*Biochimica et Biophysica Acta*, 1065 (1991) 42–48

*ADONIS* 000527369100217G

BBAMEM 75239

# Liposomes as models to study the distribution of porphyrins in cell membranes *

Fernanda Ricchelli, Giulio Jori, Silvano Gobbo and Michele Tronchin

*C.N.R. Centre of Hemocyanins and other Metalloproteins, Department of Biology, University of Padova, Padova (Italy)*

(Received 29 November 1990)

Key words: Porphyrin–liposome interaction; Hematoporphyrin; Protoporphyrin; Liposome; Membrane; Fluorescence polarization; Fluorescence quenching

**Unilamellar liposomes of dipalmitoylphosphatidylcholine (DPPC) have been choosen as suitable models of cell membranes in studies aimed at defining the influence of specific parameters on the distribution properties of selected hydrophobic photosensitizers, namely hematoporphyrin (HP) and protoporphyrin (PP), in normal and tumour tissues. To better mimic *in vivo* situations, DPPC liposomes were sometimes mixed with cardiolipin (Card) or cholesterol (Chol). Two techniques were mainly used: the quenching of porphyrin fluorescence by methyl viologen, which can discriminate different dye populations inside the vesicles as well as their degree of accessibility to the external medium, and the polarization of porphyrin fluorescence, which gives information on the dye microenvironment through its degree of rotational freedom. The nature of the porphyrin binding sites in each phospholipid monolayer is found to be a function of the degree of hydrophobicity and the concentration of the dye as well as the chemical composition of the liposomes. In DPPC and DPPC-Chol liposomes, all PP molecules are deeply embedded into very rigid, hydrophobic domains of the inner lipid monolayer. Only in the presence of cardiolipin, for [PP] > 2.5 μM, a partial shift of the dye molecules towards the outer lipid monolayer is observed. HP mostly localizes at the inner lipid/water interface in all liposomes: at very low concentrations ([HP] ≅ 0.5 μM) the dye is bound to the polar heads of the lipids through its carboxylate groups, leaving the rest of the molecule dissolved in the inner aqueous pool. At higher concentrations, HP molecules change their orientation: the ionized propionic chains still interact with the polar heads while the hydrophobic core lies in the lipid phase in DPPC and DPPC-Card vesicles. HP incorporated into DPPC-Chol mixed liposomes projects from the inner lipid phase into the aqueous compartment in all the concentration range studied by us. A very small fraction of HP population (corresponding to 5–10% of the overall fluorescence) is localized at the water/lipid external interface in DPPC and DPPC-Chol liposomes. This fraction increases in the presence of cardiolipin (up to 30% of the overall fluorescence). The possible implications of these findings for the nature of the targets of photosensitization in cell membranes are discussed.**

## Introduction

In recent years new interest is being developed in the field of photosensitized processes *in vivo*, mainly because of the variety of biomedical applications, including the photodynamic therapy (PDT) of tumors, atherosclerosis and some cutaneous diseases [1,2]. In this connection, particular attention is devoted to porphyrins and related photosensitizers absorbing in the spectral region above 600 nm, a so-called optical window where endogeneous tissular components are transparent to the incident radiation [3]. This fact minimizes the risk of photodamage at the level of cells or tissues not containing the photosensitizers.

Although PDT is being applied with increasing success in diversified clinical applications [4], its mechanism of action is still poorly understood owing to the interplay of a large number of physico-chemical, photobiological, biochemical and physiological factors which control the uptake of photosensitizers by tissues and the subsequent photoinduced necrosis [2,5]. Tumor tissues

* Dedicated to Prof. Kurt Schaffner on occasion of his 60th birthday.

Correspondence: F. Ricchelli, C.N.R. Centre of Hemocyanins and other Metalloproteins, Department of Biology, University of Padova, Via Trieste 75, 35121 Padova, Italy.

are themselves highly heterogeneous and compartmentalized systems, where the photosensitizer may possess different photoproperties [6].

Therefore, it appears important to address this problem by developing suitable model systems, mimicking specific situations occurring *in vivo* in order to define the influence of specific parameters in determining the overall photosensitizing efficiency. In this paper, we report our studies on the interaction of unilamellar liposomal vesicles with two porphyrins of medical significance, i.e. hematoporphyrin (HP), which is used in PDT of tumors [7], and protoporphyrin (PP), which is responsible for several photosensitivity phenomena in porphyric patients [8]. Actually, both HP and PP, being characterized by an octanol/water partition coefficient higher than 10, are hydrophobic compounds [9], which appear to be preferentially distributed in lipid cores of mitochondrial, lysosomal and cytoplasmic membranes [10].

In order to better mimic the composition of such membranes, liposomes were sometimes mixed with cholesterol or cardiolipin. Actually, cholesterol is a determinant of the fluidity of eukaryotic plasma membranes [11] while cardiolipin is found in the inner membrane of mitochondria.

