Biochimica et Biophysica Acta 1818 (2012) 747–752

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Kinetics of demetallation of a zinc–salophen complex into liposomes

Carla Gasbarri [a,*], Guido Angelini [a], Antonella Fontana [a], Paolo De Maria [a], Gabriella Siani [a], Ilaria Giannicchi [b], Antonella Dalla Cort [b]

[a] Dipartimento di Scienze del Farmaco, Università G. d'Annunzio, via dei Vestini, 66013 Chieti, Italy
[b] Dipartimento di Chimica, Università "La Sapienza", piazzale A. Moro, 00185 Roma, Italy

ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 7 September 2011
Accepted 18 October 2011
Available online 22 October 2011

Keywords:
Zinc-salophen
Liposomes
ESI-MS
Kinetics
Fluorescence anisotropy

ABSTRACT

A Zn–salophen complex has been incorporated into POPC large unilamellar liposomes (LUV) obtained in phosphate buffer at pH 7.4. Fluorescence optical microscopy and anisotropy measurements show that the complex is located at the liposomal surface, close to the polar headgroups. The interaction of the POPC phosphate group with Zn^{2+} slowly leads to demetallation of the complex. The process follows first order kinetics and rate constants have been measured fluorimetrically in pure water and in buffered aqueous solution.
The coordination of the phosphate group of monomeric POPC with salophen zinc also occurs in chloroform as detected by ESI-MS measurements.
The effect of the Zn–salophen complex on the stability of POPC LUV has been evaluated at 25 °C by measuring the rate of release of entrapped 5(6)-carboxyfluorescein (CF) in the presence and in the absence of Triton X-100 as the perturbing agent. It turns out that the inclusion of the complex significantly increases the stability of POPC LUV.

1. Introduction

Metal-ligand coordination is an attractive strategy for building supramolecular structures. Coordination can be induced by light excitation [1], electron transfer [2] and ion binding [3]. The formation of Lewis acid–base complexes can be easily detected if the spectral properties of a fluorescent component change as a consequence of complexation. Metallated-macrocyclic compounds, including porphyrins and salophens, have been widely studied as sensors in living organisms for light-induced reactions [4–6]. Zn–salophens are fluorescent compounds used as efficient sensors for nitroaromatics [7] and characterized by the tendency to accept one axially coordinated donor species. The formation of a pentacoordinated complex induces a strong change in the photophysical properties of the macrocycle.

It is well known [8,9] that the biological role of Zn^{2+}-complexes is essential to the processes occurring within the cellular membrane. Moreover, metal salophen complexes exhibit strong cytotoxic effects [10–12] and the understanding of their role in biological environment is essential for the prevention or treatment of cancer. Generally, metal salophen complexes are administered by means of drug delivery systems such as nanoparticles or liposomes [13].

Liposomes have been studied as drug carriers since the 1970s [14], due to their capacity to incorporate both hydrophilic and hydrophobic guests, to enhance the clinical effects of appropriate drugs, to reduce drug toxicity and to protect them from metabolism and immune responses [15–17]. An important carrier requirement is the capacity to release the entrapped drug once the target site has been reached. An effective content outflux from liposomes can be achieved in model systems by the use of surfactants [18]. The limited applications of liposomes in biotechnology are mainly due to rapid drug leakage and poor storage stability [19]. On the other hand highly stable drug-liposome systems would be ineffective due to a too slow release of the drug.

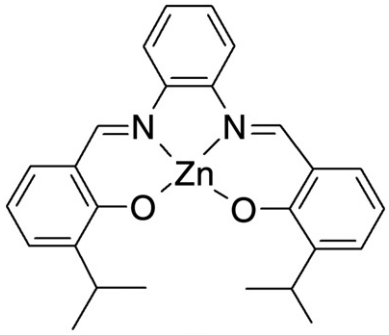
The aim of this work has been the investigation of the effects of the incorporation of the Zn–salophen complex **1** into liposomes from the natural zwitterionic surfactant 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC). The presence of the metal complex and its orientation into the phospholipid bilayer have been evaluated by fluorescence microscopy and anisotropy, while the amount of incorporation has been evaluated by UV–visible spectroscopy.

The effect of Zn–salophen on the liposome stability has been determined by following the rate of release of entrapped 5(6)-carboxyfluorescein (CF) in the presence and in the absence of the perturbing agent Triton X-100.

The affinity of zinc for the phospholipid headgroups plays a relevant role in enzyme catalysis [20–22]. The investigated POPC–Zn–salophen liposomes can be of interest in clarifying the role of Zn^{2+}-phospholipid interactions.

* Corresponding author. Tel.: +39 0871 3554786; fax: +39 0871 3554791.
E-mail address: c.gasbarri@unich.it (C. Gasbarri).

