An ESR-Study of the Peroxidation of Lecithin Multilayers

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By means of a set of fatty acid and fatty acid ester spinlabels we found that spontaneous peroxidation of lecithin multilayers increases the rigidity of all regions of the lipid bilayer. However the partition coefficient of TEMPO increased with peroxidation indicating a greater affinity of TEMPO to the modified bilayer.

Key words: Lecithin multilayers, peroxidation, ESR, spinlabel-technique.

I. Introduction

Natural phospholipids contain unsaturated double bonds and therefore are vulnerable by peroxidation. Lipid peroxidation is very important in physiology, pathology and food technology [1]. From the biophysical point of view it is interesting to know how lipid peroxidation influences the membrane structure. Contradictory results on this issue have been published. It has been reported that lipid peroxidation decreases [2–4] as well as increases [5, 6] the degree of order of natural and artificial membranes.

It seems that one reason for this discrepancy of experimental findings is the complex structure of natural membranes. The agents employed to induce peroxidation may also affect membrane proteins which in turn can influence the organization of membrane lipids. Moreover these agents and their products may also disturb the membrane structure by themselves.

The present study was devoted to the characterization of the effect of peroxidation on a simple, well defined lipid system. Multilayers of egg lecithin were subjected to spontaneous peroxidation in air.

II. Experimental

Egg lecithin was purified from fresh hen eggs according to the method of Singleton [7]. Lecithin multilayers were prepared by depositing a lecithin film on the walls of a flask from a chloroform methanol solution (3:1 volume ratio) and shaking the film with 10 mM sodium-phosphate buffer (pH 7.4). The concentration of lecithin was 30 mg/ml buffer.

Spinlabels: (Fig. 1). 5-doxylstearic acid (I), 12-doxylstearic acid (III), 16-doxylstearic acid (V) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, VI) were purchased from Sigma. Ethyl-5-doxylpalmitate (II) and methyl-12-doxylstearate (IV) were synthesized by Gwozdzinsky [8], Chair of Biophysics of the University of Łódź.

The spinlabels I–V were used at a final concentration of $1 \cdot 10^{-4}$ M and TEMPO at a final concentration of $5 \cdot 10^{-4}$ M.

From the spectrum of spinlabel I (Fig. 2) an order parameter was derived using the formula

$$S = (T_{\parallel} - T_{\perp})\,(a_N)/(T_{zz} - T_{xx})\,(a'_N). \qquad (1)$$

Here $T_{\parallel}$ and $T_{\perp}$ are hyperfine splitting elements parallel and perpendicular to z', the symmetry axis of the effective Hamiltonian (H'), and T_{xx} and T_{zz} are the hyperfine splitting elements of the static interaction tensor (T) parallel to the static Hamiltonian (H) principal nuclear hyperfine axes x and z. The x-axis is parallel to the N-O bond direction, and the z-axis is parallel to the nitrogen 2 p orbital. $a_N = 1/3\,(T_{zz} + 2\,T_{xx})$ and $a'_N = 1/3\,(T_{\parallel} + 2\,T_{\perp})$; according to [9] the values $T_{xx} = 6.1$ G and $T_{zz} = 32.4$ G were used.

From the spectra of spinlabels II–V a "motion parameter" i. e. the ratio of middle peak height to the low field peak height (h_0/h_{+1}) and the outer half-width at half-height of the high field extremum were determined [10].

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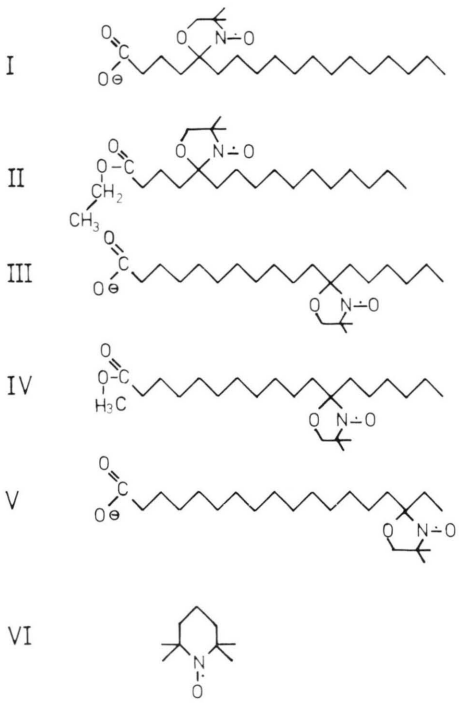

Fig. 1. Spinlabels used in this study.

