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Interaction of phthalocyanines with lipid membranes: a spectroscopic and functional study on isolated rat liver mitochondria

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Abstract

Absorption and emission spectroscopic studies on Zn(II)-phthalocyanine (ZnPc) incorporated into unilamellar liposomes of dipalmitoylphosphatidylcholine, sometimes added with cholesterol or cardiolipin, and released to rat liver mitochondria via the three types of liposomal vesicles indicated that: (a) ZnPc predominantly dissolves in all lipid domains of biological membranes with the exception of cardiolipin-containing regions; a partial localization of ZnPc in protein binding sites is also postulated; (b) the spectroscopic properties of ZnPc, although mainly determined by the aggregation state of the dye, are somewhat influenced by the physico-chemical characteristics of the lipid environment; (c) ZnPc-binding lipid domains in mitochondria are mainly localized in the outer membrane; this conclusion is clearly deduced from the trends of Arrhenius plots of the ZnPc fluorescence quantum yield in whole mitochondria and isolated inner or outer membrane in the temperature range −10°C–+45°C; (d) the nature of the ZnPc-binding site in mitochondria is not dependent on the chemical composition of the liposome carrier, contrary to what observed for other hydrophobic dyes, such as porphyrins. This has been also confirmed by photosensitization experiments. Actually, illumination of ZnPc-loaded mitochondria by 600–700 nm light causes a decline of the respiratory control ratio, which is essentially dependent on the amount of incorporated photosensitizer, irrespective of the composition of the carrier.

Keywords: Liposome; Mitochondrial membrane; Lipid phase transition; Zinc phthalocyanine; Photosensitization

1. Introduction

Phthalocyanines are a promising class of second-generation photosensitizing agents for the treatment of tumours by photodynamic therapy (PDT) [1,2]. In particular, Zn(II)-phthalocyanine (ZnPc) has been extensively investigated in our laboratory [3–5] and is now proposed for phase I/II clinical trials. Due to the insolubility of ZnPc in polar media, liposomes were used as suitable dye-carriers in vivo and also in vitro, in particular to photosensitize isolated organelles as in the present study. One peculiar feature of liposome-incorporated ZnPc is its specific association with serum lipoproteins, including low density lipoproteins (LDL) [6]; this circumstance leads to two important consequences, namely an enhanced selectivity of tumour targeting in experimental animals [7] and a preferential direct photodamage of malignant cells as compared with vascular endothelium during PDT [8]. Mitochondrial membranes of tumour cells are among the most readily attacked subcellular sites [9], resulting in the formation of swollen and optically empty mitochondria.

Abbreviations: ZnPc, zinc (II)-phthalocyanine; PP, protoporphyrin IX; PDT, photodynamic therapy; LDL, low density lipoproteins; DPPC, DL-α-dipalmitoylphosphatidylcholine; DMPC, L-α-dimirystoylphosphatidylcholine; SUV, small unilamellar vesicles; Chol, cholesterol; Card, cardiolipin; PBS, phosphate-buffered saline; SDS, sodium dodecylsulphate; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; RCR, respiratory control ratio; P, fluorescence polarization degree; T_c, critical temperature for the lipid phase transition.

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On these bases, it appears of interest to carry out a detailed investigation on the processes controlling the interaction of liposomal ZnPc with different compartments of mitochondrial membranes, since the microenvironment of ZnPc can modulate the levels of photosensitivity and the mechanisms of photodamage [10], e.g., by changing the degree of photosensitizer aggregation, its accessibility to oxygen and the probability of interaction with photoreactive targets.

2. Materials and methods

2.1. Chemicals

Zn(II)-phthalocyanine (ZnPc) (98–99% pure) was supplied by Ciba-Geigy (Basel, Switzerland). DL-α-Dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol), crystalline, 99% pure, and cardiolipin (Card) in ethanol, 98% pure, were products of Sigma (St. Louis, MO) and were used without further purification. All other chemicals were the purest commercially available products.

