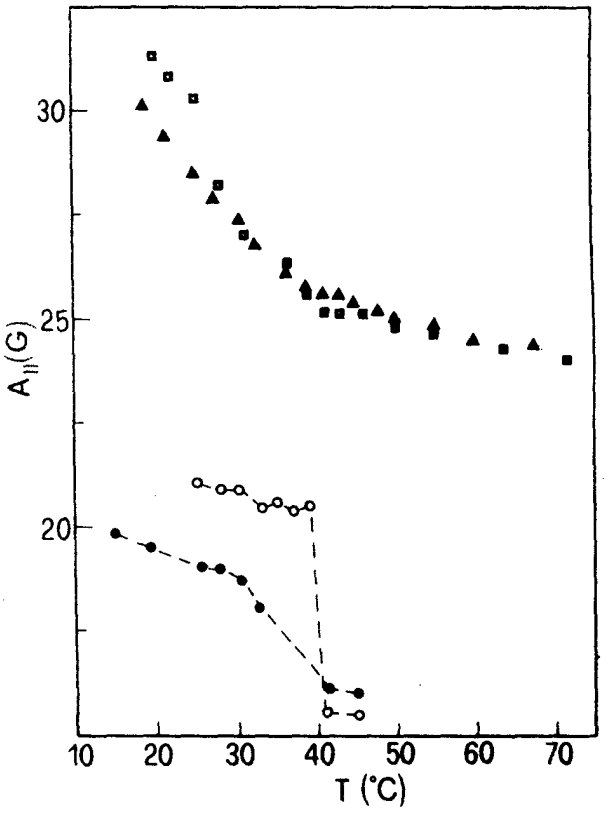

Fig.2. Temperature dependence of the maximum hyperfine $2A_{||}$ splitting of DPPC liposomes with and without αT labelled with 5 SASL and 16 SASL. 0 mol % αT, 5 SASL (□); 20 mol % αT, 5 SASL (▲); 0 mol % αT, 16 SASL (○); 20 mol % αT, 16 SASL (●).

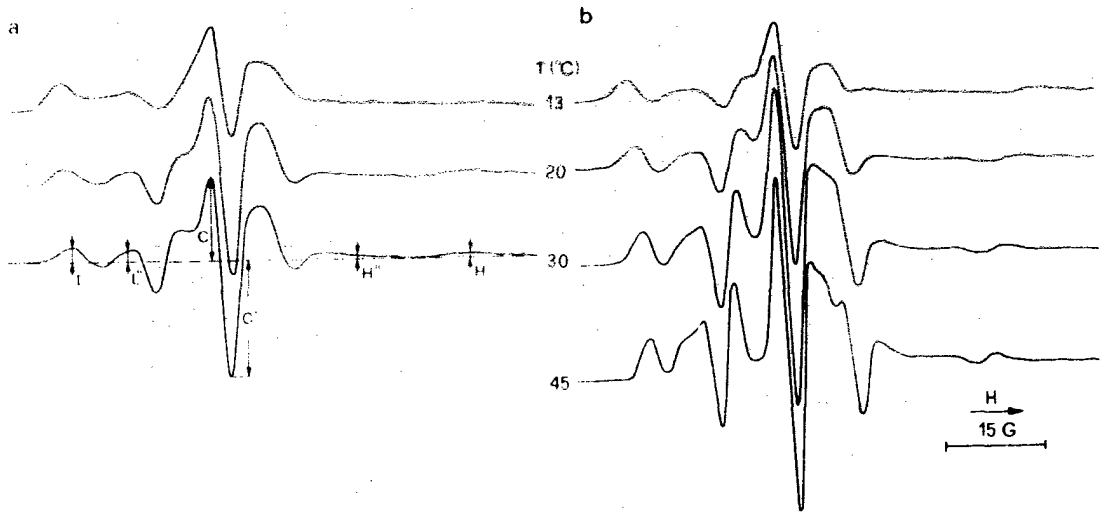

Fig.3. STESR spectra recorded at different temperature values in the second harmonic, 90° out-of-phase, absorbtion mode of DPPC liposomes without (a) and with (b) αT labelled with 5 SASL.

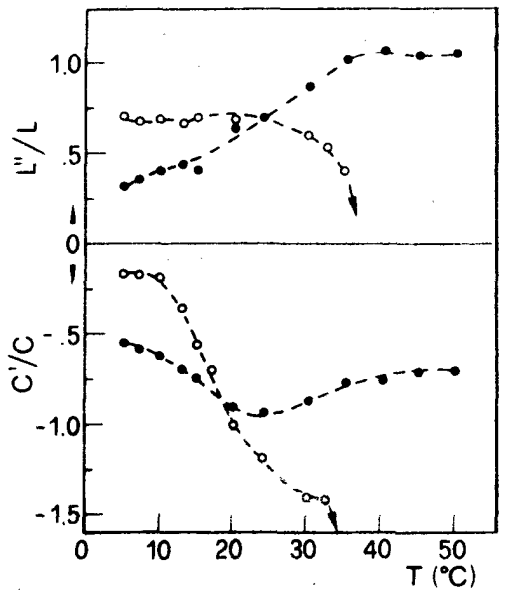

Fig.4. Comparison of the temperature dependence of L" /L and C' /C in DPPC liposomes with and without αT. The inset shows the temperature dependence of the log of the ratio of the correlation times of $\tau_c(L''/L)$ and $\tau_c(C'/C)$ obtained through the calibration curves reported in ref.[7], for pure and αT-containing DPPC liposomes: 0 mol % αT (○); 20 mol % αT (●).