0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.10.014



1

2. Experimental

2.1. Materials

Zn–salophen has been synthesized as previously described [23]. Salophen was purchased by Aldrich. POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) and NBD-PC (1-acyl-2-{12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]lauroyl}-phosphatidylcholine) were purchased from Avanti Polar Lipids (Alabaster, AL). DPH (1,6-diphenyl-1,3,5-hexatriene), Triton X-100 and 5(6)-carboxyfluorescein (95% purity) were obtained from Sigma and Fluka. All purchased materials were used without further purification.

2.2. Instruments

The fluorescence spectra and the kinetic determinations were performed by a Jasco FP-6200 spectrofluorimeter with a band pass of 5 nm. Video-enhanced microscopy measurements were carried out by a light inverse Zeiss Axiovert microscope (Zeiss, Germany), equipped with Hoffmann optic 40x and a video camera for image detection. Fluorescence images were obtained by using a Zeiss filter set number 9 that excites in the wavelength range 450–490 nm and detects fluorescence at wavelengths > 515 nm.

The Dynamic Laser Light Scattering data were extrapolated by using the Stokes–Einstein relationship for the calculation of the hydrodynamic radius with a Brookhaven (90PLUS BI-MAS) digital correlator at a scattering angle of 90°, equipped with a 35 mW He–Ne laser at the wavelength of 660 nm.

The ESI-MS measurements were performed with an ionic trap mass spectrometer operating at 2000 *m/z*. A pump driven syringe was used for direct injection of the sample into the vaporization chamber of the instrument (Thermo LCQ Advantage ionic trap operating at 2000 m/z). The spectra have been recorded by using the following instrumental setting: a positive ion mode; nitrogen as the desolvation gas (N_2); a desolvation temperature of 150 °C; a capillary voltage of 4.0 kV and a cone voltage of 30 ÷ 150 V.

2.3. Preparation of liposomes

POPC–Zn–salophen liposomes were prepared in the molar ratio 86/14 by mixing 184 μL of a POPC 13×10^{-3} M chloroform solution with 185 μL of a Zn–salophen 2.2×10^{-3} M chloroform solution. The organic solvent was then removed by evaporation under vacuum to obtain a dry film. The film was kept at 4 °C overnight and then 2 mL of a buffered aqueous solution [made of 121.5 mM NaCl, 25.2 mM Na_2HPO_4, 4.8 mM KH_2PO_4 (pH 7.4 and 578 mOsm)] were added under stirring for twenty minutes. The resulting multilamellar vesicle dispersion was extruded five times at room temperature through a polycarbonate filter (Osmonics, pore size 200 nm) mounted in an extruder from Lipex Biomembranes (Vancouver BC, Canada) and filtered through a Sephadex G-75 column. The obtained POPC–Zn–salophen 86/14 LUV were then diluted in isosmotic buffer to achieve a final concentration of 6×10^{-5} M POPC and 1×10^{-5} M Zn–salophen.

The fluorescence emission spectra were recorded by using 350 nm as the excitation wavelength.

2.4. Incorporation efficiency

Unloaded Zn–salophen remains embedded in the polycarbonate filter during extrusion, as no traces of the complex were found in the Sephadex G-75 column.

The Zn–salophen complex shows UV–vis absorption in chloroform solution in the range 200–500 nm with two peaks centered at 299 and 398 nm. The Lambert–Beer law was strictly followed at both λ_{max} up to a concentration of Zn–salophen 1.4×10^{-4} M. The molar extinction coefficients, $\varepsilon = 29{,}300$ and $\varepsilon = 16{,}200\ L\ mol^{-1}\ cm^{-1}$ have been obtained at 299 and 398 nm, respectively [23]. The concentration of Zn–salophen incorporated into POPC bilayers was calculated as the difference between the total initial concentration of Zn–salophen used for the preparation of the dry film and the amount of Zn–salophen lost during extrusion. This amount embedded into the polycarbonate filter was determined by adding 3 mL of chloroform and recording the absorbance of the yellow solution obtained. The incorporation efficiency is considerable as only $1.5\pm0.5\%$ of the total Zn–salophen used turned out to be lost during extrusion.

2.5. Determination of liposome stability

The film of POPC–Zn–salophen was hydrated with 2 mL of a 50 mM buffered 5(6)-carboxyfluorescein (CF) solution under stirring for 20 min. The obtained suspension was extruded five times and run through a Sephadex G-50 column to remove the unloaded dye and diluted to achieve a homogeneous solution of CF entrapped 1.3×10^{-5} M POPC and 0.2×10^{-5} M Zn–salophen LUV.

The stability of the prepared liposomes was evaluated spectrofluorimetrically at 25.0 ± 0.1 °C by following the increase in fluorescence at 516 nm (using 490 nm as the excitation wavelength) due to the de-quenching of CF after release from the liposomes.

