Fluidity of Liposome Membranes Doped with Metalloporphyrins: An ESR Study

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Changes in membrane fluidity of porphyrin-doped liposomes have been investigated to assess the kinetics of the fluidization process. Metal complexes of *tert*-butylphenyl *meso*-substituted porphyrin, containing ions of Mg, Mn, Fe, Co, Ni and Cu, were used as dopants. Liposomes were obtained by sonication of hen egg yolk lecithin (EYL). Electron paramagnetic resonance (ESR) was applied using two spin probes, TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and 16-DOXYL-stearic acid [2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxyl], localized at different sites within the membrane to determine the spectroscopic parameters: partition (F) and rotation correlation time (τ), related to the membrane's fluidity. It was found, that porphyrins considerably fluidize the membranes, and the dynamics of this process depends on the kind of the compound used and the membrane's area surveyed by the probes. The Cu complex proved to be the most effective one within the surface layer, whereas the Mn complex most strongly fluidized the deeper parts of the lipid double-layer. Variations in fluidity observed after the porphyrins had been introduced into the liposome were found to stabilize inside the double-layer and within the surface layer after ca. 25 and 50 h, most probably due to hydration of the hydrophilic part of the membrane.

Key words: EYL Liposomes, ESR, Metalloporphyrins

Introduction

Synthetic porphyrin derivatives and their metal complexes (Fig. 1) proved to be very attractive materials in many areas of science and technology. Owing to their very close chemical relation to natural porphyrins such as chlorophyll and heme, they are expected to show similar physicochemical properties (Berezin, 1981). Like chlorophyll, their specific structure consists of four coupled pyrrole units, which form a characteristic macrocycle, containing a unique aromatic electronic arrangement. This particular molecular set-up is the principal reason for their unusual spectral properties as well as chemical and biological activity. Therefore, these compounds are believed to find application first of all as photocatalysts and photosensitizers (Mele *et al.*, 2003, 2007). Moreover, the peculiar photoactivity of porphyrins has become a great hope for the progress in photodynamic therapy of tumours (Bonnet, 1995).

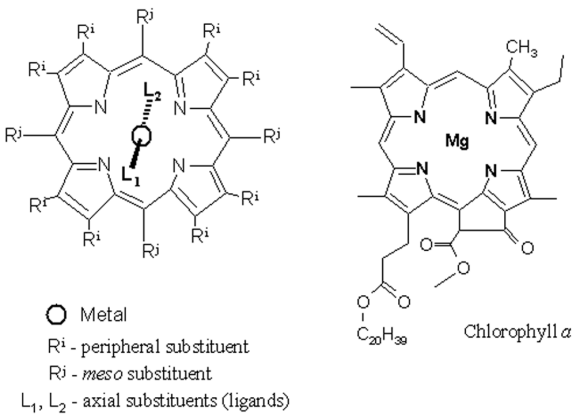

Fig. 1. General representation of metalloporphyrin derivatives (left) and the chlorophyll molecule (right). R^i and R^j may be hydrogen and/or diverse functional groups; L may be organic or inorganic ligands or void; the central metal atom may be replaced by two H atoms.

The principal objective of this work was to investigate the kinetics of processes occurring in hen egg yolk lecithin (EYL)-derived liposome membranes doped by metalloporphyrins. It is known, that organometallic compounds, when introduced into the lipid membranes, do change their physical properties (Falcioni *et al.*, 1996; Gray *et al.*, 1987;

Kleszczyńska *et al.*, 1999). Thus far, studies consisted predominantly in the observation of changes caused by differences in the concentration of the added chemicals (Podolak and Man, 2002; Podolak *et al.*, 2006; Man *et al.*, 2006; Man and Podolak, 2007). In this work, however, liposome membranes doped by an equal porphyrin quantity (2%) were investigated during a long period of time (170 h). Since the lipid double-layer presents dynamic formation with molecules being in permanent movement, one may presume that the kinetics of distribution of the introduced admixtures may be of a complex character, depending on the time and site of observation. Application of two spin probes penetrating different membrane areas should provide information on the effect of admixtures upon the changes in fluidity within the outer and inner part of the lipid double-layer.