## Materials and Methods

Hematoporphyrin IX (HP) (88% pure by HPLC analysis) and protoporphyrin IX (PP) (95% pure by HPLC analysis) from Porphyrin Products (Utah) were used as received. All aqueous solutions were prepared with doubly-distilled water in pH 7.4, 0.01 M phosphate buffer containing 0.15 M NaCl (PBS). Stock solutions of HP and PP were made in ethanol and dimethyl sulfoxide (DMSO), respectively. Small unilamellar vesicles (SUV) of DL-α-dipalmitoylphosphatidylcholine (DPPC) (average diameter $\cong 50$ nm) were prepared by the ethanol injection method, as modified by Kremer et al. [12], by co-dissolving HP or PP at the desired concentration and 9.57 mM phospholipid in ethanol. The ethanolic solution (0.7 ml) was injected into 10 ml of magnetically stirred PBS at 55°C. The injection rate was about $5 \cdot 10^{-2}$ ml/min. Mixed liposomes were prepared by adding 20% moles of cholesterol (Chol) per total moles of lipids or 20% (w/w) cardiolipin (Card) . All lipids were obtained from Sigma Chemical Co., St. Louis, MO and used as received. Stock solutions of methyl viologen ($MV^{2+}$, $\epsilon = 20\,500$ $M^{-1}$ $cm^{-1}$ at 257 nm) were prepared in PBS and stored in the dark at −15°C. The sample was purchased from Sigma. Rose bengal (from Riedel der Haen AG) was dissolved in absolute ethanol. Other chemicals were of the best available biochemical grade and used as received.

Absorption measurements were performed with a Perkin-Elmer Lambda 5 spectrophotometer. For aqueous dispersions of liposomes the contribution from light scattering was corrected using 'empty' liposomes as reference. The following extinction coefficients were used: $\epsilon = 1.4 \cdot 10^5$ $M^{-1}$ $cm^{-1}$ for HP in absolute ethanol or in liposome dispersions at 400 nm, $\epsilon = 1.46 \cdot 10^5$ $M^{-1}$ $cm^{-1}$ for PP in DMSO (at 408 nm), $\epsilon = 1.2 \cdot 10^5$ $M^{-1}$ $cm^{-1}$ for PP in liposomes (at 408 nm). Fluorescence measurements were performed with a Perkin-Elmer LS 50 spectrophotofluorimeter controlled by an Epson AX2 computer. The instrument was equipped with a water thermostatted, stirred cell holder and a polarization accessory. Fluorescence emission spectra were always excited at 440 nm, i.e. at a minimum of the absorption spectral region, in order to minimize inner filter effects. Excitation fluorescence polarization ($P$) was measured at 520 nm, after 24 h incubation of the porphyrins with liposomes, by setting the emission at 626 nm for HP and 636 nm for PP. $P$ values were calculated in a temperature range between 12 and 60 °C. Fluorescence lifetimes were measured by using an ISS phase-modulation domain fluorimeter model GREG-200. Rose bengal was used as a reference. The samples were excited at 440 nm with a xenon lamp or 442 nm with an He-Cd laser. Contribution from light scattering was eliminated using a 520 nm cut-off filter. The data were directly transferred to an IBM computer model XT and analyzed by single- and double-exponential decay curves to obtain the best fitting with the modified $\chi^2$ equation described by Lakowicz et al. [13].

Fluorescence quenching data, obtained at 25 °C by using $MV^{2+}$ as a quencher of the porphyrin fluorescence, were analyzed by the Stern-Volmer equation:

$$F_0/F = 1 + K_Q[Q]$$

which relates the decrease of fluorescence intensity ($F_0/F$) with the quencher concentration [Q] [14]. The Stern-Volmer quenching constant $K_Q$ was calculated by a modified linear regression vinculated to the point (0,1) according to:

$$M = \sum x(y-1)/x^2$$

The bimolecular quenching rate constant $K_q$, which is used as a parameter of the accessibility of the porphyrin to the external medium was calculated by the expression: $K_Q = \tau K_q$ ($\tau$ is the fluorescence lifetime in the absence of the quencher). In the case of heterogeneous emission, as indicated by a downward curvature in the Stern-Volmer plots, a modified Stern-Volmer equation, valid at low quencher concentrations, was used [14,15]:

$$F_0/(F_0 - F) = 1/(f_a K_Q[Q]) + (1/f_a)$$

where $f_a$ is the fraction of quencher-accessible fluorescence and $K_Q$ the Stern-Volmer quenching constant

associated to this fraction. All $F$ values were corrected for the dilution effects due to the added $MV^{2+}$ and for the light scattering effects of the liposomal dispersions. The fluorescence quenching measurements were performed after 24 h incubation of the porphyrins with liposomes.