2.6. Steady-state fluorescence anisotropy

The films of POPC–Zn–salophen, POPC–DPH and POPC–NBD-PC were prepared by mixing 1 mL of POPC 13×10^{-3} M with 10 μL of a 1.3×10^{-3} M probe chloroform solution. After evaporation of $CHCl_3$ the films were hydrated with phosphate buffer and filtered as described in Section 2.3 Preparation of liposomes. The final liposome suspensions were then diluted to achieve a homogeneous solution of 2.6×10^{-3} M POPC and 2.6×10^{-6} M probe. The lipid/probe molar ratio was kept constant to 1000/1 in the preparation of liposomes in order to avoid the formation of dimers into the phospholipid bilayer as well as to reduce the effect of the probe on the liposome size. The steady-state fluorescence anisotropy, *r*, was calculated according to Eq. (1):

$$r = \frac{I_{VV} - G\ I_{VH}}{I_{VV} + 2\ G\ I_{VH}} \tag{1}$$

where I_{VV} and I_{VH} are the parallel and perpendicular polarized fluorescence intensities, respectively, measured with the vertically polarized excitation light, I_{HV} and I_{HH} are the same fluorescence intensities measured with the horizontally polarized excitation light and *G* is the monochromator correction factor given by the ratio I_{HV}/I_{HH}.

The value of *r* for the POPC–Zn–salophen liposomes was calculated by using 350 nm and 540 nm as the excitation and emission wavelengths, respectively. In the case of POPC–DPH liposomes the excitation wavelength was 360 nm and the emission wavelength 430 nm;

in the case of POPC–NBD-PC liposomes the corresponding values were 460 and 534 nm.

2.7. ESI-MS measurements

The ESI-MS spectra of a 1×10^{-5} M Zn–salophen chloroform solution were acquired in the presence and in the absence of 6×10^{-5} M POPC.

3. Results and discussion

The fluorescent Zn–salophen complex **1** is soluble in organic solvents of low polarity such as chloroform, owing to the presence of two isopropyl groups [23] in positions 3 and 3′, while it is insoluble in pure water, in phosphate buffer at pH 7.4 and in NaOH aqueous solution. The incorporation into liposomes makes the Zn–salophen complex soluble in aqueous solution.

POPC–Zn–salophen liposomes in the molar ratio 86/14 were prepared as described in the Experimental section. A drop of the obtained buffered liposomal suspension on a glass slide was observed by an optical microscope (Fig. 1).

Incorporation of Zn–salophen induces fluorescence into the phospholipid bilayer. Extrusion of the turbid yellow suspension through a polycarbonate filter leads to a monodisperse population of large unilamellar vesicles. Due to its strong hydrophobicity the Zn–salophen complex is easily incorporated into POPC membrane and only 1.5 ± 0.5% of the complex is lost during extrusion.

A guest in the POPC bilayer may change the liposomal curvature [24], in particular the insertion of Zn–salophen decreases the mean diameter of POPC liposome from 200 nm to 158 ± 2 nm as measured by Dynamic Laser Light Scattering after extrusion.

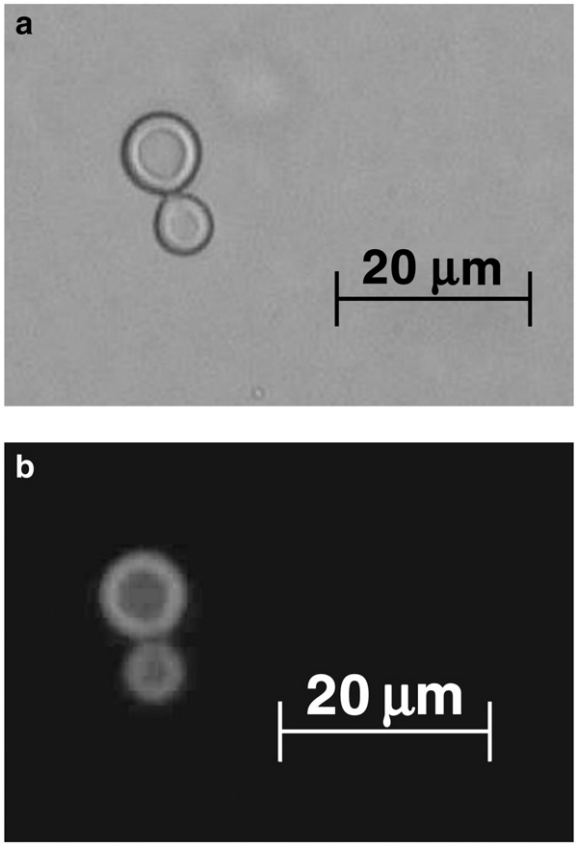


Fig. 1. POPC–Zn–salophen liposomes observed by optical microscope under visible light (a) and in fluorescence (b) before extrusion.

Table 1
Anisotropy value *r* and mean diameter for the investigated liposomes.

Liposomal system[a]	Anisotropy value (*r*)	Mean diameter/nm
POPC–Zn–salophen	0.35 ± 0.01	201 ± 2
POPC–DPH	0.08 ± 0.001	208 ± 2
POPC–NBD-PC	0.20 ± 0.01	209 ± 1

[a] The lipid/probe ratio corresponds to 1000/1.

