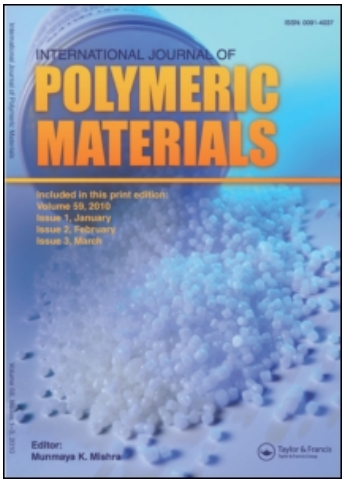

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Interaction of Zn^2 Ions with Phospholipid Multilayers

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Interaction of Zn^{2+} Ions with Phospholipid Multilayers

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Phospholipids are large natural amphipathic molecules which, in contact with water, assemble into higher molecular aggregates. The most relevant one is the bilayer for its relation to the structure, properties and functions of cell membranes. Given their structural complexity phospholipid bilayers are usually used as models for studying how biologically relevant chemicals affect the structure and functions of biomembranes. Zn is a trace element essential for the functional integrity of cell membranes. It is also a common contaminant of sea waters. Therefore, it was thought of interest to study the interaction of Zn^{2+} ions with phospholipid bilayers in order to understand the way they interact with cell membranes. With this aim Zn^{2+}, in a wide range of concentrations, was made to interact with multibilayers of the phospholipids dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE). This study was performed by X-ray diffraction. Besides, the interaction of Zn^{2+} with DMPC liposomes was studied by fluorescence spectroscopy. The results showed that indeed Zn^{2+} interacted with both phospholipids. As its concentration increased it produced first a disorder effect which was reversed at higher concentrations.

Keywords: Phospholipid; bilayers; liposomes; zinc interaction

INTRODUCTION

Phospholipids are large natural amphipathic molecules that have long hydrophobic hydrocarbon chains, saturated and/or unsaturated, and polar zwitterionic polar headgroups. In contact with water phospholipids spontaneously assemble into higher molecular aggregates if their concentration

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surpasses the critical micellar concentration. The wide range of different types of aggregates that will be formed depends on several internal and external factors. However, the most relevant phase is the bilayer for its relation to the structure, properties and functions of cell membranes. These are very complex entities. They are not only constituted by an extremely large number of different molecules but they show a very low degree of periodical order. This has led to the proposal of several different models of which that of Singer and Nicolson [1] has been accepted. In essence, the phospholipids arrange themselves in asymmetric bilayers in which the proteins are irregularly inserted. Therefore, given the complexity of cell membranes, simpler models based on phospholipid bilayers are widely used.

In our X-ray lab we have determined the structure of lecithin (diacyl phosphatidylcholine) and cephaline (diacylphosphatidylethanolamine) multilayers [2]. These are types of phospholipids that are respectively located in the outer and inner monolayers of most biomembranes [3]. Besides, we have studied the perturbing effect of water upon their structures [4]. Since then, we have been using lecithin and cephalin bilayers as models to study the way different chemicals interact with cell membranes [5–7].

The continuous discharge of wastes from mineral, industrial and agricultural activities has dramatically increased the concentration of heavy metals in the continental and sea waters [8,9]. Given their toxicity and accumulative behaviour they constitute an health risk for humans. The cell membrane, as a diffusion barrier, protects the cell interior. Therefore, its structure and functions are susceptible to be altered as a consequence of interactions with heavy metals. Unusually high concentrations of Zn have been found in water, sediment and molluscs from certain regions of Chile [9]. Zinc is also a trace element essential for the functional activity of more than 200 metalloenzymes and it is very important for the functional integrity of cell membranes [10]. NMR studies on phospholipid liposomes indicate that the phospholipid ester groups or the polar head phosphates are the most likely sites of interaction which results in changes in the phospholipid organization that stabilizes the membrane [11]. It was thought of interest to study the interaction of Zn^{2+} ions with phospholipid bilayers in order to understand the way they interact with cell membranes. With this aim Zn^{2+}, in a wide range of concentrations, was made to interact with multilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE). This study was performed by X-ray diffraction methods. In addition, the interaction of Zn^{2+} ions with DMPC liposomes was studied by fluorescence spectroscopy.

EXPERIMENTAL

Chemicals

Synthetic DMPC (Lot 80H-8371, A grade, MW 677.9) and DMPE (Lot 13H-83681, A grade, MW 635.9) from Sigma, $ZnCl_2$ p.a. from Merck, DPH and Laurdan from Molecular Probes were used without further purification.