2.2. Preparation of liposomes

Small unilamellar vesicles of DPPC (mean external radius ca. 26 nm) were prepared by the ethanol injection method [11], i.e., by co-dissolving ZnPc at the desired concentration (from a stock solution in pyridine) and 9.57 mM phospholipid in absolute ethanol. The ethanolic solution (0.7 ml) was injected (injection rate ca. $5 \cdot 10^{-2}$ ml/min) into 10 ml of magnetically stirred PBS (phosphate-buffered saline, pH 7.4) at 55°C, i.e., above the critical temperature ($T_c = 41.5$°C) of the lipid phase transition. Mixed liposomes were prepared by adding Chol (20% mol/total mol of lipids) or Card (20%, w/w) to the ethanolic solution. The liposomes so obtained were dialyzed against PBS in order to eliminate any residual pyridine.

2.3. Preparation of mitochondria and inner and outer mitochondrial membranes

Mitochondria were isolated from liver of Wistar rats, as previously described [12], and the final mitochondrial pellet was suspended in 0.5 M sucrose to give a concentration of 80–100 mg mitochondrial proteins/ml, measured according to the method of Waddel and Hill [13]. Inner and outer membranes were separated by differential centrifugation, after swelling mitochondria in a hypotonic medium, according to the procedure of Parsons and Williams [14]. The purity degree of the inner and outer membrane fractions was checked by determining the specific enzyme activity of cytochrome-*c* oxidase and monoamino oxidase, respectively, and was found in good agreement with the values reported in the literature [15].

2.4. Uptake of ZnPc by mitochondria and isolated membranes

Mitochondria or isolated membranes (0.5 mg/ml) were suspended in the respiratory medium (24 mM glycylglycine, 10 mM $MgCl_2$, 60 mM KCl, 7 mM KH_2PO_4 and 87 mM sucrose, pH 7.4) and incubated with the liposome-bound ZnPc. The amount of ZnPc taken up by whole organelles or isolated mitochondrial membranes was determined spectrophotometrically ($\epsilon_M = 241\,000$ at 672 nm), after centrifugation of the samples at $11\,600 \times g$ for 2 min ($g = 17\,000$ for the outer membrane). The dye concentration was determined both on the supernatant and the pellet after extraction with 2% SDS where the phthalocyanine is monomerically dispersed in the surfactant micelles [10]. In all experiments, the final results are the average of at least three separate runs.

2.5. Photosensitization of mitochondria by ZnPc

Irradiation of mitochondria was carried out in a thermostated (25°C) water-jacketed vessel containing 1.4 ml of magnetically stirred respiratory medium. Mitochondrial respiration was activated with succinate (14 mM) as a substrate and rotenone (5 μM) was added to block the oxidation of endogenous substrates. Mitochondria (0.5 mg/ml) were added to the medium and incubated for different times with liposome-incorporated ZnPc. Mitochondria were irradiated at 600–700 nm with a quartz/halogen lamp (250 W), equipped with a set of optical filters. The flux at the level of the reaction vessel was 80 W/m^2. Oxygen consumption was followed by using a Clark electrode connected to a recorder. Additions of ADP were made through a small port. The decline of the respiratory control ratio (RCR), i.e., the ratio between the rate of oxygen consumption in state III (in the presence of ADP) and in state IV (without ADP), was used as a measure of the photodynamic effect. Only mitochondrial preparations with an initial RCR $\geq$ 3.0 were considered for photosensitization experiments. The final results are the average of at least three measurements. Empty liposomes did not affect the functional activity of mitochondria.

2.6. Studies of the spectroscopic properties of liposome- or mitochondria-bound ZnPc

Fluorescence measurements were performed with a Perkin-Elmer LS 50 luminescence spectrometer, equipped with a water-thermostated, stirred cell holder and a polarization accessory. Absorption measurements of liposome-bound ZnPc were performed with a Perkin-Elmer Lambda 5 spectrophotometer, those for mitochondria-bound ZnPc with an Aminco DW-2000. The contribution from light scattering was corrected by using 'empty' liposomes or mitochondria as reference. Mitochondria-bound ZnPc has

been measured after removal of the unbound dye by centrifugation. For these experiments, a low centrifugation speed has been used ($9000 \times g$), in order to facilitate the resuspension of the dye-containing mitochondrial pellet. This was added with the minimal volume of the respiratory medium, then diluted up to a final concentration of 0.5 mg/ml. Dye-loaded mitochondria obtained from this procedure were structurally stable for at least 80 h after the preparation, as demonstrated by the constancy of the spectroscopic properties of the bound ZnPc. The same procedures were used for ZnPc incorporated in isolated inner or outer membranes (for this latter case, a centrifugation speed at $17000 \times g$ was always used).

