Effect of Lipid Peroxidation on Molecular Arrangement of Phospholipids in Liposomes Prepared from Egg Yolk Phosphatidylcholine or Total Rat Brain Lipids. A ^{31}P NMR Study

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Abstract. Changes in molecular arrangement of membrane phospholipids in the course of lipid autoxidation were studied by means of broad-band 31-P NMR spectroscopy. Multilamellar liposomes prepared from egg yolk phosphatidylcholine (PC) or total lipid extracts from rat brains (TL) were used as models. The initial lamellar arrangement of phospholipids of both types changed as lipid peroxidation proceeded and a narrow isotropic signal appeared in the spectra at 0 ppm, this phenomenon being more prominent for TL than for PC. Probably the isotropic signal represents some nonlamellar structures within the membranes of peroxidized lipids

Key words: Lipid peroxidation — Phospholipid arrangement — NMR spectroscopy ^{31}P

Introduction

In the last decade significant interest has focused on lipid peroxidation in biological membranes and its influence on a number of important physiological processes, since it has been recognized that free radicals and lipid peroxidation play deleterious roles in a variety of serious diseases as well as in aging (Harman 1984; Halliwell and Gutteridge 1985).

While the chemistry of lipid peroxidation has been studied in great detail (for reviews see: Porter 1984; Frankel 1985; 1987), information on changes in molecular arrangement of membrane lipids during these processes has remained limited (Barsukov et al. 1980; Van Duijn et al. 1984). It is possible that changes in membrane lipid organization may closely be related to the peroxidation-induced damage, since structural polymorphism of membrane lipids has been suggested to play an important role in membrane fusion and transmembrane transport of

cations, membrane lipids and polypeptides (Seelig 1978; Cullis and de Kruijff 1979; de Kruijff and Killian 1987; Tournois et al. 1987).

The aim of the present study was, therefore, to investigate changes in the molecular organization of phospholipids in liposomal membranes prepared from egg yolk phosphatidylcholine or from mixture of lipids isolated from rat brains, by means of ^{31}P NMR spectroscopy.

Materials and Methods

Chemicals: Egg yolk phosphatidylcholine (PC), kindly provided by E. Švajdlenka (Comenius Univ. Bratislava), was isolated according to Singleton et al. (1965). Brain total lipids (TL) were isolated from rat brains by the Folch procedure (Folch et al. 1957). All other chemicals were from commercial sources and of analytical grade.
Preparation of samples: Four hundred mg of lipids (PC or TL) were hydrated with 2 ml of saline (150 mmol/l KCl, 5 mmol/l Tris.HCl, pH 7.4). Multilamellar liposomes were prepared by vortexing the suspension for 2 minutes. The samples were incubated in a 40 °C water bath under air atmosphere. At regular time intervals, 150 μl of the suspension were taken, transferred into glass capillaries (1.5 mm i.d.), sealed and stored at −30 °C until measured. Simultaneously, lipid peroxidation was measured in the samples as conjugated diene formation, with the absorption maxima at 233 nm, using a Specord M-40 spectrometer (Zeiss Jena, Germany). Peroxidation index was evaluated as 233nm/215nm absorbancy ratio ABS (Klein 1970).
NMR measurements: Proton-decoupled ^{31}P NMR spectra were measured with a Varian VXR 300 spectrometer operating at 121.4 MHz (^{31}P). Typically, 1600 accumulations of FID's were necessary to obtain a good quality spectra at room temperature with 54° pulses and 1 s interpulse delay. Exponential multiplication corresponding to 50 Hz line broadening was applied prior to Fourier transformation.
Spectral simulation: Proton-decoupled ^{31}P NMR spectra were simulated according to Balgavý et al. (1984).

Results and Discussion

Lipid peroxidation of PC and TL liposomes, in terms of oxidation index, is illustrated on Fig. 1. For TL, a short lag-phase occurred before the onset of lipid peroxidation. The reason of the lag-phase is not known and it might be attributed to the inhibitory action of lipids with saturated acyl chains (Ondriaš, unpublished results). After the initial lag, the rates of lipid peroxidation of PC and TL were approximately identical, but higher absolute values of oxidation index were measured for PC than for TL.
Phospholipid organization: In freshly prepared liposomes PC and TL phospholipids were organized in extended bilayers, as evidenced by the low-field shoulder and a high-field peak separated by 46 and 57 ppm, for the TL and PC liposomes, respectively (Figs. 2 and 3, top spectra). This difference in chemical shift anisotropy (CSA) may be a consequence of different mobilities of PC and TL phospholipids in