## Results and Discussion

HP and PP are incorporated into DPPC liposomal vesicles in a monomeric state, as indicated by the position of the Soret peak ($\lambda_{max} = 401$ nm for HP and 408 nm for PP) and the fluorescence emission maxima (626 nm for HP and 636 nm for PP) as well as by the values of the fluorescence lifetimes (13–16 ns) [16,17]. The liposome-incorporated porphyrins remain in a monomeric state over the whole range of concentrations we have examined (0.5–15 $\mu$M corresponding to a porphyrin/lipid molar ratio of 1 : 1340–56 and an average number of about 20–600 dye molecules per liposome) and during a time interval of 48 h. This can be deduced from the following evidences: (a) the shape of the absorption and emission spectra remains constant; (b) the Lambert Beer's law is followed in all cases as it can be seen in Fig. 1 for HP in DPPC and DPPC-Chol liposomes as an example; (c) fluorescence decays are mono-exponential under all concentration conditions. At porphyrin concentrations higher than 5 $\mu$M, however, a decrease of both fluorescence quantum yield and fluorescence lifetime is observed (Figs. 1 and 2) probably indicating a singlet state-deactivation by neighboring ground state molecules. Inner filter effects have been corrected for, while trivial reabsorption of emitted fluorescence by porphyrins is negligible under our conditions. The distribution properties of HP and PP inside the lipid bilayer as a function of the porphyrin concentration and the chemical composition of the liposome can be deduced from the values of the fluorescence quenching parameters $K_q$ and $f_a$. The results obtained are summarized in Figs. 3 and 4 for HP and in Table I for PP. In the case of heterogeneous emissions the parameters of both readily and poorly quencher-accessible fractions of fluorescence are reported. These have been calculated after analyzing the fluorescence quenching data at low quencher concentrations (where the accessible class of porphyrin first interacts with the quencher) by the modified Stern-Volmer equation (see Materials and Methods). The $K_q$ value of the poorly accessible porphyrin fraction can be obtained approximately from the slope of the linear portion of the titration curve at higher quencher concentrations (see Fig. 5). The approximation requires that all the accessible porphyrin fluorescence is already quenched in that concentration range. This is likely since the $K_q$ values of the two porphyrin populations are drastically different, the order of magnitude being $10^{11}$ and $10^9$ $M^{-1}$ $s^{-1}$

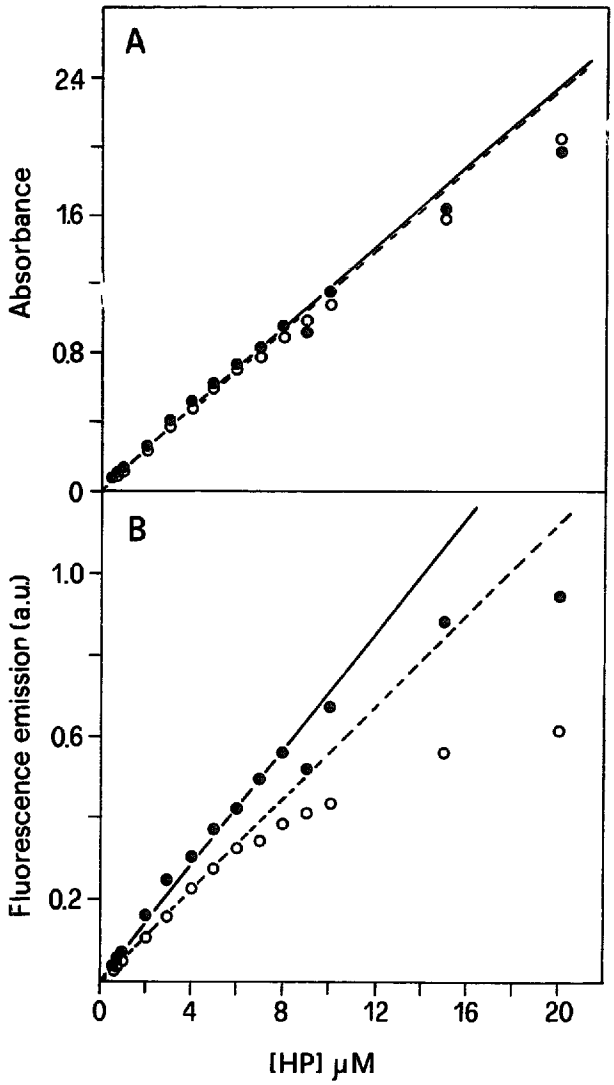


Fig. 1. Change of absorbance (A) and fluorescence emission (B) as a function of concentration of HP in DPPC (●) and DPPC-Chol (○) liposomes. Fluorescence intensity is given in arbitrary units.

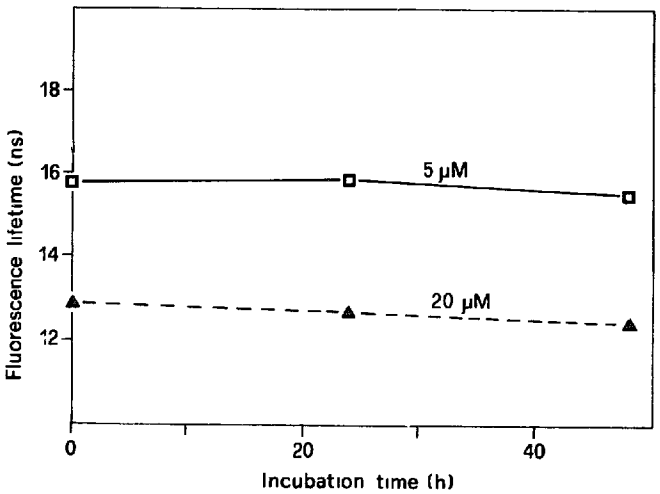


Fig. 2. Change of fluorescence lifetime as a function of the incubation time with DPPC liposomes for 5 $\mu$M (□) and 20 $\mu$M (▲) PP.