3. Results

3.1. Spectroscopic properties of liposome-bound ZnPc

As previously observed, ZnPc incorporated into small unilamellar vesicles (SUV) of DPPC is monomeric at stoichiometric concentrations below 0.5 μM, corresponding to an effective dye concentration inside the lipid membrane of approx. 1 mM [1]. At higher concentrations, some aggregation of ZnPc occurs. The same behaviour is observed upon enriching the lipid vesicles of DPPC with Chol or Card (data not shown).

Fluorescence quantum yields (Φ_F), relative to that reported for ZnPc in DPPC liposomes ($\Phi_F = 0.14$) [10], are: 0.13 and 0.16 in liposomes of DPPC-Card and DPPC-Chol, respectively (for $\lambda_{exc} = 610$ nm).

In Fig. 1, we report the fluorescence excitation and the polarized fluorescence excitation spectra of ZnPc in liposomes in the red spectral region: the degree of polarization (P) is 0.15 and is constant throughout the wavelength interval examined by us. P is defined as the ratio: $(I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the emitted intensities

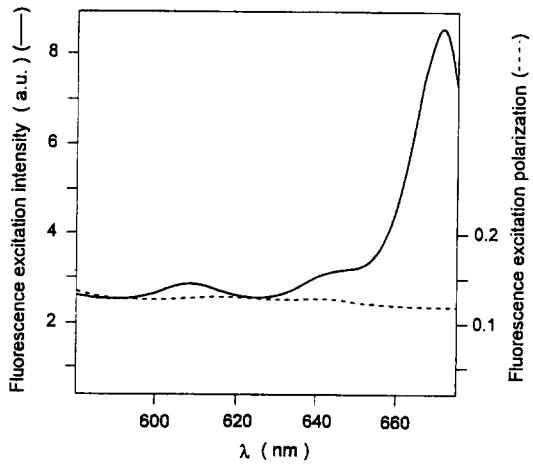

Fig. 1. Fluorescence excitation (–) and polarized fluorescence excitation (- - -) spectra of 0.4 μM ZnPc in DPPC liposomes (emission $\lambda = 680$ nm).

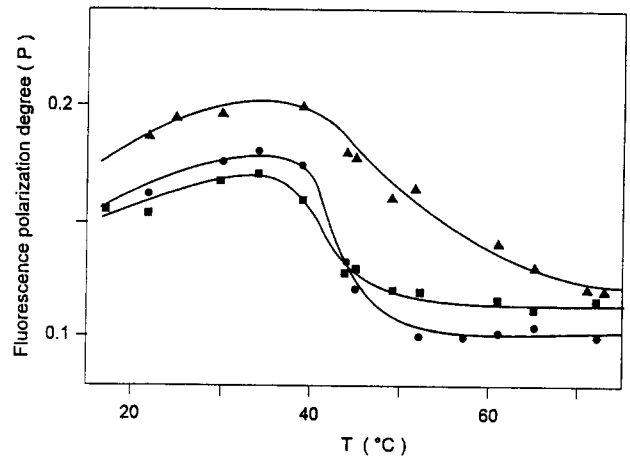

Fig. 2. Temperature dependence of the polarization degree at 610 nm (emission $\lambda = 680$ nm) of 0.4 μM ZnPc in liposomes of DPPC (●), DPPC-Chol (▲) and DPPC-Card (■).

parallel to and perpendicular to the linearly polarized exciting light. The P values are corrected for instrumental artifacts [16].

Fig. 2 shows the temperature-dependence of the polarization degree P for the dye embedded in the three types of liposomal systems. The mid-point of the observed transitions in DPPC and DPPC-Card liposomes is 41.5–42°C, which corresponds with the value of the critical temperature for the gel-liquid transition phase of DPPC lipids [16,17]. In the presence of Chol, the mid-point temperature is shifted to 51°C.