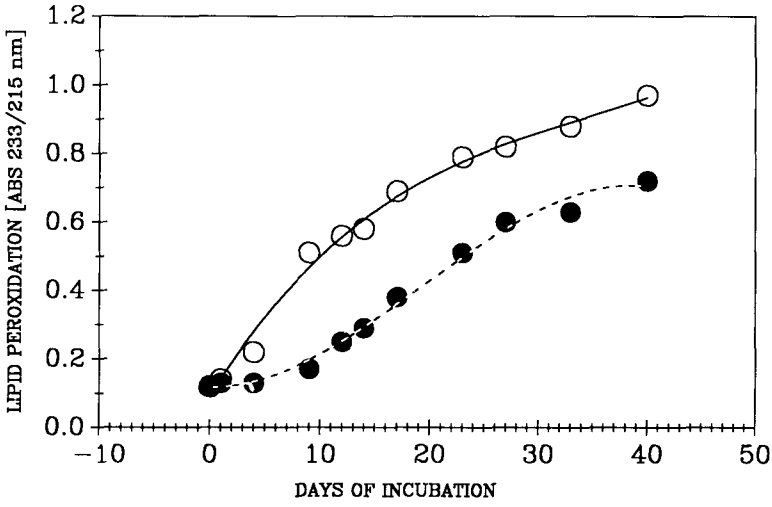

Figure 1. Time course of lipid peroxidation of incubated liposomes expressed as oxidation index (ABS 233/215 nm). Full line – liposomes from egg yolk lecithin (PC), broken line – liposomes from total brain lipids (TL).

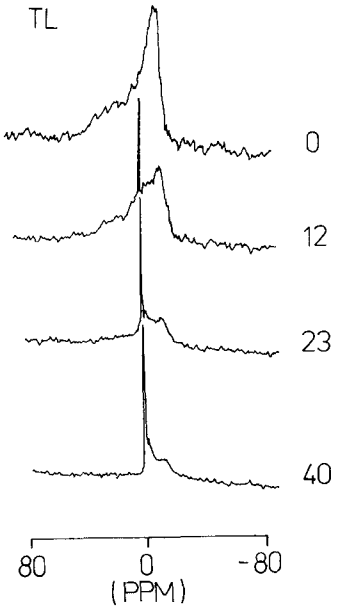

Figure 2. Proton-decoupled ^{31}P NMR spectra of TL phospholipids in liposomes taken on different days of sample incubation. The figures indicate the duration of incubation (days).

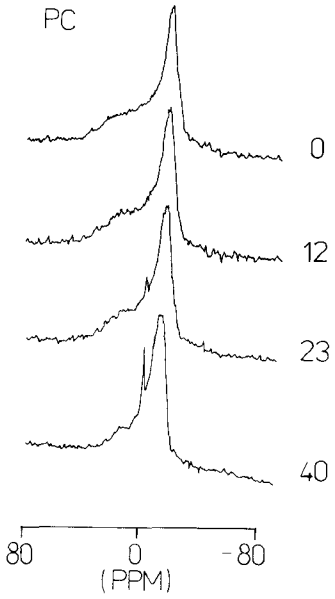

Figure 3. Proton-decoupled ^{31}P NMR spectra of PC phospholipids in liposomes taken on different days of sample incubation. The figures indicate the duration of incubation (days).

liposomal membranes (Smith and Ekiel 1984; Ondriaš et al. 1991). In the course of lipid peroxidation a decrease in CSA was observed for PC (Figs. 3 and 6). For TL, CSA could not be determined from the spectra in later phases of lipid peroxidation.

There is no simple explanation for the PC CSA decrease: different reasons, enhanced movement of phospholipids in the membrane (swinging and tumbling or a lateral diffusion), decreased liposome diameter can lead to the same effect (McLaughlin et al. 1975; Smith and Ekiel 1984). Moreover, a change in the individual line width can also result in a CSA change. This is documented in Fig. 4, constructed from simulated data (CSA[ppm] = $0.38 \times \delta + 50$). From spectra simulation a relationship was obtained allowing estimation of the individual line width from the spectra. Individual line width was found to be proportional to that of the high field peak at the spectrum maximum halfheight ("halfwidth"): $\delta_{(1/2)max} = 3.39 \times \delta$ (Fig. 5). However, as we can see from Fig. 6, the halfwidth of highfield maxima did not change significantly in the spectra of PC liposomes during 40 days of lipid peroxidation, whereas a significant decrease of the chemical shift anisotropy was observed during this period. We may thus conclude that i) the individual line width, which is mainly influenced by the relaxation time T_2^* of the 31-phosphorus nuclei, did not change significantly in the course of lipid peroxidation, and ii) the change in individual line width did not contribute significantly to the decrease observed in the chemical shift anisotropy.

