Comparison of Dynamics of Lecithin Liposomes and Brain Total Lipid Liposomes with Synaptosomal Membranes. An EPR Spectroscopy Study.

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Abstract. Dynamics and/or order of the hydrophobic part of phosphatidylcholine (PC) liposomes and rat brain total lipid (TL) liposomes and synaptosomes were studied and compared by EPR spectroscopy using the spin probes 5 or 16-doxyl stearic acid and 14-doxyl phosphatidylcholine. The dynamics and/or order of the hydrophobic part of TL liposomes or synaptosomes were similar but differed largely from those of PC liposomes. The dynamics of the hydrophobic part of the liposomes decreased gradually with the increasing TL/PC ratio in the sample. To obtain in TL liposomes or synaptosomes the same EPR spectrum parameters as in PC liposomes at 37 °C, the formers have to be heated to temperatures of approximately 50-60 °C. The dynamics and/or order of the hydrophobic part of lecithin liposomes at 5-10 °C were comparable with those of TL liposomes or synaptosomes at 37 °C. The results emphasize the role of the lipid composition in studies concerning drug-lipid and protein-lipid interactions in model and biological membranes.

Key words: Lipid membrane — Synaptosomal membrane — Membrane dynamics — EPR spectroscopy

Introduction

Lipid membranes are widely used as a model for the lipid part of biological membranes. The functions of proteins incorporated in either to liposomes or planar lipid membranes depend on the physical properties of the lipid used (Sanderman 1978; Montal et al. 1981).

Drug-protein and drug-lipid interactions in the ternary model system containing lipid-protein-drug also depend on the properties of the lipids (Neal et al. 1976; Hershkowitz et al. 1982; Feinstein et al. 1975; Baláž et al. 1986). The lipids used most frequently for reconstituted systems are single phospholipids. Membranes prepared from these lipids can have different physical properties with comparison to either total lipids isolated from biological membranes or lipid domains in biological membranes.

Therefore the aim of the present work was to compare the dynamics and/or order of the hydrophobic part of lipid liposomes prepared either from single phospholipid lecithin or from total lipids isolated from rat brain or lipid domains in synaptosomal membranes. We found the dynamics and/or order of synaptosomal membranes to considerably differ of those artificial lecithin membranes.

Materials and Methods

Spin probes 5- and 16-doxyl stearic acid (5-SA and 16-SA) were from Aldrich, U.S.A., and 14-doxyl phosphatidylcholine (14-PC) was provided by courtesy of Dr. L. I. Horvath (Biolog. Res. Center, Szeged, Hungary).

Samples. TL were extracted from rat brain (Folch et al. 1957) and PC from egg yolk (Singleton et al. 1965). PC and TL in chloroform/methanol were mixed at the weight ratios of 4:0, 3:1, 2:2, 1:3 and 0:4. The spin probe was added in ethanol at a lipid/probe ratio of 100. The solvent was evaporated in a stream of nitrogen, followed by evacuation. The dry lipids were hydrated with a buffer containing (in mmol/l) NaCl 145, KCl 5, $MgCl_2$ 1.4, $CaCl_2$ 1, Hepes.HCl 20, 7.4 pH. The lipid/buffer weight ratio in the samples was 0.1. In order to prepare liposomes the samples were sonicated five times for 20 seconds with 30 seconds intervals in a bath at room temperature, and repeatedly subjected to freeze-thaw-vortex cycles. Macala et al. (1983) reported the following composition of rat whole brain lipids (weight per cent): cholesterol 18.6, sphingomyelin 2.8, phosphatidylcholine 22.6, PE 24.1, phosphatidylinositol 2.4, PS 14.6, cerebrosides 11.3 and sulfatides 3.6. Isolated synaptosomes (Krueger et al. 1979) (3 mg of proteins) in the buffer (100 μl) were mixed with the 7.5 μg of the spin probes and equilibrated for 60 min at 4 °C.