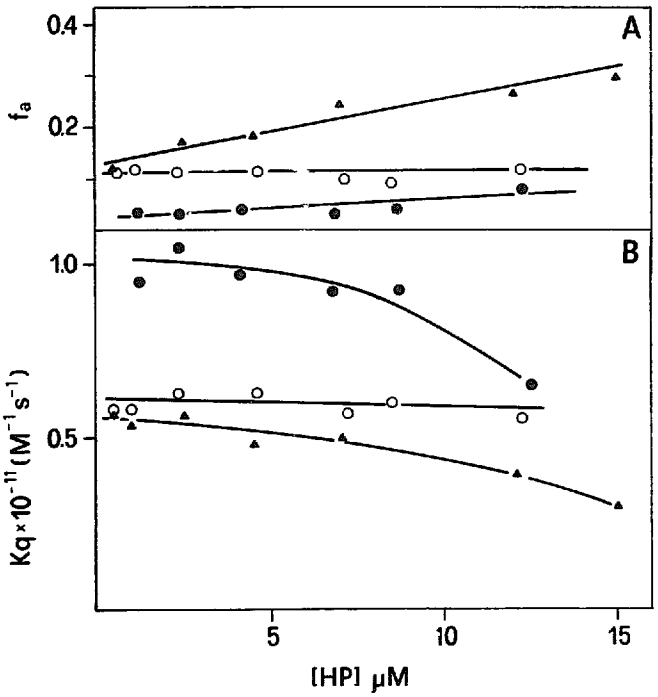


Fig. 3. Dependence of the quenching parameters $K_q$ (B) and $f_a$ (A) on HP concentration. The values refer to the largely quencher-accessible fraction. HP in DPPC (●——●), in DPPC-Chol (○——○) and DPPC-Card (▲——▲) liposomes.

for the readily and poorly accessible fractions (Figs. 3 and 4 and Table I).

The $K_q$ values of the largely quencher-accessible porphyrin fractions are of the same order of magnitude as those reported for porphyrins freely dissolved in aqueous solutions ($K_q = (2.3–2.6) \cdot 10^{11}$ $M^{-1}$ $s^{-1}$ [18]); thus, this population can be considered as being localized in some regions of the outer monolayer, relatively exposed to the external solvent. On the other hand, the drastic drop of the $K_q$ values for the poorly quencher-accessible fractions suggest a deep burial of porphyrin

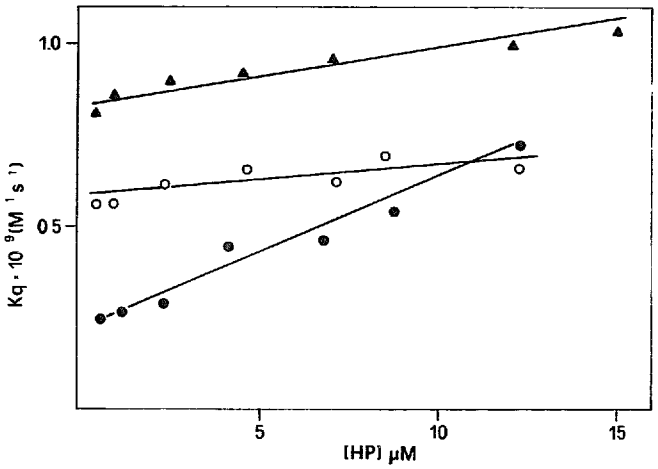


Fig. 4. Dependence of the $K_q$ value on the HP concentration for the poorly quencher-accessible fraction. Symbols are as in Fig. 3.

TABLE I

*Fluorescence quenching parameters of PP in liposomes*

| PP concn. (μM) | Medium | | | |
|---|---|---|---|---|
| | DPPC | DPPC + Chol | DPPC + Card | |
| | $K_q \times 10^{-9}$ (a) ($M^{-1} s^{-1}$) | $K_q \times 10^{-9}$ (a) ($M^{-1} s^{-1}$) | $K_q \times 10^{-9}$ (b) ($M^{-1} s^{-1}$) | $f_a$ |
| 0.5 | 0.22 | 0.21 | 0.22 | 1.00 |
| 1.0 | 0.33 | 0.40 | 0.30 | 1.00 |
| 2.5 | 0.34 | 0.42 | 13.60 | 0.09 |
| 5.0 | 0.32 | 0.39 | 15.60 | 0.15 |
| 7.5 | 0.31 | 0.38 | 14.00 | 0.27 |
| 12.0 | 0.31 | 0.39 | 11.30 | 0.24 |

[a] Linear plots ($f_a = 1.0$) are obtained for all concentrations.

[b] At [PP] ≥ 2.5 μM, the Stern-Volmer plots are heterogeneous. The $K_q$ values, calculated from the linear portion of the $MV^{2+}$-titration curves at high quencher concentrations, range between $0.43 \cdot 10^9$ and $0.51 \cdot 10^9$ $M^{-1} s^{-1}$.

in the internal regions of the liposomal structure, hence hindered from the contact with the quencher.