3.2. Uptake of ZnPc by mitochondria

Kinetic studies of ZnPc uptake by whole mitochondria or isolated mitochondrial membranes indicate that the maximal amount of dye delivered to mitochondria from the different types of liposomes is reached within 1 min (data not shown). On these bases, the effect of dye concentration in the various carriers on the ZnPc uptake was studied after 2 min incubation (see in Fig. 3 the results obtained for the whole mitochondria). The final ZnPc concentrations were in the range 0.05–1.0 μg of ZnPc added per mg of mitochondrial protein. The highest uptake of ZnPc by mitochondria is obtained when the lipid dye-carrier is added with cholesterol, the lowest uptake when cardiolipin is present in the lipid vesicles (Fig. 3). Similar results are obtained for the uptake of ZnPc by the isolated inner and outer membranes. The isolated outer membrane, however, exhibits a degree of dye incorporation approx. 30% lower than that shown by whole mitochondria or isolated inner membrane (data not shown).

3.3. ZnPc photosensitization of mitochondria

The photosensitizing efficiency of ZnPc was determined by following the decline of RCR after 2 min incubation of

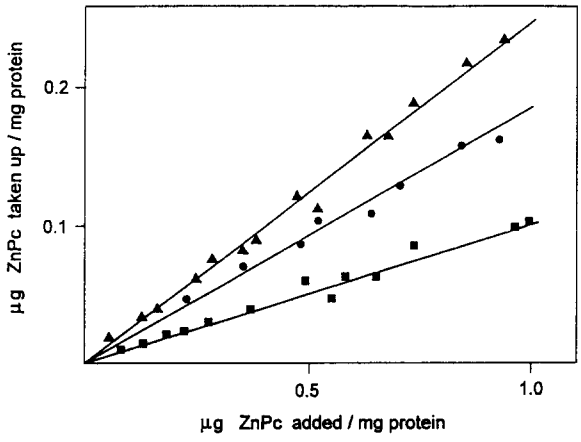

Fig. 3. Dependence of ZnPc uptake by mitochondria (0.5 mg mitochondrial proteins/ml) on the dye concentration. ZnPc was added to mitochondria after incorporation in liposomes of DPPC (●), DPPC-Chol (▲) and DPPC-Card (■). Incubation time was 2 min. All the data are the average of at least three independent experiments.

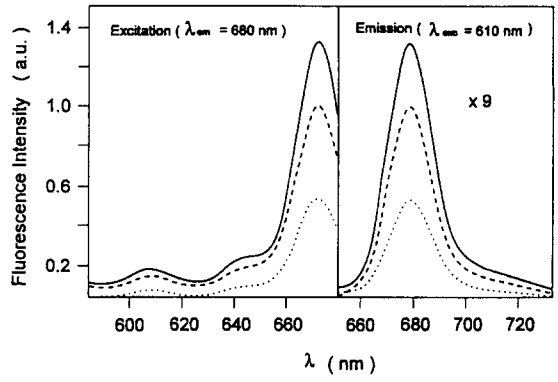

Fig. 5. Excitation and emission fluorescence spectra of ZnPc in mitochondria (0.5 mg mitochondrial proteins/ml) after release from liposomes of DPPC (- - -), DPPC-Chol (· · ·) and DPPC-Card (–). The initial concentration of the dye was 0.25 μM, corresponding to 0.29 μg ZnPc added/mg mitochondrial proteins. Emission spectra were 9 times magnified.

mitochondria with the dye incorporated into the three types of lipid carriers. The comparisons were made on the basis of the amount of ZnPc taken up by mitochondria, after 45 s (A) and 60 s (B) irradiation (Fig. 4). RCR values, expressed as percentage of the RCR control (without irradiation), were obtained according to the relationship: $\% = (RCR_{light} - 1) \times 100/(RCR_{dark} - 1)$. No dark effect of ZnPc on mitochondrial function was observed in the range of concentrations we have used.