X-ray Diffraction Analysis of Phospholipid Multilayers

About 1–2 mgs of each phospholipid were introduced into 1.0 mm diam. special glass capillaries. They were then filled with about 200 μL of a) distilled water, and b) 10^{-5} M up to 10^{-2} M $ZnCl_2$ aqueous solutions and sealed. The specimens were X-ray diffracted 2 and 14 days after preparation in flat plate cameras provided with rotating devices. Specimen-to-film distances were 8 or 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Philips PW1140 X-ray generator was used. The relative reflection intensities were obtained from films by peak-integration in a Joyce-Loebl MKIIICS microdensitometer interfaced to a PC. No correction factors were applied. The experiments were performed at 17 ± 2°C, which is below the main transition temperatures of both DMPC and DMPE multilayers.

Fluorescence Measurements of Large Unilamellar Vesicles (LUV)

The influence of Zn ions in the physical properties of DMPC LUV was studied by fluorescence spectroscopy using DPH and Laurdan fluorescent probes. DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its fluorescence steady-state anisotropy measurements were used to investigate the structural properties of DMPC as it provides a measure of the rotational diffusion of the fluorophore, restricted within a certain region such as a cone, due to the lipid hydrocarbon chain packing order. On the other hand Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra to the physical state of membranes. With the fluorescent moiety within a shallow position in the bilayer, Laurdan provides information of the dynamic properties in the zone of DMPC liposomes which is related to the lipid polar organization. The quantitation of Laurdan fluorescence maximum shift was done using the general polarization (GP)

concept [12]. A detailed description of the techniques and instruments employed have already been described elsewhere [13].

RESULTS AND DISCUSSION

X-ray Studies

Table I and Figure 1 present the results obtained after DMPE was mixed and allowed to interact with water and Zn^{2+} solutions. As it has previously been reported, water did not affect very much the structure and, therefore, the X-ray pattern of DMPE. This is because DMPE molecules pack very tightly with a number of hydrophobic and hydrophilic interactions and hydrogen bonds [14], being its multibilayer arrangement very compact and difficult to perturbate [7]. However, it was observed that Zn^{2+} in a concentration as low as 10^{-5} M produced a considerable diminishing of DMPE reflection intensities, disappearing many of them. This effect increased with higher concentrations of Zn^{2+}. Nevertheless, when the Zn^{2+} concentration

TABLE I Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE specimens with water and Zn^{2+} aqueous solutions (a, b)

DMPE + H_2O		*DMPE + Zn^{2+} 10^{-5} M*		*DMPE + Zn 10^{-3} M*		*DMPE + Zn 10^{-2} M*	
do(Å)	*Io rel.*	*do(Å)*	*Io rel.*	*do(Å)*	*Io rel.*	*do(Å)*	*Io rel.*
51.4*	2550*	51.5*	462	51.5*	146*	51.1*	823*
25.1	18	–	–	–	–	25.0	5
17.0	22	16.9	2	–	–	16.9	4
12.6	25	12.7	8	–	–	12.7	6
5.92	37	5.95	3	5.91	3	5.94	8
5.68	31	–	–	–	–	5.60	6
5.47	12	–	–	–	–	–	–
5.22	7	–	–	–	–	5.22	5
5.05	19	–	–	–	–	–	–
4.78	42	–	–	4.75	10	4.78	12
4.66	33	4.67	2	4.62	4	4.63	8
4.21	8	–	–	–	–	4.22	2
4.04	311	4.06	53	4.04	42	4.04	100
3.91	30	–	–	3.91	3	3.91	2
3.78	156	3.82	20	3.79	26	3.80	48
3.63	14	3.67	4	–	–	3.65	3
3.49	11	–	–	–	–	–	–
3.39	14	–	–	–	–	–	–
3.16	11	–	–	–	–	–	–

a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. 8 and 14* cm specimen-to-film distance.
b) Reflections that were either too weak or with do values below 3.0 Å are not included.