Materials and Methods

Liposomes were obtained by sonication of EYL in distilled water, using a ultrasonic disintegrator UD-20 (TECHPAN, Warsaw, Poland). The total sonication time for a single sample of the volume 1.5 ml was 5 min, and the process consisted of a sequence of 60 s cycles of sonication followed by 60 s of cooling. The EYL concentration in the sample was 0.04 M, and that of the probe was 0.01 M relative to the lecithin. The lipophilic complexes of 5,10,15,20-tetrakis(4-*tert*-butylphenyl)porphyrin containing divalent ions of Mg, Co, Ni, Cu and trivalent ions of Mn and Fe were prepared and characterized as described by Mele *et al.* (2007). For clarity, the compounds have been denoted MPp, where M is the appropriate metal and Pp stands for the porphyrin moiety, $[C_{60}H_{60}N_4]^{2-}$. In general, their molecular structures are similar to the one presented in Fig. 2 (Słota *et al.*, 2007).

The porphyrins were added to samples containing liposomes previously formed in water. The content of the admixtures was 2% relative to the molar content of EYL. ESR spectroscopy was applied in the investigations, and the spectra were recorded within 180 h starting from the moment the porphyrins were introduced into the liposomes at a constant temperature of 24 °C. Between the measurements the samples were dark-stored at the same temperature. Two spin probes, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxyl (16-DOXYL-stearic acid) located at different sites inside the liposome membrane, were used. The TEMPO probe dissolves both in the hydrophobic part of the membrane as well as

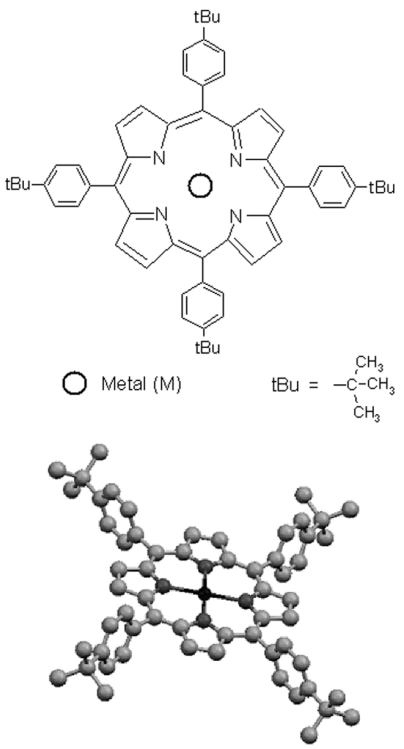

Fig. 2. Chemical structure and 3-D view of the MPp molecule.

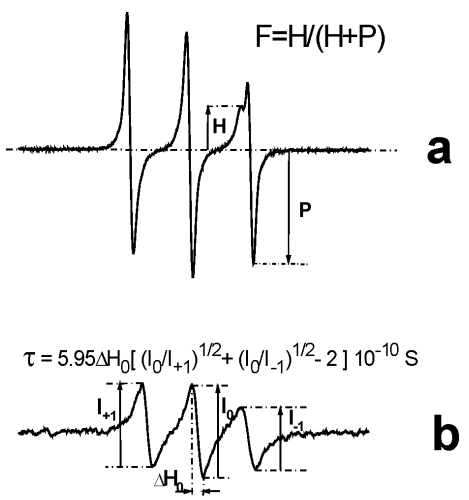

Fig. 3. ESR spectra of spin probes placed in the membrane of EYL liposome: (a) TEMPO probe; (b) 16-DOXYL-stearic acid.

in the water ambient, whereas the 16-DOXYL-stearic acid probe is placed deeply within the hydrophobic layer of the membrane. From the ESR spectrum of the TEMPO probe the spectroscopic partition parameter (F) reflecting the probe distribution between the membrane and its ambient was determined. Measure of the parameter F is the ratio of the high-field line amplitude in the ESR spectrum of the probe dissolved in the water ambient (P) to the amplitude of the low-field line (H) in the lipid environment (Fig. 3a) The value of F, among other parameters, is connected with the membrane fluidity (Shimshick and McConnell, 1973). From the spectrum of the 16-DOXYL-stearic acid probe the spectroscopic parameter τ was determined. Its value depends, among other things, on the degree of the membrane fluidity and is the greater, the more rigid (better ordered) is the ambient of the probe (Hemminga, 1983). In case of an isotropic environment, τ is the rotation correlation time of the probe (Fig. 3b).