As shown in Fig. 4, the extent of ZnPc-induced photodamage depends only on the amount of dye bound to mitochondria, irrespective of the composition of the carrier.

3.4. Spectroscopic properties of mitochondria-bound ZnPc

The excitation and emission fluorescence spectra of 0.25 μM ZnPc (corresponding to 0.29 μg ZnPc added/mg of mitochondrial proteins), released to mitochondria from liposomes of DPPC, DPPC-Card, and DPPC-Chol are shown in Fig. 5. The spectra are typical of the monomeric form of ZnPc (excitation $\lambda_{max} = 672$ nm; emission $\lambda_{max} = 680$ nm), as one would expect since ZnPc in lipid environments at such low concentrations is known to be completely monomeric [1]. The fluorescence quantum yields (Φ_F) of mitochondria-bound ZnPc, calculated on the basis of the amount of dye taken up by the organelles and referred to that of ZnPc in DPPC liposomes, are approx. 0.09 in the three cases, i.e., independent of the chemical composition of the carrier. Similar quantum yields (Φ_F = 0.09–0.10) are exhibited by ZnPc in isolated outer membrane while in the inner membrane the values of Φ_F are somewhat different ranging between 0.12–0.14 in dependence of the ZnPc-carrier.

Absorption spectra (data not shown) also confirm the occurrence of dye monomeric species inside mitochondria or isolated membranes.

The changes of fluorescence emission intensity of mitochondria-bound ZnPc were studied in the temperature range −10°C–+45°C. The experiments were performed after adding 20% ethylene glycol to prevent freezing. Ethylene glycol did not affect the ZnPc fluorescence as deduced by the similarity of the spectra obtained in the absence and in the presence of the cryoprotectant above 0°C. Mitochondria in glycol have intact inner and outer membranes, showing only some loss of matrix material [18]. Heating the mitochondrial suspension does not cause a monotonic decrease of the fluorescence yield, as usually expected for fluorophores dissolved in homogeneous solutions. As already observed for other hydrophobic dyes embedded in lipid membranes [16], the trends of the fluorescence curves probably follow the phase transitions of the lipids of the dye microenvironment. The experimental data were analyzed in terms of Arrhenius plots (Fig. 6) which, according

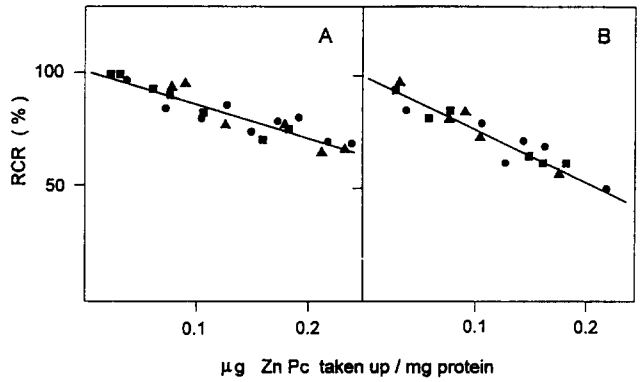

Fig. 4. Decline of RCR (in %) as a function of the amount of mitochondria-bound ZnPc after 2 min incubation of the dye with mitochondria and 45 s (A) or 60 s (B) irradiation. Conditions and symbols as in Fig. 3.