The distribution of an entrapped molecule into different regions of a liposome depends on hydrophobic and electrostatic interactions. Generally the hydrophobic part of a guest molecule inserts deeply into the bilayer, in a microenvironment of low polarity, while the hydrophilic part is oriented towards the surface of the liposomes to interact with the polar heads of the lipids.

Two of the most commonly used fluorescent probes for anisotropy are DPH [25] and NBD-PC [26]. DPH is a rigid, linear, rodlike fluorophore, characterized by the absence of polar groups, and accordingly, it partitions spontaneously into the hydrophobic acyl chain region of the membrane. On the contrary, NBD-PC, consisting of a fluorophore attached to a phospholipid headgroup, is located preferentially in the rigid region of the liposomal membrane. Since anisotropy, *r*, Eq. (1) is directly related to the fluidity of the bilayer, low values of *r* are associated to DPH, while high values of *r* are associated to NBD-PC. The *r* values for the investigated POPC–Zn–salophen, POPC–DPH and POPC–NBD-PC liposomes in the lipid/probe molar ratio 1000/1 are reported in Table 1 together with the mean diameter measured by Dynamic Laser Light Scattering.

The mean diameter of the investigated liposomes approximately corresponds to the pore size of the filter used for extrusion, showing that the curvature of large unilamellar POPC liposomes is not affected by the presence of the probe at low concentration.

The high fluorescence anisotropy, *r*, measured for the POPC–Zn–salophen liposomes suggests that the metal complex is located in the ordered region of the membrane, i.e. at the surface of the liposomes. This orientation allows Zn–salophen to establish Lewis acid–base interactions with the neighboring phosphate group of POPC. It is well known that zinc ions [27] bind to the negatively charged phosphate groups of zwitterionic lipids to form a 2:1 or 1:1 coordination complex.

The fluorescence emission spectra of Zn–salophen incorporated into POPC liposomes and in chloroform solution (Fig. 2) show the same band centered at 540 nm, suggesting that the guest is present as monomer at the investigated concentration. Analogous spectra

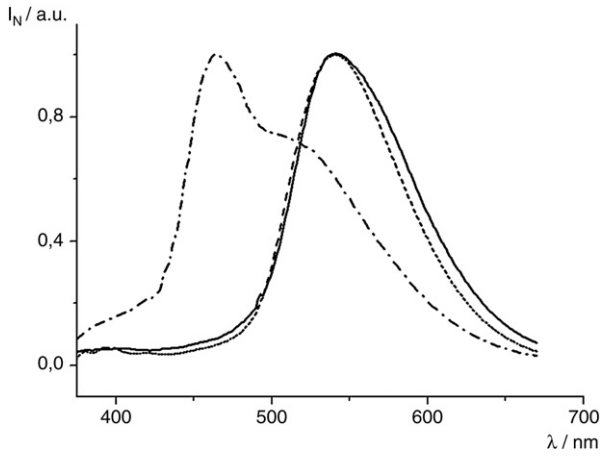


Fig. 2. Normalized fluorescence emission spectra of 1×10^{-5} M Zn–salophen in $CHCl_3$ (dashed line), in POPC liposomes (solid line) and in POPC liposomes after 120 h from LUV preparation (dash dotted line).

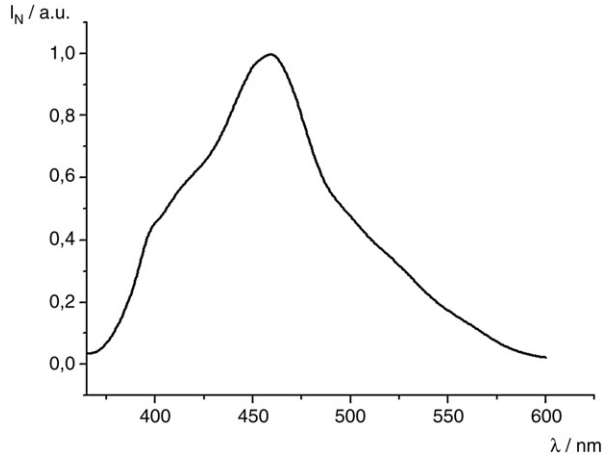


Fig. 3. Normalized fluorescence emission spectra of 1×10^{-5} M salophen in 86/14 POPC–salophen liposomes.

have been obtained by Knapp and coworkers for several Zn–salophen complexes in acetonitrile [28].

The ability to incorporate hydrophobic and amphiphilic molecules in the monomeric form within the bilayer is one of the typical properties of liposomes as carriers [29,30].

However, the same fluorescence emission spectrum of the POPC–Zn–salophen changes dramatically after 120 h, while that of Zn–salophen in chloroform remains essentially unchanged (see Fig. 2).

The Lewis acid–base interaction of Zn–salophen can slightly shift the maximum [31] or decrease the intensity of the emission band in the fluorescent spectra [23]. The new band at 460 nm is typical of the fluorescence spectra of salophen in conventional organic solvents [32]. Apparently coordination between Zn and the POPC phosphate group slowly leads to demetallation of Zn–salophen. Demetallation is confirmed by comparison of the spectrum of POPC–Zn–salophen after 120 h shown in Fig. 2 with that of POPC–salophen liposomes shown in Fig. 3.