On the basis of the fluorescence quenching parameters, HP and PP appear to be mostly partitioned in the inner phospholipid monolayer ($f_a \leq 10\%$). Only in the presence of cardiolipin, at concentrations higher than 5 μM, does a significant fraction of both porphyrins ($f_a \cong 30\%$) distribute into the external monolayer (Figs. 3 and Table I).

The fluorescence quenching data described above can provide only limited information about the local microenvironments of HP and PP. Actually, each phospholipid monolayer will exhibit a variety of organizational domains depending on the bilayer asymmetry, lateral phase separation, headgroup interactions and

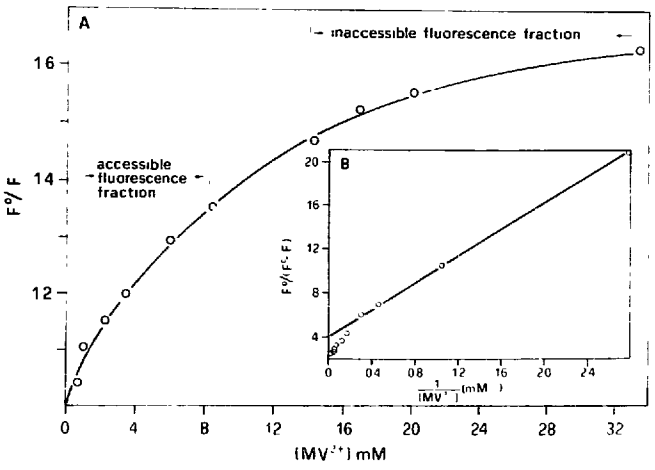


Fig. 5. Selective quenching by $MV^{2+}$ of the fluorescence of 15 μM PP in DPPC-Card liposomes. The data al low quencher concentrations (A) have been treated according to the modified Stern-Volmer equation (inset B), as described in Materials and Methods, in order to obtain the quenching parameters of the largely quencher-accessible fraction. The $K_q$ value for the poorly quencher-accessible fraction has been obtained from the slope of the curve at high $MV^{2+}$ concentrations.

lipid–nonlipid interactions [19]. Different localizations of the two porphyrins in both monolayers can be already deduced from slight changes in their $K_q$ values in the same experimental conditions: in particular, the accessible fraction of HP in DPPC-Card liposomes shows $K_q$ values 5-times higher than those obtained for PP (Fig. 3, Table I) indicating a greater proximity of the porphyrin to the external medium. Moreover, the porphyrin distribution should change, at least for HP, as a function of the porphyrin concentration and the nature of the liposome (Figs. 3 and 4).

Fluorescence polarization results, performed at selected porphyrin concentrations in the three types of liposomes, help us to get some insight on the HP and PP localization in the lipid medium. Figs. 6, 7 and 8 show the changes of fluorescence polarization at increasing temperatures for HP and PP at 0.5, 5 and 12 μM, respectively. On the basis of the quenching data previously discussed, the polarization results mainly reflect the situation of the porphyrin population incorporated into the inner phospholipid monolayer of the different liposomes, except for the case of high HP and PP concentrations in DPPC-Card mixed vesicles.

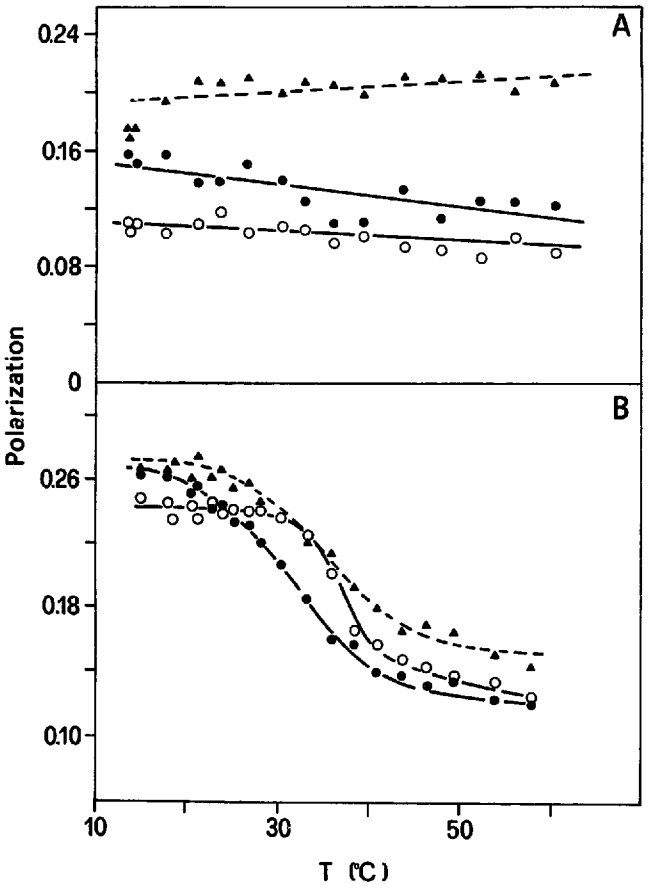


Fig. 6. Excitation fluorescence polarization of 0.5 μM HP (A) and 0.5 μM PP (B) as a function of the temperature. Porphyrins are in DPPC (○), DPPC-Chol (▲) and DPPC-Card (●) liposomes. Emission λ was set at 626 nm for HP and 636 nm for PP. Polarization was observed at 520 nm.