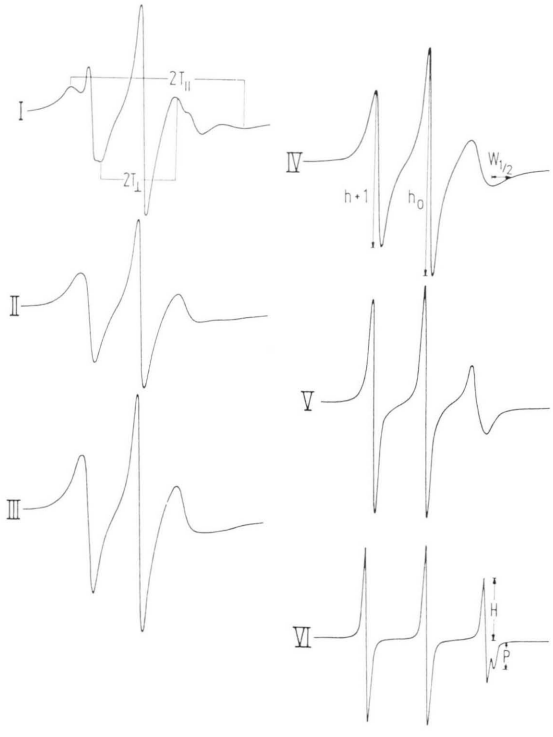

Fig. 2. ESR-spectra of spinlabels I–VI in lecithin multilayers.

From the spectrum of TEMPO the partition coefficient between the lipid and the water phases $f = H/H + P$ was obtained [11].

The lecithin multilayers were subjected to spontaneous peroxidation in air in a closed container saturated with water vapor in order to keep a constant concentration of lipid in the buffer at room temperature up to seven days.

Lipid peroxidation was determined by the spectrophotometric method [12].

III. Results and Discussion

Application of a set of spinlabels of similar structure (doxylgroup located at different positions along the fatty acid chain, Fig. 1) allowed to probe the fluidity in different regions of the lecithin bilayers.

The spectrum of spinlabel I shows the rigidity of the fatty acid chain close to the bilayer surface (Figure 2). From this spectrum the order parameter could be derived. In the spectra of the spinlabels II–V the outer hyperfine extrema were not resolved. The motional behaviour of these spinlabels was characterized by determining the h_0/h_{+1} peak height ratio and the outer half-width at the halfheight of the high field extremum.

All these parameters decrease with increasing temperature (Fig. 3), which confirms their usefullness for estimation of the bilayer fluidity. Figure 4 shows the temperature dependence of the partition coefficient of TEMPO, which increases with increasing temperature.

In agreement with literature [13] we observed that the motional freedom of fatty acid esters is much higher than that of the corresponding fatty acids (Fig. 2, Table 2).

Exposure of lecithin multilayers to air at room temperature induced progressive peroxidation of the polyunsaturated fatty acid residues as demonstrated by increasing values of the absorption ratio measured spectrophotometrically at two wavelengths of 233 and 215 nm (Table 1).

Table 1. A_{235}: A_{215} values, mean ± S.D., $n = 5$.

Time (days)	0	2	7
A_{235}: A_{215}	0.19 ± 0.01	0.36 ± 0.03	0.75 ± 0.05

Table 2. Effect of peroxidation on the spectral parameters of various spinlabels in lecithin multilayers measured at 30 °C ($n = 5$, mean values ± S.D.).