The observed demetallation follows first order kinetics and the rate constants (k_{obs}) have been fluorimetrically measured in phosphate buffer solution (pH 7.4) and in pure water (pH 5.5) at 25 °C by following the increase of the emission, I, at 460 nm with time (Fig. 4) by means of Eq. (2):

$$\mathrm{Ln}(I_\infty - I_t) = k_{obs}t \qquad (2)$$

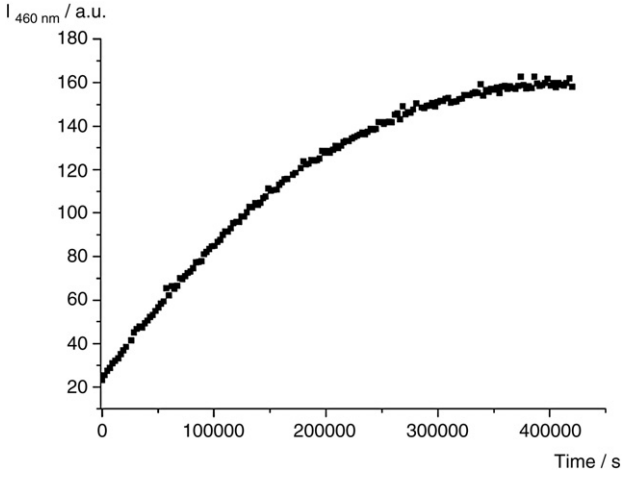


Fig. 4. Increase of the emission at 460 nm due to demetallation of Zn–salophen in POPC liposomes.

Table 2
k_{obs} values for the coordination between POPC and Zn–salophen.

Experimental conditions	$k_{obs}/10^{-6}\,s^{-1}$
Phosphate buffer solution (pH 7.4)	3.75 ± 0.01
Pure water (pH 5.5)	13.1 ± 0.1

where I_∞ is the emission value after 120 h (corresponding to 432,000 s as shown in Fig. 4).

The k_{obs} values obtained in phosphate buffer solution and in pure water are reported in Table 2.

The strong binding properties in ethanol of Zn–salophen with inorganic phosphates PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ and adenosine nucleotides AMP^{2-}, ADP^{3-} and ATP^{4-} have recently been investigated [33]. In POPC liposomal system the PO_4^{3-} ions of the buffer solution acting as Lewis bases can in principle coordinate the Zn atom of the complex instead of or together with the phosphate group of the phospholipid. The coordination process is followed by demetallation of Zn–salophen due to protonation of the phenolic groups. Demetallation occurs easier at lower pH in water (Table 2) probably due to an increase of the degree of protonation which leads to free salophen and $Zn(OH)_2$ [34].

The ESI-Mass acquisition spectrum of 86/14 POPC–Zn–salophen in $CHCl_3$ solution (Fig. 5) confirms the existence of a Lewis acid–base complexation of the POPC phosphate group with Zn–salophen. This spectrum remains essentially unchanged after 120 h in agreement with the fluorescence emission spectrum of Fig. 2 in the same solvent.

In the ESI-Mass spectrum the peak at m/z = 1224 corresponds to the formation of the pentacoordinated complex POPC–Zn–salophen. The peak at m/z = 761 corresponds to the $[POPC+H]^+$ monomer, while the peak at m/z = 1520 corresponds to the $[2POPC+H]^+$ dimer. The peak at 401 corresponds to the bare salophen. (Molecular weight of POPC = 760.1; Molecular weight of Zn–salophen = 463.4).

The effect of the incorporation of Zn–salophen on the stability of POPC liposomes has been spectrofluorimetrically evaluated by following the release of an hydrophilic fluorescent dye, 5(6)-carboxyfluorescein (CF), entrapped into the liposomal core during the hydration process of the POPC–Zn–salophen film. The leakage of CF from the liposomes may occur spontaneously, owing to the presence of pores and defects in the bilayer [35] and is promoted by Triton X-100, a well known destabilizing agent for cellular and liposomal membranes [36,37]. A large excess of Triton X-100 over the liposome concentration leads to saturation of the liposomal membrane and causes the transition of liposomes into mixed micelles [38].

The spontaneous and Triton X-100 induced leakage of CF from the investigated 86/14 POPC–Zn–salophen liposomes has been followed at 25 °C in phosphate buffer solution. In the presence of large excess of Triton X-100 pseudo first-order rate constants k'_{obs} have been measured according to the rate law of Eq. (3):

$$k'_{obs} = k[\mathrm{TritonX-100}]^n \qquad (3)$$

where n represents the order in Triton X-100 and k is a constant which includes a contribution from the binding constant of the surfactant to the bilayer [39]. The non-linearity of the plot of k'_{obs} versus the concentration of Triton X-100 shows that n is › 1 indicating that a critical number n of surfactant molecules has to assemble in the membrane to trigger the formation of a new hydrophilic pore or to stabilize the pre-existing channels (see Supplementary material for further information). The results shown in Table 3 indicate a slower leakage of CF from the POPC–Zn–salophen liposomes than that for pure POPC liposomes [40] both in the absence and in the presence of Triton X-100.