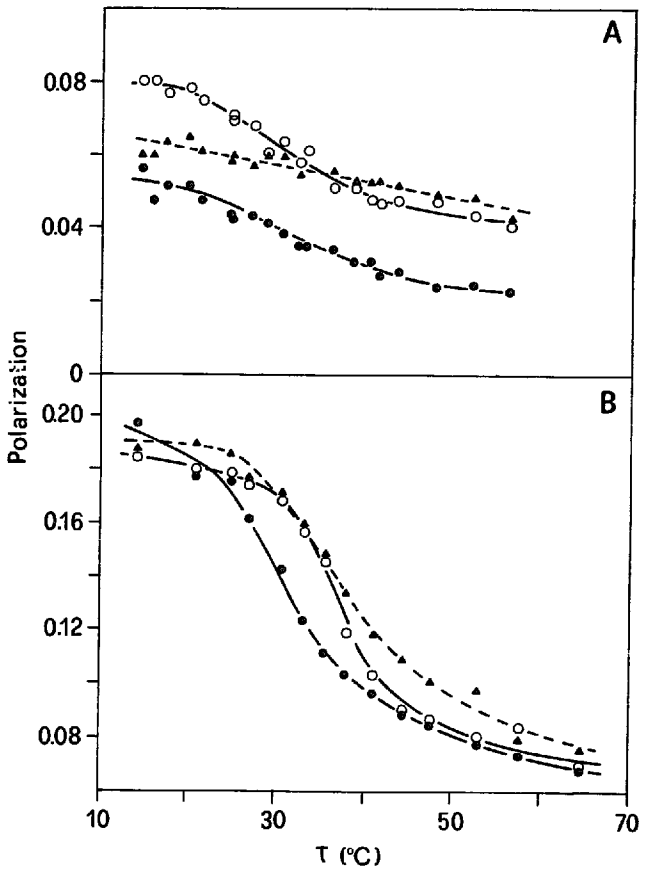


Fig. 7. Excitation fluorescence polarization of 5 μM HP (A) and 5 μM PP (B) as a function of the temperature. Experimental conditions and symbols are as in Fig. 6.

The polarization plots as a function of temperature for PP show a trend typical of the gel-liquid phase transition of the lipids. The transition mid-point is at 38°C for DPPC liposomes, slightly below that typical for DPPC lipids (41.5°C), probably as a consequence of PP incorporation. Addition of 20 mol % of cholesterol has a weak fluidifying effect: $T_c = 35$°C (see Figs. 6B–8B). It must be pointed out that an appropriate amount of cholesterol has been chosen in order to avoid drastic changes of the liposome main properties: at Chol concentration lower than 33 mol % of total lipid moles, no effect is observed on the size of liposomes and the phase transition is not inhibited [20]. Moreover, cholesterol is homogeneously distributed between the inner and outer monolayers [21]. As deduced from the polarization data of PP, addition of cardiolipin to DPPC liposomes leads to a strong increase of the lipid fluidity, the $T_c$ for the phase transition shifting to 31°C (Figs. 6B–8B).

Similar to the fluorescence quenching data, the strong degree of polarization suggests that PP is deeply embedded into the hydrophobic core of the lipid bilayer in all three types of liposomes. Likely, at very low concentrations (0.5 μM), PP occupies an inner apolar domain rather close to the internal head groups, which is particulary rigid owing to the high curvature of the

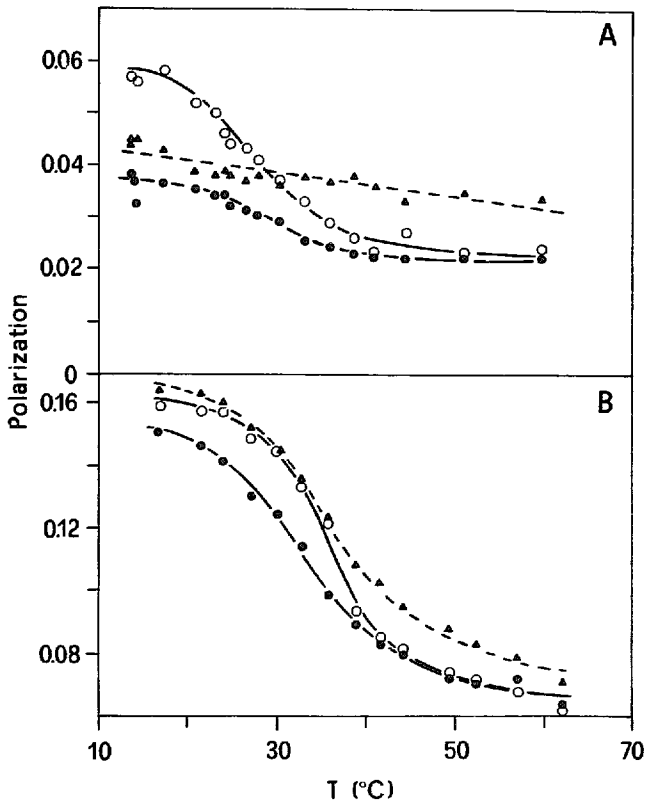


Fig. 8. Excitation fluorescence polarization of 15 μM HP (A) and 15 μM PP (B) as a function of the temperature. Experimental conditions and symbols are as in Fig. 6.