EPR measurements. Liposomes or synaptosomes (55 μl) were filled into a glass capillary and EPR spectra were recorded by a BRUKER ER 200 D-SRC spectrometer. Typical instrumental settings were: 9.9 mW microwave power, modulation amplitudes 0.2, 0.05 and 0.1 mT for the spin probe 5-SA, 16-SA and 14-PC, respectively. To compare the two kinds of liposomes the outer (A_{max}) and the inner splittings (A_{min}) were evaluated from the spectra and the apparent order parameter S was calculated according to Marsh (1981). Decrease of parameter S or A_{max} or increase of parameter A_{min} indicates either

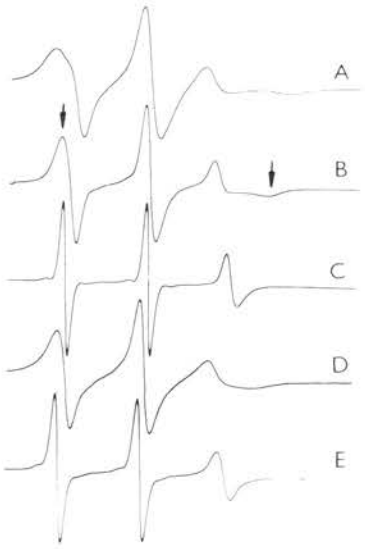

Figure 1. EPR spectra of the probe 16-SA in TL liposomes at 0 °C (A), 37 °C (B), 80 °C (C) and in PC liposomes at 0 °C (D) and 37 °C (E). Spectrum width 6 mT. The arrows indicate A_{max} splitting.

a higher disordering and/or a higher dynamics of the hydrophobic part of the membranes (Gaffney 1976). The membrane order and dynamics were not distinguished from the obtained EPR parameters.

Parameter P was used to estimate dynamics and/or order of synaptosomes or liposomes prepared from the different lipids at a temperature scale (Ondriaš et al. 1983). The parameter P expresses the temperature effect required for a sample studied to reach the same value of the EPR spectrum parameter as that for a sample with another lipid composition. The EPR parameters were estimated to be within the relative error of ± 3%.

Results

The EPR spectra of the spin probes depended strongly on the lipid composition of the liposomes. Examples are shown in Figs. 1 - 3, where EPR spectra of the probes in TL and PC liposomes are compared at various temperatures. Similar results were obtained with the 16-SA and 14-PC spin probe. The spectra of the TL liposomes and synaptosomes are anisotropic, with the A_{max} splitting being clearly resolved even at 37 °C (arrow). On the other hand, the spectra of the PC liposomes are more fluid, with A_{max} being not resolved even at 0 °C. It is also seen

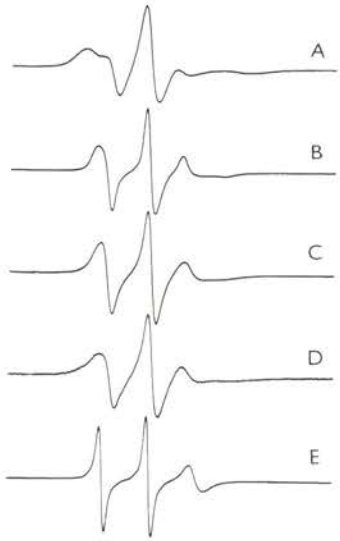

Figure 2. EPR spectra of the probe 14-PC in TL liposomes at 0 °C (A), 37 °C (B), 60 °C (C), and in PC liposomes at 0 °C (D) and 37 °C (E). Spectrum width 10 mT.

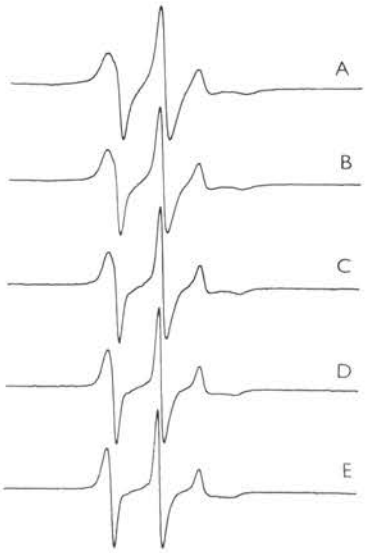

Figure 3. EPR spectra of the probe 16-SA in synaptosomal membranes at 0 °C (A), 10 °C (B), 20 °C (C), 30 °C (D), and 37 °C (E). Spectrum width 10 mT.