It is well known that the binding of divalent metal ions to negatively charged lipids induces rigidity in the bilayer and dehydration of the liposomal surface [41,42] which decrease the number of

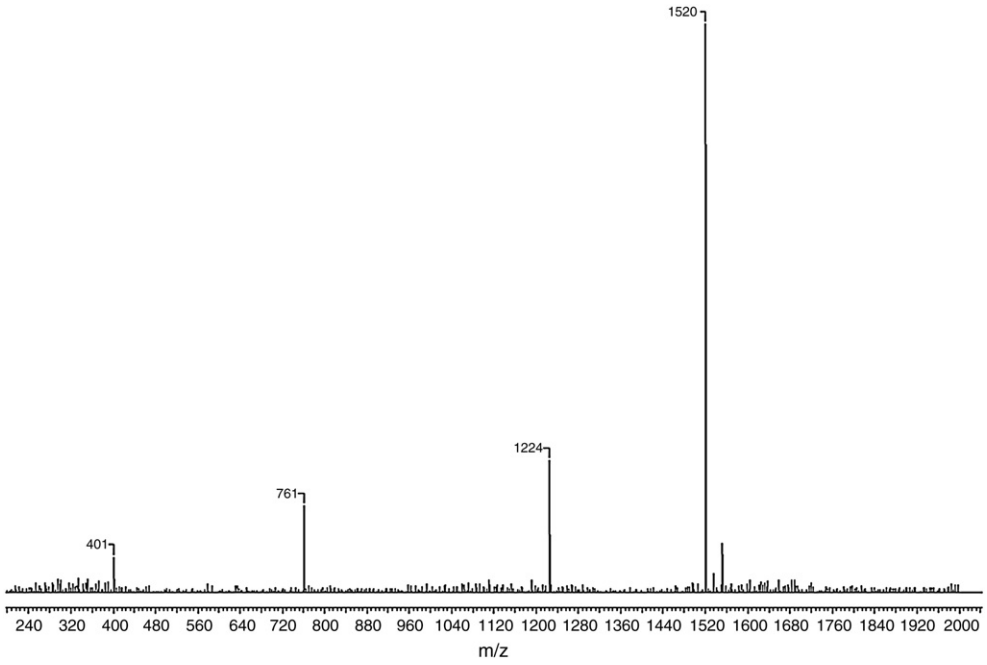


Fig. 5. ESI-Mass acquisition spectrum of 86/14 POPC–Zn–salophen in CH_3Cl solution.

transient pores within the bilayers thus limiting the insertion of Triton X-100. Analogously the presence of Zn–salophen enhances the stability of the liposomes due to the coordination between the metal and the POPC phosphate groups. Interestingly, the k'_{obs} measured for the POPC–Zn–salophen liposomes (Table 3) are similar to the values obtained in the presence of fullerene C_{60} [40], cholesterol [43] and cardanol [43] incorporated as guests in the POPC bilayer.

4. Conclusion

The investigated Zn–salophen complex can be incorporated into POPC liposomes prepared in phosphate buffer solution. The insertion of Zn–salophen into the liposomal bilayer decreases the size and enhances the stability of the POPC liposomes.

The complex tends to be located at the liposomal surface near to the phospholipid headgroups. Interaction between zinc and the phosphate groups slowly leads to demetallation of the complex.

This process changes the structural properties of metal salophen and should be taken into account for pharmaceutical formulations of these complexes.

Acknowledgement

This work has been supported by MIUR (PRIN 2008, prot. 20085M27SS) and Università "G. d'Annunzio" of Chieti-Pescara.

Table 3
Pseudo-first order rate constants for CF release from pure POPC and 86/14 POPC–Zn–salophen liposomes in the presence and in the absence of Triton X-100, in phosphate buffer solution at 25 °C.

[Triton X-100] (10^{-5}/M)	[a]Pure POPC Liposomes k'_{obs} ($10^{-5}/s^{-1}$)	86/14 POPC–Zn–salophen liposomes k'_{obs} ($10^{-5}/s^{-1}$)
0	6.61 ± 0.5	2.87 ± 0.1
2.40	21.1 ± 0.1	4.48 ± 0.2
3.20	25.2 ± 0.1	8.50 ± 0.2
4.40	43.7 ± 0.2	11.7 ± 0.7
5.20	66.8 ± 0.5	12.8 ± 0.5
6.00	83.3 ± 0.1	15.5 ± 0.9
6.40	104 ± 1	21.1 ± 0.1

[a] Ref. [40] in the text.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bbamem.2011.10.014.