vesicle. This agrees with the very low $K_q$ value observed for this system (Table I), indicative of a large interaction distance between the quencher and the fluorophore. At higher PP concentrations, more flexible domains of the inner phospholipid monolayer become populated, as suggested by the increase of the $K_q$ value for the masked PP fraction (Table I) and the decrease of fluorescence polarization (Figs. 7B and 8B). A concentration-dependent depolarization effect might also occur: however, the constancy of the $K_q$ values in the 2–13 μM range indicates a homogeneous distribution of PP inside the inner monolayer thus limiting the contact between molecules. The similar behaviour in the presence of Chol or Card demonstrates that PP homogeneously dissolves into the hydrophobic part of these liposomes, irrespective of their lipid composition.

Fluorescence polarization data of HP suggest a quite different environment for this porphyrin in the inner lipid monolayer. The polarization values are noticeably lower than those obtained for PP at all concentrations used thus indicating an interaction of HP with relatively flexible domains. A limiting situation is shown at a HP concentration of 0.5 μM (Fig. 6A): no phase-transition is observed for all three types of liposomes. This fact can be justified only by supposing that HP binds to the polar heads of the lipids through one or two carboxylate groups leaving the rest of the molecule dissolved into the internal aqueous compartment. This particular HP accommodation at the inner lipid/water interface cannot be influenced by the variations of the physical state of the lipid chains. Such an interaction mode of HP with liposomes was already hypothesized by Brault [22]. The orientation mode of the cholesterol molecules in the bilayer leads to a partial immobilization of those regions of the hydrocarbon chains closest to the polar headgroups [20]: this can be reflected in the higher value of polarization degree for 0.5 μM HP incorporated into DPPC-Chol vesicles (Fig. 6A). The effect appears to be smaller for HP in DPPC-Card liposomes.

Due to the tendency of HP to localize in these specific surroundings, a drastic depolarization occurs at high porphyrin concentrations. This effect is much more pronounced in the presence of cholesterol and cardiolipin. Likely, the insertion of the Chol hydroxyl groups close to the polar headgroups of the phospholipid molecules determines an overcrowding of HP molecules in a more limited space. A similar effect may occur with cardiolipin. At high HP concentrations (Figs. 7A and 8A), the lipid transition phase is present for DPPC-Chol and DPPC-Card mixed vesicles. This suggests that at increasing HP concentrations, most of the molecules orient differently in the inner lipid monolayer: the ionized propionic chains still interact with the polar heads of the membrane while the hydrophobic core lies in the lipid phase. Correspondingly, the increase of the $K_q$ values at increasing HP concentrations (Fig. 4) indicates a gradually closer proximity of the HP molecules to the quencher. It is interesting to notice that the $T_c$ for the phase transition of DPPC lipids, as monitored by the temperature-dependent polarization changes of HP, is only 31°C. Since the lipid domains with higher curvature show lower $T_c$ values, as observed by Michels et al. [23] for dimirystoylphosphatidylcholine (DMPC) liposomes, our observations support what previously suggested for the localization of HP at the inner lipid/water interface.

HP incorporated into DPPC-Chol mixed liposomes does not monitor any transition phase, even at high concentrations (Figs. 7A and 8A). Thus, HP shows no tendency to dissolve into cholesterol-rich regions. The constancy of the $K_q$ values (Fig. 4) confirms that, even at high concentrations, HP molecules occupy the specific domain of the inner lipid monolayer strictly in contact with the water compartment.

The limited information available from the $K_q$ values of the largely quencher-accessible porphyrin fraction (Figs. 3 and Table I) indicate that HP and PP distribute into the outer lipid monolayer analogously to what observed for the inner monolayer. The fraction of PP molecules in the outer monolayer accomodates into the hydrophobic core of DPPC-Card vesicles (the $K_q$ values are 5-times lower than the corresponding values for HP) and gives an homogeneous distribution (the

constancy of the $K_q$ value in the whole range of concentrations is an indication that $K_q$ weights the average of the different $K_q$ values of the various lipid domains of the outer monolayer). In contrast, HP populates outer lipid domains which are more accessible to the solvent (higher $K_q$ values) (Fig. 3). Upon increasing HP concentrations, the interaction involves also more internal lipid regions, as deduced by the decreased values of $K_q$. HP in DPPC-Chol liposomes, however, shows no modification of the overall distribution.

## Conclusions

The data obtained in the present paper suggest that the distribution of porphyrins in liposomal vesicles is a complex process, which is controlled by a variety of factors, including the degree of hydrophobicity imparted by the side chains protruding from the tetrapyrrolic macrocycle, the acid strength of the carboxylic groups, and the composition of the liposome.