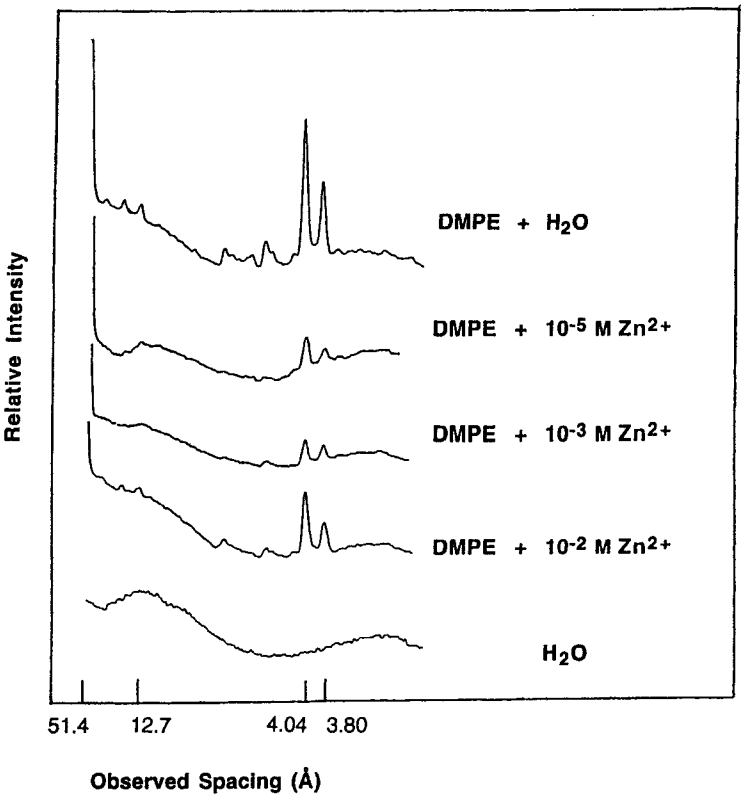

FIGURE 1 Microdensitograms from DMPE in Zn^{2+} aqueous solutions. Flat-plate-cameras; specimen-to-film distance 8 cm.

was 10^{-2} M, this effect was reversed and an increase of the reflection intensities was observed. On the other hand, the bilayer width of DMPE remained practically constant at about 51.5 Å under all Zn^{2+} concentrations. The above results can be explained in terms of Zn^{2+} ions interacting with the negatively charged phosphates of DMPE polar groups. At low Zn^{2+}/PO_4^- ratios, the scanty Zn^{2+} ions would attach to a few phosphates resulting in a generalized disorder of the Zn^{2+}-free polar groups. However, as the Zn^{2+}/PO_4^- ratio increased, each Zn^{2+} ion would be linked to two neighboring PO_4^- groups producing a cooperative ordering of most of DMPE molecules.

Table II and Figure 2 show the results observed when Zn^{2+} aqueous solutions were made to interact with DMPC multilayers. In the first place it was observed, as expected, that pure water affected the structure of DMPC. Its bilayer width expanded from about 55 Å when dry [4] to 65 Å when

TABLE II Comparison of observed interplanar spacings (do) and relative intensities (lo rel) of DMPC specimens with water and Zn^{2+} aqueous solutions (a, b)

DMPC $+H_2O$		*DMPC* $+Zn^{2+}10^{-5}M$		*DMPC* $+Zn^{2+}10^{-4}M$		*DMPC* $+Zn^{2+}10^{-3}M$		*DMPC* $+Zn^{2+}10^{-2}M$	
do(Å)	*lo rel.*	*do(Å)*	*lo rel.*	*do(Å)*	*lo rel.*	*do(Å)*	*lo rel.*	*do(Å)*	*lo rel.*
65.0*	501*	65.0*	372*	diffuse halo		diffuse halo		52.6*	48*
32.5	315	32.5	159	–	–	–	–	35.0*	14*
21.2	4	21.2	4	–	–	21.0	6	20.6	3
4.2	100	4.2	86	4.2	23	4.2	18	4.2	23

a) The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras. 8 and 14* cm specimen-to-film distance.
b) Very weak reflections are not included.