References

[1] T. Muraoka, K. Kinbara, T. Aida, Mechanical twisting of a guest by a photoresponsive host, Nature 440 (2006) 512–515.
[2] V. Amendola, L. Fabbrizzi, L. Linati, C. Mangano, P. Pallavicini, V. Pedrazzini, M. Zema, Electrochemically controlled assembling/disassembling processes with a bis-imine bis-quinoline ligand and the Cu^{II}/Cu^{I} couple, Chem. Eur. J. 5 (1999) 3679–3688.
[3] M. Barboiu, G. Vaughan, N. Kyritsakas, J.M. Lehn, Dynamic chemical devices: generation of reversible extension/contraction molecular motion by ion-triggered single/double helix interconversion, Chem. Eur. J. 9 (2003) 763–769.
[4] B.W. Henderson, T.J. Dougherty, How does photodynamic therapy work? Photochem. Photobiol. 55 (1992) 145–157.
[5] R. Bonnett, Photosensitizers of the porphyrins and phtalocyanine series for photodynamic therapy, Chem. Soc. Rev. 24 (1995) 19–33.
[6] D.N. Silverman, S. Lindskog, The catalytic mechanism of carbonic anhydrase: implications of a rate-limiting protolysis of water, Acc. Chem. Res. 21 (1988) 30–36.
[7] M.E. Germain, M.J. Knapp, Discrimination of nitroaromatics and explosive mimics by a fluorescent Zn (salicylaldimine) sensor array, J. Am. Chem. Soc. 130 (2008) 5422–5423.
[8] D.W. Christianson, Structural biology of zinc, Adv. Protein Chem. 42 (1991) 281–355.
[9] N. Gresh, J. Sponer, Complexes of pentahydrated Zn^{2+} with guanine, adenine and the guanine–cytosine and adenine–timine base pairs. Structures and energies characterized by polarizable molecular mechanics and ab initio calculations, J. Phys. Chem. B 103 (1999) 11415–11427.
[10] K.I. Ansari, S. Kasiri, J.D. Grant, S.S. Mandal, Apoptosis and anti-tumor activities of manganese (III)-salen and salphen complexes, Dalton Trans. 40 (2009) 8525–8531.
[11] H. Annegret, G. Ronald, Influence of methoxy groups on the antiproliferative effects of $[Fe^{III}$(salophene-OMe)Cl] complexes, Eur. J. Med. Chem. 45 (2010) 5486–5492.
[12] S.S. Mandal, Metallo-salen complexes show promise towards treatment of leukemia, Leuk. Res. 35 (2011) 571–572.
[13] S. Patel, A.A. Bhirde, J.F. Rusling, X. Chen, S.J. Gutkind, V. Patel, Nano delivers big: designing molecular missiles for cancer therapeutics, Pharmaceutics 3 (2011) 34–52.
[14] G. Gregoriadis, B.E. Ryman, Lysosomal localization of β-fructofuranosidase-containing liposomes injected into rats. Implications in the treatment of genetic disorders, Biochem. J. 129 (1972) 123–133.
[15] N. Oku, Y. Namba, S. Okada, Tumor accumulation of novel RES-avoiding liposomes, Biochim. Biophys. Acta 1126 (1992) 255–260.
[16] E. Reddi, Role of delivery vehicles for photosensitizers in the photodynamic therapy of tumors, J. Photochem. Photobiol. B 37 (1997) 189–195.
[17] A. Fontana, M. Viale, S. Guernelli, C. Gasbarri, E. Rizzato, M. Maccagno, G. Petrillo, C. Aiello, S. Ferrini, D. Spinelli, Strategies for improving the water solubility of new antitumour nitronaphthylbutadiene derivatives, Org. Biomol. Chem. 8 (2010) 5674–5681.

