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POSTER

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FTIR studies of vitamin E-cholesterol-DPPC membrane interactions in CH_2 region

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Abstract Binary and ternary mixtures of α-tocopherol (αT), cholesterol and dipalmitoyl phosphatidylcholine (DPPC) in the form of multilamellar liposomes have been investigated by Fourier Transform Infrared Spectroscopy (FTIR). Investigation of frequencies, bandwidths and band shapes of CH_2 stretching and scissoring bands indicate that the effect of αT is dominant in comparison with cholesterol and αT decreases the interaction of cholesterol with phospholipid membranes.

Introduction

The use of vitamin E in the treatment and prevention of several diseases, including cardiovascular disorders, has been mainly explained, so far, by considering its role in prevention or minimization of free radical damage [1]. The alpha-form of vitamin E with its prominent antioxidant activity is the major biological dietary component [2]. Recently it has been proposed that there may be a correlation between the structural and dynamical membrane properties of vitamin E and its antioxidant potency [3]. However, the exact molecular mechanism behind such diverse biological functions of vitamin E is not clearly known. An understanding of the interaction between αT, cholesterol and phospholipids will be important to understand this mechanism. To elucidate this binary and ternary mixture of αT, cholesterol and dipalmitoyl phosphatidylcholine (DPPC) multilamellar liposomes (MLV) were investigated by Fourier Transform Infrared (FTIR) spectroscopic technique.

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Materials and methods

d-α-Tocopherol, cholesterol and dipalmitoyl-L-α-phosphatidylcholine (DPPC) were purchased from Sigma (ST. Louis, Mo) and were used without further purification. Pure phospholipid multilamellar liposomes were prepared according to the procedure reported by Severcan and Cannistraro [4], but with a reduced amount (80%) of hydration. Infrared spectra were obtained using a Nicolet 510 FT-IR spectrometer. Samples suspension (20 µl) were placed between CaF_2 windows with 12 µm sample thickness. Interferograms were averaged for 100 scans at 2 cm^{-1} resolution. Temperature was regulated by a Unicam Specac digital temperature controller unit. Samples were incubated for 10 min at each temperature before the scan of the spectrum.

Results and discussion

Infrared spectra of lipids have been studied in detail and most bands have been assigned [5–6]. More commonly employed IR parameters are the frequency and the bandwidths of the individual vibrational modes. Infrared spectra of DPPC multilamellar liposomes, both pure and those containing 20 mol% αT and/or 20 mol% cholesterol were investigated as a function of temperature. The CH_2 stretching and the scissoring modes were considered. The results presented here refer to the effect of cholesterol and αT on the DPPC spectra since the actual amounts of αT and cholesterol are much smaller compared to DPPC.

Figure 1 shows the IR spectra of the C–H stretching region of DPPC liposomes in the absence and presence of cholesterol and/or αT at 30 °C. The strong bands around 2920 and 2850 cm^{-1} correspond to the CH_2 antisymmetric and symmetric stretching modes of acyl chains, respectively, which exhibit shifts in the frequency and changes in the bandwidth after the addition of αT and/or cholesterol.

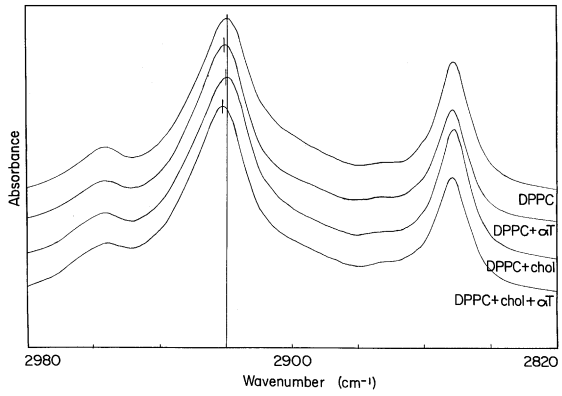

Fig. 1 Infrared spectra of the C–H stretching region of DPPC liposomes containing a) 0 mol% αT and cholestrol, b) 20 mol% αT, c) mol% cholesterol, d) 20 mol% αT and 20 mol% cholesterol, at 30 °C

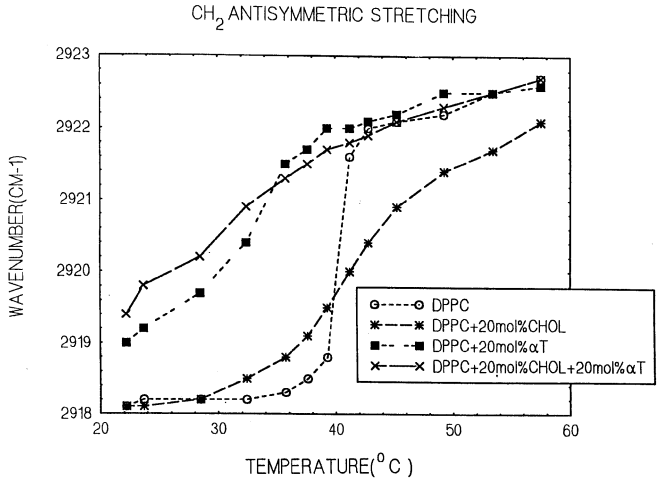

Fig. 2 Temperature dependence of the frequency of the CH_2 antisymmetric stretching mode of pure DPPC multilamellar liposomes in the absence and presence of 20 mol% cholesterol and/or 20 mol% αT