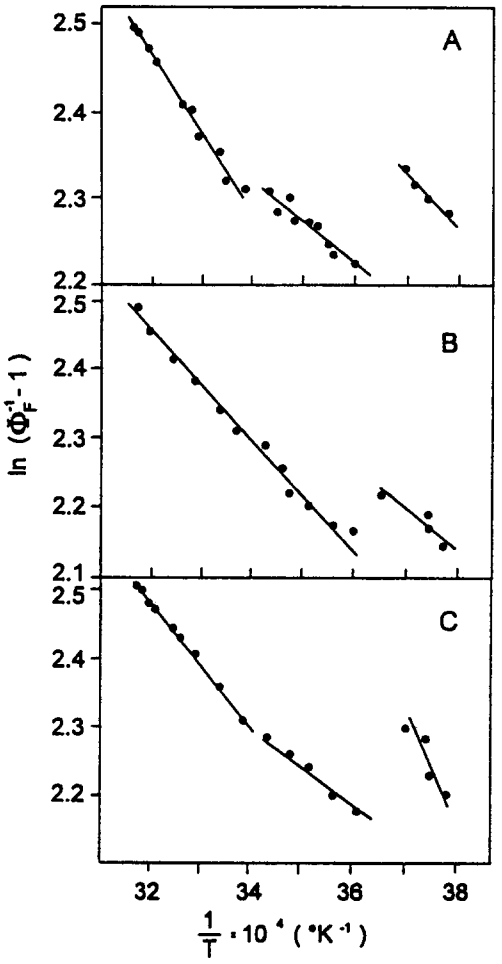

Fig. 6. Arrhenius plots of ZnPc quantum yields in the whole mitochondria (A) and isolated inner (B) and outer (C) membranes. ZnPc was released to mitochondria and isolated membranes from DPPC liposomes. Similar results were obtained when the dye-carrier were liposomes of DPPC-Chol or DPPC-Card.

to Kirby and Steiner [19], can be expressed by the relationship:

$$\ln\left(\Phi_F^{-1} - 1\right) = \ln f_i/k_f - E_i/RT$$

where R is the gas constant (in kcal/mol per K), T is the absolute temperature, k_f is the temperature-independent, first-order rate constant for the direct emission of fluorescent radiation by the excited state, E_i and f_i are the activation energy and the frequency factor, respectively, for the various radiationless deactivation processes of the first excited singlet state. Arrhenius plots of ZnPc quantum yield in whole mitochondria show discontinuities at about 0°C and 24°C (Fig. 6A). The values for the Arrhenius activation energies are 0.97 and 1.80 kcal/mol, respectively, for the T ranges 2–20°C and 25–45°C. The same experiments, repeated on the isolated inner and outer membrane, show a discontinuity in the Arrhenius plots at 0°C for both membranes; the inner membrane, however, shows no change in the slope above 0°C; the calculated E_a is 1.6 kcal/mol (Fig. 6B). The outer membrane, on the contrary, shows a discontinuity at approx. 25°C, the E_a for the two slopes being 1.0 and 1.78 kcal/mol (Fig. 6C).

Polarization experiments, performed in the interval of T: 10–45°C for the whole mitochondria, confirm a change of the physical state of the lipids starting from 25°C (Fig. 7): the temperature range of the transition is rather wide with a mid-point at a T not lower than 35°C.

4. Discussion

Previous spectroscopic studies of ZnPc in different lipid environments [1,10] indicate a strong tendency of this phthalocyanine to form aggregates even in non-polar me-

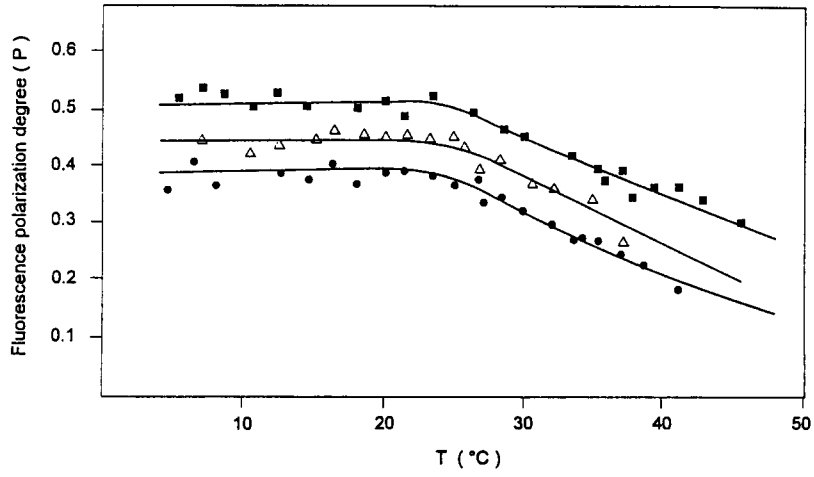

Fig. 7. Temperature dependence of fluorescence polarization degree at 610 nm (emission λ = 680 nm) of ZnPc released to mitochondria from liposomes of DPPC (●), DPPC-Chol (△) and DPPC-Card (■). The data were the average of at least three separate experiments.

dia. This generally causes a drop in photosensitizing efficiency as compared with monomeric ZnPc, since aggregated species are characterized by a lower triplet quantum yield and/or shorter triplet state lifetime [1]. To minimize aggregation, the concentration of the dye as well as the size of the lipid structure are critical factors [1,10]. In all our experimental conditions, ZnPc is predominantly present in a monomeric state.