Thus, the preferential interaction of HP with the polar heads of the phospholipid bilayer appears to be due to the combined effect of the presence of two relatively polar secondary alcoholic functions in the peripheral substituents and the extensive ionization of the two propionic groups. The latter are characterized by p$K$ values of 5.0 and 5.5 in aqueous solution; the interaction of HP with the vesicles shifts such p$K$ values upwards by about 1 pH unit [24], hence over 90% deprotonation occurs at pH 7.4 used in our experiments. A similar shift of the propionic p$K$ value to about 6.5 was observed upon incorporation of PP into neutral surfactant micelles [25], hence the degree of ionization of this porphyrin in the liposomes should be closely similar to that of HP. The deeper burial of PP can thus be ascribed to the larger hydrophobicity caused by the replacement of two hydroxyethyl substituents with two vinyl groups.

This interpretation is not in contrast with the results obtained for HP at high concentrations: though some molecules are oriented towards inner areas of the liposomes, HP is still preferentially localized at the water/lipid interface.

A peculiar finding is the low affinity of HP for cholesterol-rich domains of the vesicles. This would suggest some degree of specificity in the interaction of porphyrins with lipid systems. Therefore, the concentration of a porphyrin may appreciably change in different regions of a membrane.

This finding can have profound implications for achieving a 'fine tuning' of the photosensitization process. Since the overall photoprocess occurs within a very limited spatial range around the photosensitizer binding site in a cell owing to the short lifetime and high reactivity of the photogenerated transients [26], the possibility exists to modulate the nature and number of photomodified sites through an appropriate choice of the porphyrin and its concentration. Thus, cholesterol is known to be a critical target of porphyrin-photosensitized membrane damage [27], hence the level of its photomodification can alter both the efficiency and the mechanism of cell killing. Experiments are in progress in our laboratory in order to test the feasibility of such approach toward selectivity of photosensitization by studying the PP- and HP-photosensitization of more complex biological systems.

## References


1 Spikes, J.D. and Jori, G. (1987) Lasers Med. Sci. 2, 3–15.
2 Zhou, C.N. (1989) J. Photochem. Photobiol. B Biol. 3, 299–318.
3 Svaasand, L.O., Boerslid, T., Oeveraasen, M. (1985) Lasers Surg. Med. 5, 598–602.
4 Dougherty, T.I. (1984) CRC Crit. Rev. Oncol. Hematol. 2, 83–116.
5 Kessel, D. (1984) Photochem. Photobiol. 39, 851–859.
6 Jori, G. (1990) Photochem. Photobiol. 52, 439–443.
7 Jori, G. and Spikes, J.D. (1984) in Topics in Photomedicine (Smith, K.C., ed.), pp. 183–318, Plenum Press, New York.
8 Schmid, R., Schwarz, S. and Watson, C.J. (1954) Arch. Intern. Med. 93, 167–190.
9 Garbo, G.M., Kramer, J.B., Keck, R.W., Selman, S.H. and Kreimer-Birnbaum, M. (1985) Anal. Biochem. 151, 70–81.
10 Dougherty, T.I. (1987) Photochem. Photobiol. 45, 879–889.
11 Margolis, L.B. (1988) in Liposomes as Drug Carriers (Gregoriadis, G., ed.), pp. 75–111, Wiley, Chichester.
12 Kremer, J.M.H., De Esker, M.W.J., Pathmamanohoran, C. and Wiersema, P.H. (1977) Biochemistry 16, 3932–3935.
13 Lakowicz, J.R., Gratton, E., Cherek, H., Maliwal, B.P. and Lackzo, G. (1984) J. Biol. Chem. 259, 10967–10972.
14 Eftink, M.R. and Ghiron, C.A. (1976) J. Phys. Chem. 80, 486–493.
15 Lehrer, S.S. (1971) Biochemistry 10, 3254–3263.
16 Andreoni, A., Cubeddu, R., Jori, G., Laporta, P. and Reddi, E. (1983) Z. Naturforsch. 38c, 83–89.
17 Rodgers, M.A.J. (1985) in Photodynamic Therapy of Tumours and other Diseases (Jori, G. and Perria, C., eds.), pp. 21–35, Libreria Progetto, Padova.
18 Ricchelli, F., Jori, G., Moreno, G., Vinzens, F. and Salet, C. (1990) J. Photochem. Photobiol. B Biol. 6, 69–77.
19 Williams, B.W. and Stubbs, C.D. (1988) Biochemistry 27, 7994–7999.
20 Machy, P. and Leserman, L. (1987) in Liposomes in Cell Biology and Pharmacology (Machy, P. and Leserman, L., eds.), pp. 7–13, John Libbey Eurotext/INSERM, London, Paris.
21 Ford, W.E. and Tollin, G. (1984) Photochem. Photobiol. 40, 249–259.
22 Brault, D. (1990) J. Photochem. Photobiol. B Biol. 6, 79–86.
23 Michels, B., Fazel, N. and Cerf, R. (1989) Eur. Biophys. J. 17, 187–190.
24 Brault, D., Vever-Bizet, C. and Le Doan, T. (1986) Biochim. Biophys. Acta 857, 238–250.
25 Savitski, A.P., Vorobyova, E.V., Berezin, I.V. and Ugarova, N.N. (1981) J. Colloid Interface Sci. 84, 175–181.
26 Moan, J. (1990) J. Photochem. Photobiol. B Biol. 6, 343–345.
27 Girotti, A.W. (1990) Photochem. Photobiol. 51, 497–509.