Results and Discussion

The relationship between the spectroscopic parameter F and time of the TEMPO probe dissolved in an aqueous suspension of EYL liposomes doped with metalloporphyrins is presented in Fig. 4a. It follows from the plot, that all of the studied compounds produced an increase of F relative to the reference sample (labeled by O) which may indicate for enhanced fluidity of the liposome membranes (Shimshick and McConnell, 1973). The least activity in interaction with the membranes was demonstrated by Mn and Mg porphyrins and the highest activity was shown by the complexes of Cu and Co. However, the dynamics of variation of F was not constant in time, as shown in Fig. 4a. The most significant changes were observe during the first 50 h of measurements. Within this time range the parameter F changed, respectively, from 0.227 to 0.310 (ca. 37% of the initial value) for the reference sample, from 0.254 to 0.368 (ca. 45% of the initial value) for the sample doped by MnPp and from 0.285 to 0.410 (ca. 44% of the initial value) for the sample containing CuPp. Similarly, the average dynamics of the parameter F variation for the other samples was ca. 45% of the initial value, and thus it was somewhat greater than for the reference one. After 50 h almost a complete stabilization of the F parameter occurred for the reference; however,

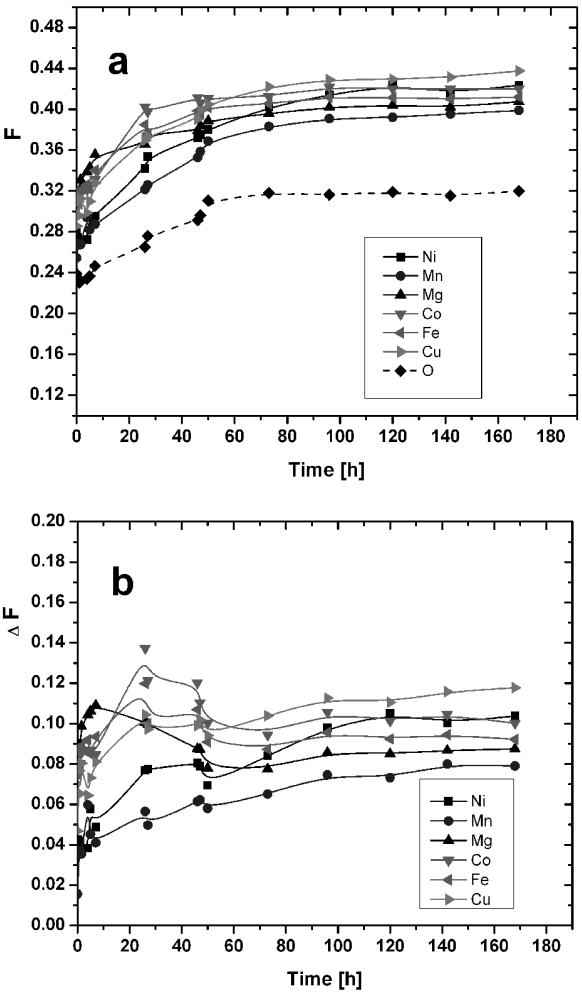

Fig. 4. Variations in spectroscopic parameters F and ΔF vs. time for MPp-doped liposomes marked with the TEMPO probe (M = Ni, Mn, Mg, Co, Fe, Cu). (a) The reference sample with no MPp admixture is labeled by O. (b) ΔF stands for the difference between the F parameter of a tested sample and the corresponding F value of the reference.

in the case of the other samples some raise in the F value was observed. This may count for a slight increase in fluidity of the porphyrin-doped membranes in the course of time. Dynamics of this process is very similar for all of the tested porphyrins and amounts to ca. 8% for the tested period of time, from 50 to 170 h. Time variations in F relative to the reference sample have been highlighted in Fig. 4b as the relationship of ΔF and time. The value of ΔF was computed as the difference between F for the given sample and the appropriate