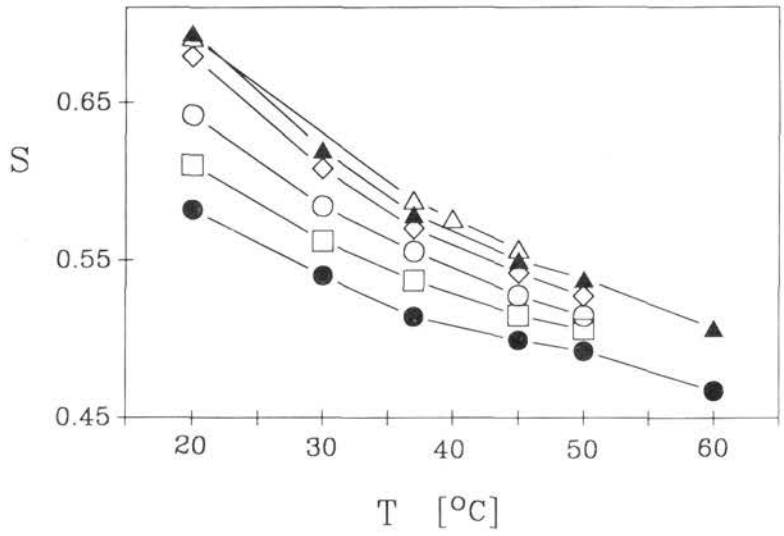

Figure 4. The temperature dependence of parameter S of the probe 5-SA in liposomes at different PC/TL molar ratios: (•), 4:0; (□), 3:1; (○), 2:2; (◇) 1:3; (▲), 0:4; and in synaptosomes, (△).

that PC spectrum obtained at 37 °C is roughly comparable with the TL spectrum at 80 °C.

The apparent order parameter S of the spin probe 5-SA in lipid liposomes increased gradually with the increasing TL/PC ratio in the sample (Fig. 4). The parameter S for synaptosomal membranes was similar to that for TL liposomes. The differences in S between the individual samples expressed in terms of temperature (parameter P) can be evaluated from Fig. 4. For example, the values of S for lecithin liposomes obtained at 20, 30 and 37 °C were comparable with those for TL liposomes at 36, 49 and 58 °C, respectively. For TL liposomes P_S values corresponding to those for PC liposomes at 20, 30 and 37 °C were obtained at 16, 19, and 21 °C, respectively. Qualitatively similar results were obtained for the temperature dependence of parameter A_{min} of the probe 5-SA (data not shown). From this evaluation parameter P_A for TL liposomes with comparison to PC liposomes at 20, 30 and 37 °C, was 14, 15, and 15 °C, respectively.

The differences in the hydrocarbon membrane core obtained using the probes 16-SA (Fig. 5) and 14-PC (Figs. 6 and 7) were more pronounced than those found by the probe 5-SA. Parameter A_{min} for the 16-SA decreased gradually with the increasing TL/PC ratio in the sample, and was similar for synaptosomes and TL within 0–50 °C (Fig. 5). For example, values of A_{min} obtained for lecithin liposomes

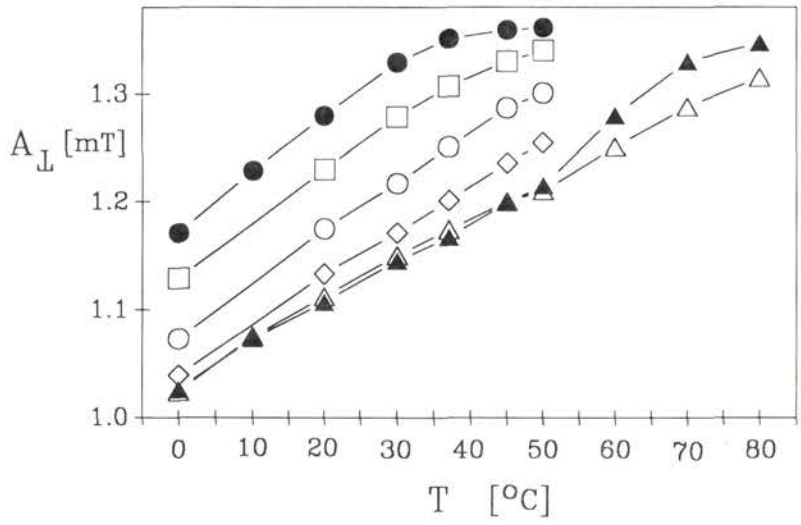

Figure 5. The temperature dependence of parameter A_{min} ($A_{\perp}$) of the probe 16-SA in liposomes at the same PC/TL molar ratios as indicated in legend to Fig. 4.