In addition to the changes of the CSA another, even more apparent, feature

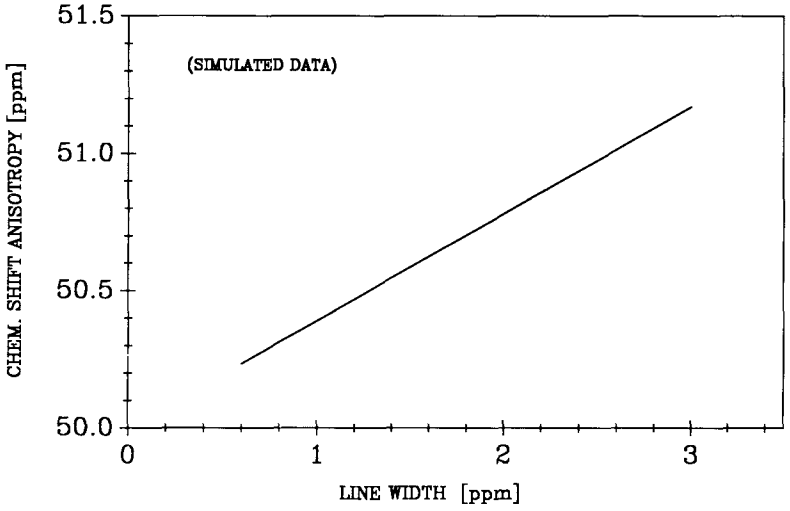

Figure 4. The chemical shift anisotropy (σ_{EFF}) plotted vs. individual line width, δ, (constructed from simulated data).

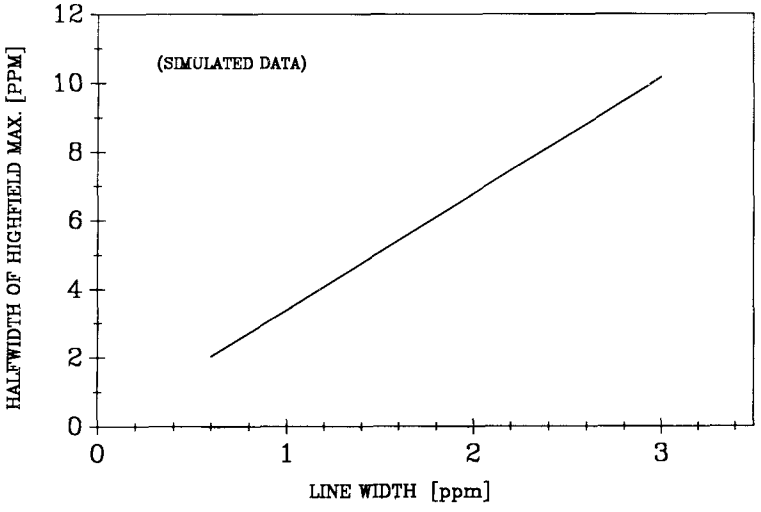

Figure 5. The "halfwidth" of highfield maxima ($\delta_{(1/2)max}$) in the proton decoupled ^{31}P NMR spectra plotted vs. individual line width, δ, (constructed from simulated data).