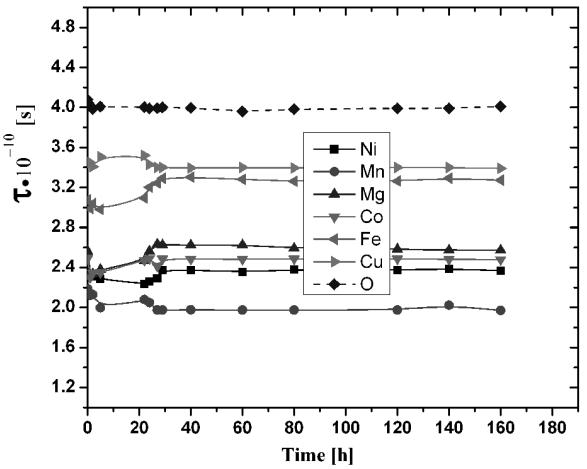

Fig. 5. Variations in the spectroscopic parameter $\tau \cdot 10^{-10}$ vs. time for MPp-doped liposomes marked with the 16-DOXYL-stearic acid probe (M = Ni, Mn, Mg, Co, Fe, Cu). The reference sample with no MPp admixture is labeled by O.

F value for the reference. It follows from the graph, that in most cases a local maximum has appeared after 20–25 h, however for MgPp it has emerged considerably earlier, after 10 h. The time after which the local maxima have been formed (except for MgPp) correlates well with the stabilization time of the τ parameter for the 16-DOXYL-stearic acid probe penetrating the interior of the lipid double-layer.

In Fig. 5 the relationship between the spectroscopic parameter τ and time has been shown for the 16-DOXYL-stearic acid probe dissolved in an aqueous suspension of EYL liposomes containing of MPps. It follows, that all of the studied admixtures have produced a considerable decrease of the *F* parameter in comparison with the reference sample (labeled by O) which may indicate increasing of the liposome's membrane fluidity due to the effect of porphyrin (Hemminga, 1983). Unlike in the case of the TEMPO probe, on interaction with membranes the manganese complex, MnPp, proved the most active one, while the least activity was displayed by CuPp. Dynamics of variations in the τ parameter of the 16-DOXYL-stearic acid spin probe was much less pronounced than for the *F* parameter in the case of the TEMPO probe. Slight changes may be observed during the first 25 h of measurements followed by stabilization of the τ parameter, which is evidenced by the almost parallel orientation of the plot against the time axis. Although the dynamics of variations was small, from Fig. 5 it is clear, however, that during the initial stage of measurements the changes of τ relative to the reference sample were considerably bigger, than in the case of the *F* parameter. This may indicate a strong fluidization of the inside part of the lipid double-layer due to the effect of the porphyrins located within the membranes.

The biggest change was shown by the sample containing MnPp and amounted to 50% relative to the reference (τ changed from ca. $4 \cdot 10^{-10}$ s to ca. $2 \cdot 10^{-10}$ s), whereas in case of the TEMPO probe the biggest change observed in *F* was ca. 12% (for CuPp). The least changes in τ (ca. 15%) were observed for the porphyrin admixtures containing ions of Fe and Cu. The other porphyrins produced changes of the τ parameter of about 40–45% relative to the reference.

The following may be concluded from the analysis of the results obtained in this work: All studied porphyrins considerably fluidized the liposome membranes. The dynamics of this process depended on the metal ion complexed by the porphyrin and on the membrane's part penetrated by the spin probes. The effect of porphyrin on the fluidity of the double-layer interior was considerably greater than on the surface layer. The TEMPO probe showed that CuPp most strongly fluidized the membrane and MnPp did most weakly. In the case of the 16-DOXYL-stearic acid probe a reversed process was observed, *i. e.* the least fluidization was for CuPp and the strongest for MnPp, which may indicate that the Mn complex penetrates deeper into the lipid double-layer, whereas the Cu one is localized closer to the surface layer. Stabilization in fluidity fluctuations of the membrane was confirmed for the double-layer interior ca. 20 h and within the surface layer ca. 50 h after the porphyrin admixtures had been introduced. Such considerable discrepancy may be due to hydration of the hydrophilic part of the membrane, *i. e.* creation of dynamic defects, which allow the TEMPO probe to easier penetrate the membrane, thus increasing the value of the *F* parameter (Iwkow and Bieriestowskij, 1982).

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