In this paper, some fluorescence properties have been investigated for ZnPc incorporated into lipid vesicles of DPPC, sometimes modified by addition of cholesterol or cardiolipin in order to better simulate the peculiar characteristics of the mitochondrial outer and inner membranes [20]. From these simplified models, the behaviour of the dye in different lipid microenvironments can be better understood. Thus, the nature or physical state of the lipid domains do not influence the shapes of the fluorescence spectra of ZnPc (data not shown); these parameters, however, somewhat affect the fluorescence quantum yields of the dye, being the influence higher in the presence of cholesterol (see Results). Accordingly, different Φ_F values have been calculated for ZnPc embedded in lipid microenvironments of different chemical composition and fluidity properties such as vesicles of dimiristoylphosphatidylcholine (DMPC) ($\Phi_F = 0.17$) or mitochondrial membranes (range of $\Phi_F = 0.09–0.14$). On the other hand, Jori et al. [10] found that the fluorescence quantum yield of monomeric ZnPc in DPPC liposomes ($\Phi_F = 0.14$) consistently differs from that of monomeric ZnPc in low density lipoproteins ($\Phi_F = 0.08$). The significant decrease of Φ_F for ZnPc in lipoproteins and in the whole mitochondria may indicate a partial distribution of the dye in some protein binding sites.

Studies of the changes of the physical state of the liposomal systems as a function of the temperature provide some insight into the solubilization properties of ZnPc in different lipids. As known, [17,20] the addition of 20% (w/w) cardiolipin to DPPC liposomes strongly increases lipid fluidity, due to its high content (90%) of unsaturated fatty acids. This results in a lowering of the critical temperature (T_c) for the gel-to-liquid crystal phase transition of the lipids. When protoporphyrin (PP) is used as a probe of lipid fluctuations, a 10°C shift to lower T is observed [17]. ZnPc, however, does not monitor any variation of DPPC liposome fluidity in the presence of cardiolipin, the T_c value remaining constant at 41–42°C (see Fig. 2). This should suggest a preferential distribution of ZnPc in DPPC-rich domains and its exclusion from Card-containing domains. Cholesterol is inserted into the lipid bilayer with its -OH groups located between the phospholipid polar heads while steroid rings interact with those parts of the hydrocarbon chains which are nearer the head groups [17]. At 20% mol/mol concentration, such cholesterol distribution causes a partial immobilization of the liposomal regions near the lipid/water interfaces, while the remaining part of the vesicle is relatively flexible. If the probe is inserted in the former lipid domains, it should monitor a relative decrease of fluidity. On these bases, the shift of T_c to 47°C for DPPC-Chol liposomes suggests a localization of ZnPc also in Chol-containing lipid domains.

The properties of ZnPc binding sites in mitochondrial membranes have been studied after release of the dye from liposomes of different composition. Actually, a modulation of the mitochondrial binding sites depending on the nature of the lipid carrier has been observed for highly hydrophobic dyes such as PP [20]. In particular, the enrichment of the carrier with Chol or Card led to a preferential delivery of the dye to the outer or inner membrane, respectively. This resulted in an increased photobiological response, as measured by the RCR parameter, for PP transported to mitochondria by Card-containing liposomes [20]. Actually, photodamage appears to be mainly due to the impairment of the inner membrane mitochondrial carriers [21]. In the case of ZnPc, the photosensitizing efficiencies depend only on the amount of dye incorporated into mitochondria (Fig. 4). This, as well as the similarity of fluorescence quantum yields (see Results), indicate that ZnPc is mostly located in the same microenvironment, independently of the nature of the carrier.