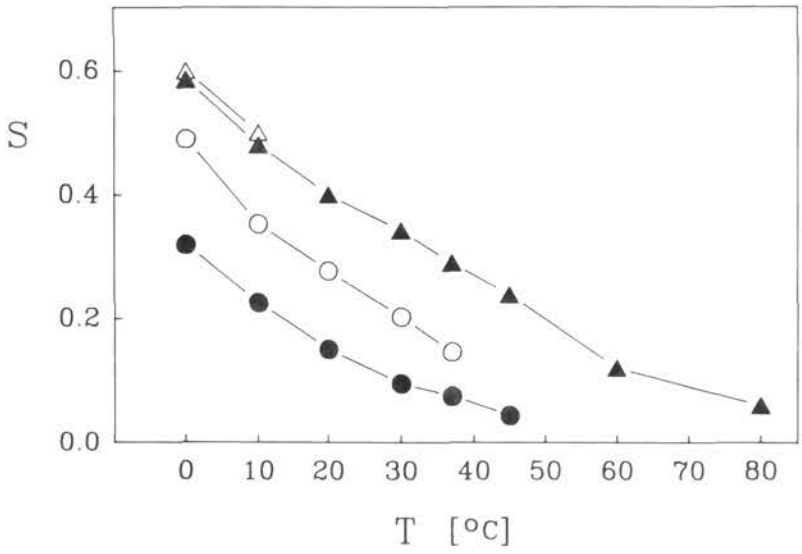

Figure 6. The temperature dependence of parameter S of the probe 14-PC in liposomes: PC (•), TL (▲), PC/TL=1:1 molar ratio (○), and in synaptosomes (△).

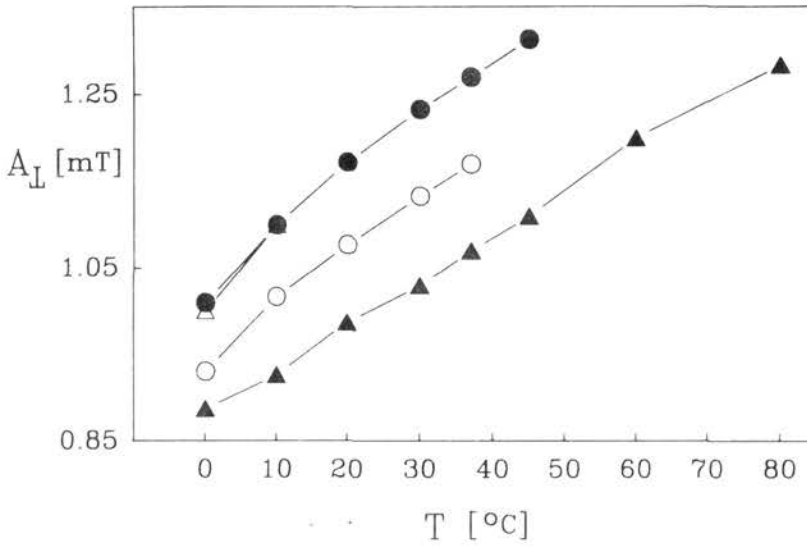

Figure 7. The temperature dependence of parameter A_{min} ($A_{\perp}$) of the probe 14-PC in the same samples as indicated in legend to Fig. 6.

at 0, 10, 20, 30 and 37 °C were comparable with those for TL liposomes (or in synaptosomes) at 35 (35), 53 (53), 60 (67), 70 (>80) and 83 (>87) °C, respectively. Thus, parameter P_A for TL liposomes (synaptosomes) with comparison to PC liposomes at 0, 10, 20, 30, and 37 °C was 35 (35), 43 (43), 40 (47), 40 (>50) and 43 (>50) °C, respectively.

Qualitatively similar results were obtained when the spin probe 14-PC was used. Again, the values of parameter S for TL liposomes were similar to that for synaptosomes, and higher ($P_s \approx 35$ °C) than in PC liposomes (Fig. 6). Similar results were obtained when parameter A_{min} was evaluated from EPR spectra of the 14-PC spin probe (Fig. 7).