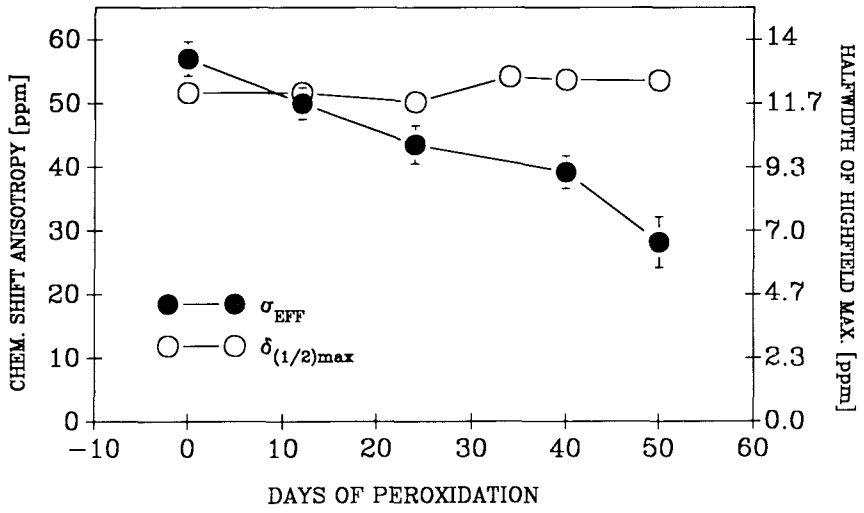

Figure 6. Changes in chemical shift anisotropy (σ_{EFF}) and in "halfwidth" of the highfield maxima ($\delta_{(1/2)max}$). Data were obtained from the spectra shown in Fig. 3.

was observed in the spectra of peroxidized lipids. Besides the maximum at about 17 ppm, which is typical for lamellar arrangement of lipids, a narrow line appeared in the spectra at 0 ppm. The intensity of this "isotropic" signal increased in the course of lipid peroxidation, and was more prominent in TL than in PC. The isotropic signal could be ascribed to lipid aggregates with effectively isotropic and fast motion of lipid molecules, such as in mixed lipid micelles, cubic and tetraedric phases (Lindblom and Rilfors 1989), and in lipid bilayers of unilamellar vesicles with a diameter smaller than 150 nm (Burnell et al. 1980). An isotropic signal in the ^{31}P NMR spectra has been observed after addition of lyso-PC (15 mol %) to PC liposomes (Barsukov et al. 1980). Isotropic signal in the ^{31}P NMR spectra of oxidized phosphatidylethanolamine (PE) has been reported by van Duijn et al. (1984). In their system, oxidation led to the stabilization of PE in a lamellar phase, and after prolonged oxidation an isotropic signal was observed. As evidenced by HPLC and freeze-fracture electron microscopy, this isotropic signal did not originate either from lyso-compounds or from small highly curved vesicles. However, in contrast to our results these authors did not observe any isotropic signal during autoxidation of PC, perhaps due to the lesser extent of lipid oxidation in their system.

It is conceivable that the observed isotropic signal originates from some non-bilayer structures within the membrane. This possibility is supported by several facts. Gast et al. (1982) reported the diameter of PC vesicles to increase during

autoxidation. According to these authors products of lipid autoxidation may act as fusogens. In the process of fusion, nonlamellar structures form in membranes (Cullis and de Kruijff 1979; Gast et al. 1982), e.g. inverted micelles with a typical isotropic signal (de Kruijff et al. 1982). Moreover, the rates of lipid flip-flop, which increase several times in the presence of nonlamellar structures in the membrane (Cullis and de Kruijff 1979), increased during lipid peroxidation (Kornberg and McConnell 1971; Barsukov et al. 1980; Shaw and Thompson 1982).

The formation of nonlamellar structures during lipid peroxidation may be explained on the basis of the molecular shape concept (Cullis and de Kruijff 1979). During peroxidation of unsaturated fatty acyl chains of lipids polar hydroperoxy groups form in the hydrophobic part of membranes. For instance, in the course of oxidation of substituted linoleic acid peroxy groups were localized on the C-9 and C-13 carbons of the fatty acid (Logani and Davies 1980). As a consequence of the presence of the polar hydroperoxy groups the effective diameter of peroxidized lipids increases in the hydrophobic part. Thus the dynamic shape of peroxidized lipids becomes more cone-like, with the effective diameter at the hydrocarbon moiety of the lipid molecule being larger than that at the polar lipid head portion. At this molecular shape, phospholipids prefer nonlamellar arrangement in the membrane (Wieslander et al. 1980). The difference observed in the isotropic signal intensity can be understood within the framework of the shape concept as well. Besides containing the cylindrical PC (which prefers lamellar arrangement), total brain lipids are also rich in inverted cone-shaped unsaturated PE which prefers nonlamellar arrangement in the membrane. Thus the lamellar structure of TL can easily be converted into nonlamellar structure when hydroperoxy groups are formed in the process of lipid peroxidation, a process which raises the content of inverted cone-shaped lipids in TL membranes.

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