3a) and presence (Fig.3b) of 20 mol % αT.It is seen that the spectra are very dependent on temperature. Motion of 5 SASL is restricted by the addition of αT as seen in Fig. 3b. C, C' , L and L'' parameters (shown in the figure) were measured according to ref.[10] and the effective correlation times were obtained by using the reference curves reported in ref.[7].

Temperature dependence of (L''/L) and (C'/C) parameters in DPPC liposomes with and without addition of αT have been depicted in Fig.4 and the corresponding effective correlation times have been listed in Table I. By looking at Fig.4 and Table I, it can be inferred that, in liquid crystalline phase, αT notably decreases the rate both of the spin label rotation around the long axis (variation in C'/C) and of the rotation of axis itself (variation in L''/L). On the other hand, in the gel phase,when αT is added a slight increase both in the wobbling motion and in the rate of motion around the long axis are registered.

Since our findings related to changes in membrane fluidity induced by addition of αT, conflict with those of some authors [8,11], we decided to obtain additional information on the reorientational dynamics of a small, almost spherical spin probe, PDDTBN, in the presence of αT. PDDTBN molecules located in the lipidic milieu give an almost isotropic ESR spectrum whose components permit us to estimate the rotational correlation time (τ_c) of the spin probe [12]. Variation of τ_c in pure DPPC bilayers and in the presence of 20 mol % αT concentration is shown as a function of temperature in Fig.5. Above the phase transition higher values are obtained for τ_c in the presence of αT. This is indicative again of an αT induced decrease in the bilayer fluidity in the liquid crystalline phase. Our results on the lipid dynamics in the presence of αT are in agreement with those of other authors [10,13].

TABLE I. Effective rotational correlation times of the 5-SASL in DPPC liposomes in the absence and in the presence of 20 mol % αT deduced from calibration curves of STESR line-height ratios of spin labelled hemoglobin.

	τ_c (L"/L) s									
Lipid	5°C	10°C	15°C	20°C	24°C	30°C	35°C	40°C	45°C	50°C
DPPC	1.6×10^{-5}	1.6×10^{-5}	1.6×10^{-5}	1.6×10^{-5}	1.6×10^{-5}	1.2×10^{-5}	0.6×10^{-5}	$< 10^{-6}$	$< 10^{-6}$	$< 10^{-6}$
DPPC+αT	4.6×10^{-6}	6.3×10^{-6}	6.3×10^{-6}	1.5×10^{-5}	1.9×10^{-5}	3.0×10^{-5}	5.0×10^{-5}	5.5×10^{-5}	5.3×10^{-5}	5.3×10^{-5}
	τ_c (C'/C) s									
DPPC	2.5×10^{-6}	2.4×10^{-6}	4.0×10^{-7}	0.4×10^{-7}	$\sim 0.1 \times 10^{-7}$	$\sim 0.1 \times 10^{-7}$	$< 10^{-8}$	$< 10^{-8}$	$< 10^{-8}$	$< 10^{-8}$
DPPC+αT	4.0×10^{-7}	3.0×10^{-7}	1.0×10^{-7}	0.4×10^{-7}	0.4×10^{-7}	0.63×10^{-7}	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}

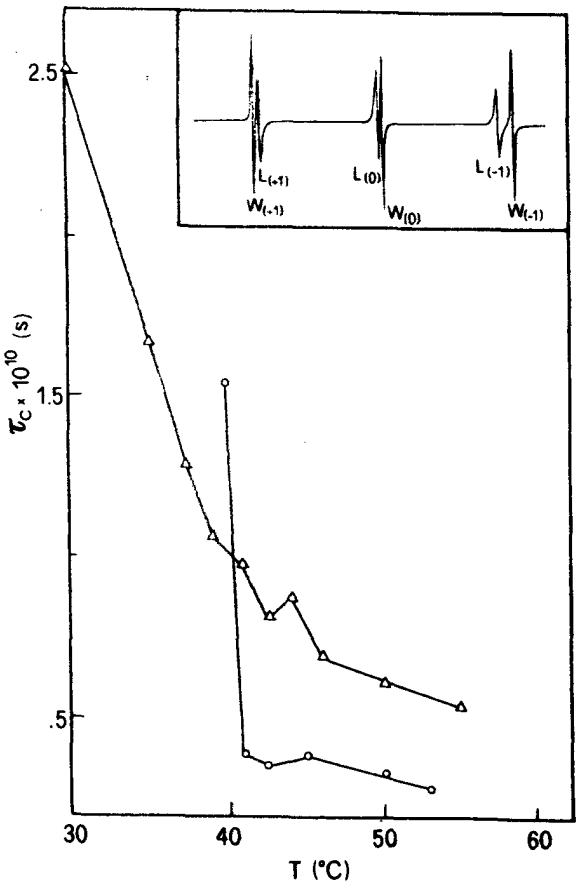

Fig.5 Temperature dependence of the rotational correlation time of PDDTBN in pure (○) and 20 mol % αT containing (△) DPPC liposomes. The inset shows the general appearance of the ESR spectrum of PDDTBN in the liposomes, above the main phase transition.

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