Figure 2 shows the temperature dependence of the frequencies of the asymmetric CH_2 stretching mode for DPPC liposomes in the absence and presence of cholesterol and/or αT. As seen from the figure, in the gel phase, addition of αT into the DPPC system increases the frequency which implies that the number of **gauche** conformers (disordering) increases in the system. Cholesterol has a negligible effect. However, when both of them are present together in the system, an increase in the frequency with respect to pure DPPC is observed. In the liquid crystalline phase ($T > 41$ °C) cholesterol decreases the frequency. The effect of αT on the frequency is negligible. When αT is added to cholesterol containing DPPC liposomes the wavenumber values are very close to those of DPPC indicating that the system behaves as if there is no cholesterol in the system.

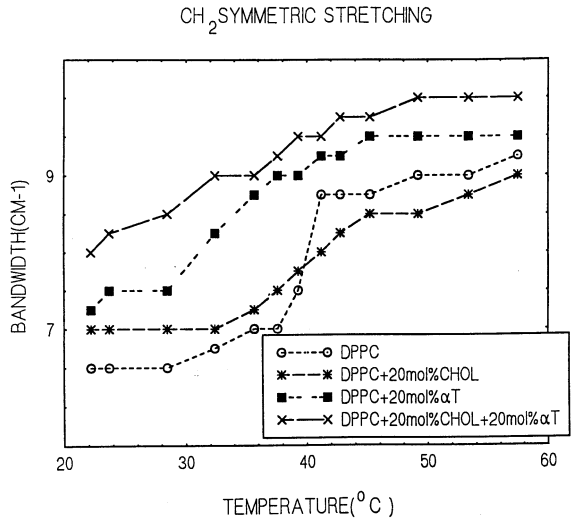

Fig. 3 Temperature dependence of the bandwidth of $0.75 \times$ peak height of CH_2 symmetric stretching mode of pure DPPC multilamellar liposomes in the absence and presence of 20 mol% cholesterol and/or 20 mol% αT

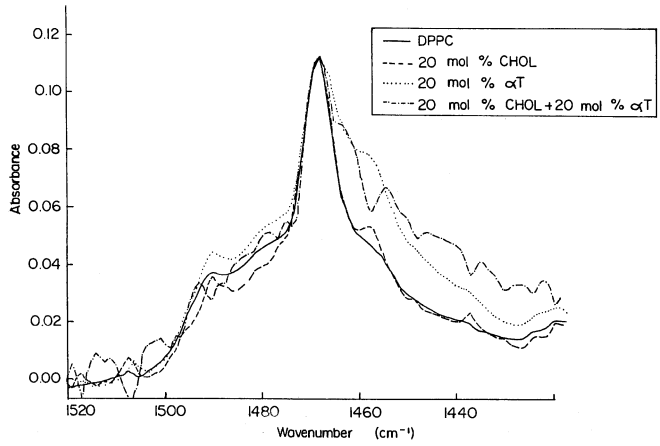

Fig. 4. Infrared spectra of the CH_2 scissoring mode of DPPC at 39 °C in the absence and presence of 20 mol% cholesterol and/or 20 mol% αT

Variation of bandwidth values of the CH_2 symmetric stretching mode at 75% peak height as a function of temperature are shown in Fig. 3, which indicates that cholesterol increases the bandwidth (mobility) in the gel and decreases the bandwidth (mobility) in the liquid crystalline phase. On the other hand, αT increases the bandwidth both in the gel and in the liquid crystalline phase. However, when cholesterol and αT are present in the system together, similar to the effect of αT, an increase in the bandwidth is observed.

Figure 4 shows the infrared spectra of the CH_2 scissoring mode of DPPC, pure and containing αT and/or cholesterol liposomes at 39 °C. As can be seen, inclusion of cholesterol does not change the width of the band. An increase in the bandwidth is observed with

the inclusion of αT in the absence and presence of cholesterol, suggesting an increase in the conformational disorder and chain rotation. Similar effects are also observed at other temperatures.

In conclusion, the present results indicate that, in a ternary mixture of αT, cholesterol and DPPC, the effect of αT on DPPC liposomes is dominant and, especially in the liquid crystalline phase, αT decreases the effect of cholesterol on DPPC liposomes.

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