Discussion

The spin probes 5-SA and 16-SA or 14-PC reflect the dynamics and order of liposomes at the region close to the polar membrane part and the hydrocarbon membrane core, respectively. The 5-SA probe with its bulky doxyl group can significantly disturb a membrane due to steric interactions (Taylor and Smith 1983). The probe may be shifted vertically depending on the lipid composition. For these reasons the dynamics and/or order of the hydrophobic part of liposomes and synaptosomes using different spin probes were estimated on the temperature scale using

parameter P (Ondriaš et al. 1983; 1987). Qualitatively similar results concerning the physical properties of the hydrocarbon membrane core in dependence on the lipid composition were obtained using both spin probes which contain COOH (16-SA) and phosphatidylcholine (14-PC) head groups. This indicates that changes of EPR spectra observed with different lipids are not connected with specific interactions of the spin probes with the lipids.

Membrane order and/or dynamics and lipid composition. Lipid composition of membrane (lecithin, total lipids and synaptosomes) considerably modulated the dynamics and the order of the hydrophobic part of the membrane. The differences between PC liposomes and TL liposomes or synaptosomes expressed on temperature scale were 15-20 °C as detected by the spin probe 5-SA, and >30 °C as revealed by the spin probes 16-SA or 14-PC. The different values of P obtained with the different spin probes indicate that the membrane dynamics and order of e. g. PC liposomes, cannot be restored to levels measured for TL liposomes or synaptosomes by simply cooling the sample down. In other words, the dynamics and order of the membrane as modulated by lipid composition does not correspond to membrane modulation by temperature. The considerable difference in the physical properties between model lecithin membranes and bilogical synaptosomal membranes emphasizes the role of the lipid composition in studies concerning protein-lipid interactions, e.g. when studying properties of reconstituted proteins in lipid liposomes or planar lipid membranes. So, proteins from synaptosomes incorporated into PC liposomes at 37 °C "perceive" a temperature of approximately 70 - 80 °C in the hydrocarbon membrane core and this may alter the protein function or make the proteins to denature.

EPR spectral parameters of the probes used in the present experiments were similar for TL liposomes and synaptosomal membranes. This suggest that the lipid domains in synaptosomal membranes are sufficiently large and are not pronouncedly affected by membrane proteins; the physical properties of their lipid domains are similar to the properties of liposomes prepared from brain total lipids.

One major reason for the decreased dynamics and/or increased order of the hydrophobic part of TL liposomes or synaptosomes in comparison to PC liposomes can be the presence of cholesterol and lipids with hydrogen bond donor and acceptor groups. Brain total lipids contain PE, PS, cholesterol and cerebrosides which can form hydrogen bonds (Boggs et al. 1986; Hanpft and Mohr 1985; Wong et al. 1989). Cevc (1987) showed that besides the lipid chain type, length, and degree of unsaturation, the major factors determing the physico-chemical state of a membrane are surface polarity and interfacial hydration, including the effects of head group dipole and multipole moments as well as the head group ability to bind hydrogen. Boggs et al. (1986) reported that single-chain compounds (e. g. palmitic

acid) capable of intemolecular hydrogen bonding interaction with dipalmitoylphosphatidylcholine raised the temperature of the main phase transition by >20 °C, comparable to the phase transition of pure dipalmitoylphosphatidylethanolamine which enters into intermolecular hydrogen bonding interactions with lipids. Non-hydrogen bonding compounds, (e.g. methyl palmitate) had no pronounced effect on the phase transition of dipalmitoylphosphatidylcholine. The authors suggested that the high temperature of the membrane phase transition is a result of intermolecular hydrogen bonding interactions.

Cholesterol as an almost rigid planar steroid ring system was found to have an ordering effect on lecithin membranes (for review see Finean 1990). Recently, the hydroxyl group of cholesterol was found to act as a proton donor forming strong hydrogen bonding to the sn-2 chain carbonyl group of dipalmitoylphosphatidylcholine (Wong et al. 1989). The total lipids and synaptosomes in our study contained cholesterol (19% of total lipids), which can make a significant contribution to the membrane dynamics decrease and/or membrane order increase as observed in synaptosomal and TL membranes. It can be supposed that cholesterol, either as a rigid steroid molecule and/or through its hydrogen binding properties, contributes to this effect.

Abbreviations used: 5-SA and 16-SA, stearic acid labeled with dimethyloxazolidinyl (doxyl) group at carbon 5 and 16, respectively; 14-PC, lecithin labeled with the doxyl group at carbon 14; PC, egg yolk lecithin; TL, rat brain total lipids; Hepes, 4-(2-hydroxyethyl) -1-piperazineethanesulphonic acid; EPR, electron paramagnetic resonance; PE, phosphatidylethanolamine; PS phosphatidylserine

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