Spinlabel	Parameter	Time of Peroxidation		
		0	2	7 Days
I	S	0.459 ± 0.010	0.469 ± 0.004	0.485 ± 0.009
II	h_0/h_{+1}	1.515 ± 0.008	1.525 ± 0.010	1.627 ± 0.002
III	h_0/h_{+1}	1.383 ± 0.004	1.459 ± 0.006	1.520 ± 0.010
IV	h_0/h_{+1}	1.199 ± 0.001	1.235 ± 0.002	1.244 ± 0.003
V	h_0/h_{+1}	1.038 ± 0.002	1.038 ± 0.001	1.048 ± 0.010
II	$W_{1,2}$ (G)	6.9 ± 0.1	7.6 ± 0.1	8.4 ± 0.2
III	$W_{1,2}$ (G)	4.7 ± 0.2	5.2 ± 0.3	5.8 ± 0.6
IV	$W_{1,2}$ (G)	2.5 ± 0.1	3.1 ± 0.1	3.4 ± 0.1
V	$W_{1,2}$ (G)	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
TEMPO	f	0.758 ± 0.001	0.782 ± 0.0035	0.808 ± 0.005

Table 3. Slopes of Arrhenius plots (Fig. 3) for spectra parameters of spin labels in fresh and peroxidized lecithin multilayers.

Spinlabel	Parameter	Slope [K] $*10^{-3}$ Time of Peroxidation	
		0	7 Days
I	S	1.547	0.977
II	h_0/h_{+1}	1.285	1.156
III	h_0/h_{+1}	2.007	1.156
IV	h_0/h_{+1}	1.107	1.040
V	h_0/h_{+1}	0.351	0.187
TEMPO	f	− 0.627	− 0.593

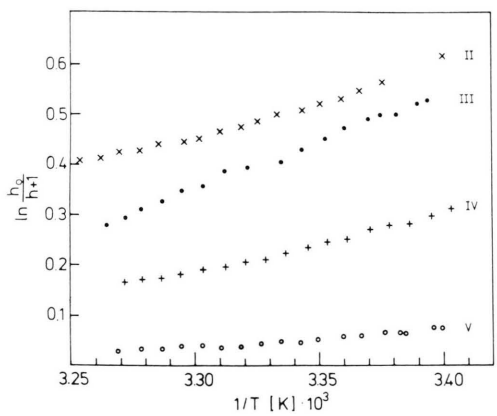

Fig. 3. Temperature dependence of the ratio h_0/h_{+1} of spinlabels II–V in lecithin multilayers plotted in Arrhenius coordinates.

No detectable hydrolysis took place during the incubation period employed, as checked by thin layer chromatography [14].

We observed an increase of the motion parameter ratio h_0/h_{+1} and the halfwidth of the outer extremum with increasing peroxidation, as shown in Table 2. The changes in lipid fluidity upon peroxidation depended on the depth within the bilayer at which they were measured, maximal rigidification being noted in the middle of the constituent monolayers (spinlabels III, IV: depth about 12 carbon atoms). These results are in agreement with those of Bruch and Thayer [15] derived from a spinlabel study of soybean lipid liposomes subjected to iron-ascorbate induced peroxidation, and are in contrast with those of Dobretsov et al. [16], who reported a homogeneous transversal decrease in fluidity in peroxidized biological and model membranes using fluorescent labels.

However the partition coefficient of TEMPO increased rather than decreased with peroxidation. This result is unexpected, because higher values of the partition coefficient of TEMPO are believed to reflect higher bilayer fluidity [10].

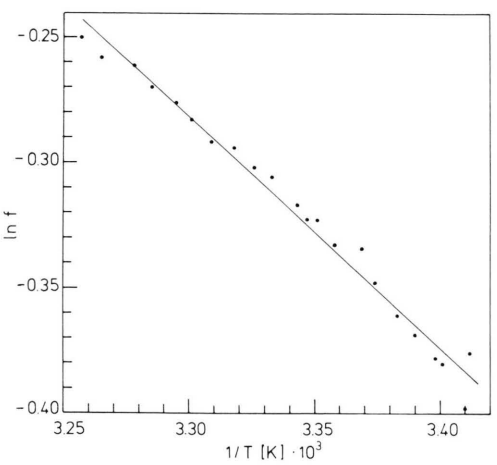

Fig. 4. Temperature dependence of the partition coefficient of TEMPO.

Apparently this molecule may have higher affinity for a lipid bilayer modified by peroxidation although the reason for this effect remains unclear.

In Table 3 the slopes of Arrhenius plots of parameters derived from spin label spectra for 0 and 7 days are listed. These slopes decrease after peroxidation of the multilayers, indicating that the fluidity is less temperature dependent after peroxidation.

In summary, we conclude that peroxidation increases the rigidity of all regions of lecithin bilayers, with preference of the middle of the chains.

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