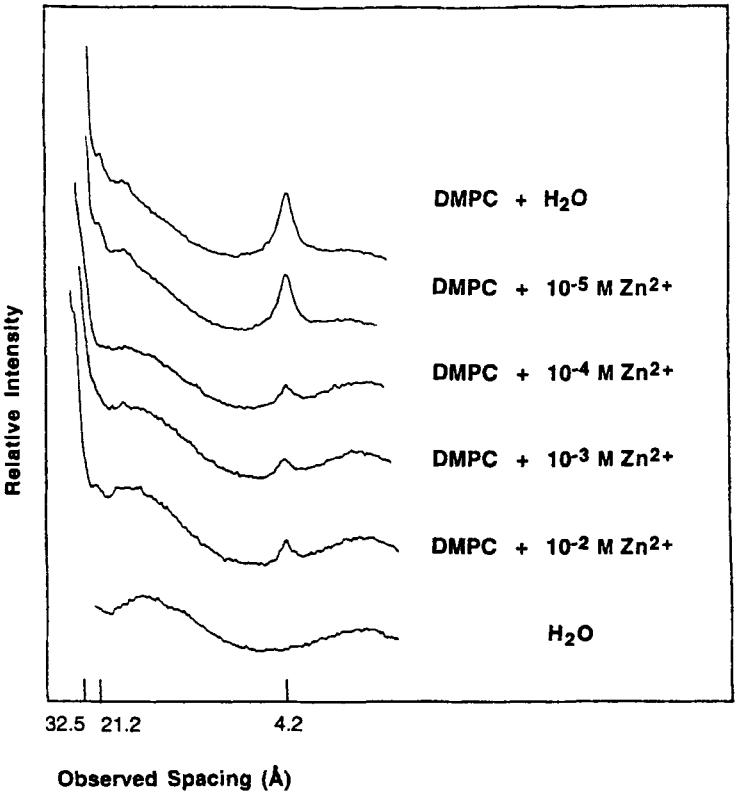

FIGURE 2 Microdensitograms from DMPC in Zn^{2+} aqueous solutions. Flat-plate-cameras; specimen-to-film distance 8 cm.

immersed in water and the observed reflections were reduced to only the first three orders of the bilayer width. On the other hand, a new and strong reflection of 4.2 Å showed up. The appearance of this reflection was indicative of the fluid state reached by DMPC multilayers. In fact, it corresponds to the average separation of the fully extended hydrocarbon chains organized with rotational disorder in an hexagonal lattice [15]. The different effect of water upon DMPE and DMPC is due to the fact that DMPC multilayer arrangement is more open than that of DMPE [2]. This allows water and other molecules an easier penetration into DMPC and, consequently, its structural perturbation.

The effects of Zn ions upon DMPC were the following: 10^{-5} M produced only a mild diminishing of the reflection intensities. When its concentration was increased to 10^{-4} M it was observed a) the disappearance of the three low angle reflections and their replacement by a diffuse halo, and b) a lower intensity of the 4.2 Å reflection. This pattern remained almost the same at 10^{-3} M except by the fact the 21.0 Å reflection showed up again. The pattern observed when the Zn^{2+} concentration was 10^{-2} M showed two new reflections of 52.6 Å and 35.0 Å that did not were observed before. The observed disorder-order effect of Zn^{2+} upon DMPC might have the same cause as explained above for DMPE. On the other hand, the appearance of new reflections observed in the "reordered" DMPC is most likely due to a "ripple" arrangement of the phospholipid multilayers [16].

The described effects of Zn^{2+} ions upon DMPE and DMPC multilayers can explain several effects observed in cell membranes. In fact, there are evidences that Zn^{2+} plays an important role for the proper maintenance and stabilization of cells and biomembranes [11,17,18]. Moreover, it has been reported that the cell membrane is protected and stabilized by increasing levels of Zn^{2+}, and the inverse occurs when its levels are decreased [18].

Fluorescence Studies on Vesicles

The interaction of Zn^{2+} ions with DMPC large unilamellar vesicles was studied evaluating DPH steady state fluorescence anisotropy (r) and Laurdan general polarization (GP). The results are presented in Table III. The presence of increasing concentrations of Zn^{2+} up to 10^{-2} M produced a mild increase in the fluorescence anisotropy of DPH and a more pronounced one in the general polarization of Laurdan. These results can be explained in the above mentioned terms of Zn^{2+} interaction with the phospholipid polar groups and the acyl chains. In fact, there is a clear decreasing effect on the dynamics of the water dipolar relaxation at the

TABLE III Effects of Zn^{2+} on the anisotropy (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) and the general polarization (GP) of Laurdan embedded in large unilamellar dimyristoylphosphatidylcholine (DMPC) vesicles

$ZnCl_2$ conc.	*r DPH*	*GP Laurdan*
0.0 M	0.270	0.520
10^{-5} M	0.271	0.519
10^{-4} M	0.273	0.520
10^{-3} M	0.274	0.555
10^{-2} M	0.279	0.579

hydrophilic/hydrophobic interface sensed by Laurdan and a lesser ordering effect on the acyl chain packing organization sensed by DPH. Therefore, these results agree with those obtained by X-ray diffraction on DMPC multibilayers.

Acknowledgements

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