The ZnPc microenvironment in mitochondrial membranes must be mostly a lipid domain, as indicated by the presence of phase transitions in the curves showing the fluorescence changes at increasing temperatures (Fig. 6).

The value of the critical temperature for the lipid phase transition should provide an indication as regards the location of ZnPc-binding lipid domains. A reversible broad gel to liquid crystalline phase transition occurs at 0°C in rat liver whole mitochondria [18]. On the other hand, a narrower membrane lipid transition centered at +10°C has been reported by Madden et al. [22]. Lipid phase transitions were detected in a wide range of low temperatures (−10°C to 0°C) for the inner mitochondrial membrane of different mammals [22–25], while these occur at higher temperatures (9°C, according to Hackenbrock et al. [23]) in isolated outer membrane fraction. The extent of the thermotropic transition region is quite narrow (11°C) in the inner membrane, due to a higher degree of cooperative lipid transition, as compared to the outer membrane (24°C) [23]. The observed differences for the two membranes are apparently related to their distinct lipid and protein compositions. In particular, inner mitochondrial membrane exhibits a higher content of unsaturated phospholipids (mainly cardiolipin) which are known to lower the T_c of the lipid transition, a negligible Chol content and a high content of integral proteins. Other authors [26], however, rule out a significant difference in the temperature of the phase transitions for the inner and outer mitochondrial membranes. Major lipid phase transitions occurring at higher temperatures are revealed from spin labelling experiments and Arrhenius plots of enzyme activation energies [27–29]: these occur at about 8–17°C and 23–27°C for mitochondria of homeothermic animals, including rats. Recent

studies, however, rule out the occurrence of lipid phase transitions at temperatures above 4°C for the inner mitochondrial membrane which is predominantly fluid at that temperature; on the contrary, the apparent breaks in the Arrhenius plots, observed at high temperatures, were attributed to changes in conformation of the membrane-associated enzymes [25]. Due to the inconsistency of the literature data, we decided to study the lipid temperature-dependent transitions of whole mitochondria as well as of the two separate mitochondrial membranes by following the fluorescence changes of the same dye, ZnPc, whose localization properties inside mitochondria were of interest for us.

The Arrhenius plots clearly demonstrate a differential response to the temperature of the two isolated membranes, as monitored by ZnPc fluorescence. For both, a transition below 0°C is present in the plots. At higher temperatures, the inner membrane exhibits a linear decrease of Q with increasing temperature over the entire T range examined, with an unique value of E_a, thus confirming the results of Pehowich et al. [25]. On the contrary, a lipid transition with an onset temperature around 23°C clearly occurs for the outer membrane. The extent of this phase transition is very wide. A comparison with the data obtained for the whole mitochondria suggests a preferential localization of ZnPc in the outer mitochondrial membrane. Actually, the trends of Arrhenius plots and the values of activation energies of ZnPc in the whole mitochondria nearly overlap those found for the isolated outer membrane. It is reasonable to hypothesize that the nonradiative pathways for dissipating the excitation energy are reduced when the fluorophore is located in a rather rigid environment thus decreasing the value of the activation energy E_a, as actually found for ZnPc located in the outer mitochondrial membrane. When the ZnPc lipid surroundings become more flexible, due to a change of the physical state (Fig. 6C) or to a change in the chemical composition (Fig. 6B), thus increasing the frequency of the nonradiative deactivation processes, an increase of the E_a value is observed.

A comparison of the values of Φ_F in whole mitochondria and isolated membranes (see the Results) also suggests a preferential localization of ZnPc in the outer mitochondrial membrane. The low content of cardiolipin of the outer membrane favours the binding of ZnPc which, as previously discussed for the case of liposome-incorporated drug, shows a low tendency to dissolve in Card-rich regions. Moreover, the presence of cardiolipin in the carrier strongly reduces the uptake of ZnPc by mitochondria (Fig. 3) or isolated membranes. Since a mechanism of lipid exchange has been postulated to explain the drug transport from liposomal to mitochondrial membranes [20], the low tendency of ZnPc to interact with Card-rich regions clearly reduces the amount of dye released to mitochondria from a Card-containing carrier.

Acknowledgements

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