[18] J. Lasch, Interaction of detergents with lipid vesicles, Biochim. Biophys. Acta 1241 (1995) 269–292.
[19] M.C. Woodle, Sterically stabilized liposome therapeutics, Adv. Drug Delivery Rev. 16 (1995) 249–266.
[20] D. Leung, G. Abbenante, D.P. Fairlie, Protease inhibitors: current status and future prospects, J. Med. Chem. 43 (2000) 305–341.
[21] J.H. Exton, Cell signaling through guanine-nucleotide-binding regulatory proteins (G proteins) and phospholipases, Eur. J. Biochem. 243 (1997) 10–20.
[22] E. Hough, L.K. Hansen, B. Birknes, K. Jynge, S. Hansen, A. Hordvik, C. Little, E. Dodson, Z. Derewenda, High-resolution (1.5 Å) crystal structure of phospholipase C from *Bacillus cereus*, Nature 338 (1989) 357–360.
[23] A. Dalla Cort, L. Mandolini, C. Pasquini, K. Rissanen, L. Russo, L. Schiaffino, Zinc–salophen complexes as selective receptors for tertiary amines, New J. Chem. 31 (2007) 1633–1638.
[24] C. Gasbarri, S. Guernelli, S. Boncompagni, G. Angelini, G. Siani, P. De Maria, A. Fontana, Fine-tuning of POPC liposomal leakage by the use of β-cyclodextrin and several hydrophobic guests, J. Liposome Res. 20 (2010) 202–210.
[25] J. Shobha, D. Balasubramanian, Microenvironmental differences among micelles of various shapes and structures, Proc. Ind. Acad. Sci. (Chem. Sci.) 98 (1987) 469–478.
[26] L.M.S. Loura, F. Fernandes, A.C. Fernandes, J.P.P. Ramalho, Effects of fluorescent probe NBD-PC on the structure, dynamics and phase transition of DPPC. A molecular dynamics and differential scanning calorimetry study, Bioch. Biophys. Acta 1778 (2008) 491–501.
[27] H. Binder, K. Arnold, A.S. Ulrich, O. Zschörnig, Interaction of Zn^{2+} with phospholipid membranes, Biophys. Chem. 90 (2001) 57–74.
[28] M.E. Germain, T.R. Vargo, P.G. Khalifah, M.J. Knapp, Fluorescent detection of nitroaromatics and 2,3-dimethyl-2,3-dinitrobutane (DMNB) by a zinc complex: (salophen) Zn, Inorg. Chem. 46 (2007) 4422–4429.
[29] N.G. Angeli, M.G. Lagorio, E.A. San Román, L.E. Dicelio, Meso-substituted cationic porphyrins of biological interest. Photophysical and physicochemical properties in solution and bound to liposomes, Photochem. Photobiol. 72 (2000) 49–56.
[30] A. Blum, L.I. Grossweiner, Singlet oxygen generation by hematoporphyrin IX, uroporphyrin I and hematoporphyrin derivative at 546 nm in phosphate buffer and in the presence of egg phosphatidylcholine liposomes, Photochem. Photobiol. 41 (1985) 27–32.
[31] M.E. Germain, T.R. Vargo, B.A. McClure, J.J. Rack, P.G. Van Patten, M. Odoi, M.J. Knapp, Quenching mechanism of Zn (salicyldimine) by nitroaromatics, Inorg. Chem. 47 (2008) 6203–6211.
[32] W. Bi, T. Wei, X. Lue, Y. Hui, J. Song, S. Zhao, W.K. Wong, R.A. Jones, Hetero-trinuclear near infrared (NIR) luminescent Zn_2Ln complexes from salen-type Schiff-base ligands, New J. Chem. 33 (2009) 2326–2334.
[33] M. Cano, L. Rodriguez, J.C. Lima, A. Dalla Cort, C. Pasquini, L. Schiaffino, Specific supramolecular interactions between Zn^{2+}-salophen complexes and biologically relevant anions, Inorg. Chem. 48 (2009) 6229–6235.
[34] S.J. Wezenberg, E.C. Escudero-Adan, J. Benet-Buchholz, A.W. Kleij, Colorimetric discrimination between important alkaloid nuclei mediated by a bis-salphen chromophore, Org. Lett. 10 (2008) 3311–3314.
[35] D. Kashchiev, D. Exerowa, Bilayer lipid membrane permeation and rupture due to hole formation, Biochim. Biophys. Acta 732 (1983) 133–145.
[36] O. Lopez, A. de la Maza, L. Coderch, C. López-Iglesias, E. Wehrli, J.L. Parra, Direct formation of mixed micelles in the solubilization of phospholipid liposomes by Triton X-100, FEBS Lett. 426 (1998) 314–318.
[37] R.W. Egan, M.A. Jones, A.L. Lehninger, Hydrophile-lipophile balance and critical micelle concentration as key factor influencing surfactant disruption of mitochondrial membranes, J. Biol. Chem. 251 (1976) 4442–4447.
[38] K. Edwards, M. Almgren, Kinetics of surfactant-induced leakage and growth of unilamellar vesicles, Prog. Colloid Polym. Sci. 82 (1990) 190–197.
[39] Y. Nagawa, S.L. Regen, Surfactant-induced release from phosphatidylcholine vesicles. Regulation of rupture and leakage pathways by membrane packing, J. Am. Chem. Soc. 114 (1992) 1668–1672.
[40] P. De Maria, A. Fontana, C. Gasbarri, D. Velluto, Effects of fullerene guests on the stability of 1-palmitoyl-2-oleoylphosphatidylcholine liposomes, Soft Matter 2 (2006) 595–602.
[41] H.L. Casal, H.H. Mantsch, H. Hauser, Infrared studies of fully hydrated saturated phosphatidylserine bilayers. Effect of lithium and calcium, Biochemistry 26 (1987) 4408–4416.
[42] G. Laroche, E.J. Dufourc, J. Dufourcq, M. Pezolet, Structure and dynamics of dimyristoylphosphatidic acid/calcium complexes by deuterium NMR, infrared and Raman spectroscopies and small-angle X-ray diffraction, Biochemistry 30 (1991) 3105–3114.
[43] P. De Maria, P. Filippone, A. Fontana, C. Gasbarri, G. Siani, D. Velluto, Cardanol as a replacement for cholesterol into the lipid bilayer of POPC liposomes, Coll. Surf. B 40 (2005) 11–18.