

INTERACTION OF METALLOPORPHYRINS WITH LIPID BILAYERS, A CALORIMETRIC STUDY

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The interaction of three metalloporphyrins, containing manganese, iron and cobalt atoms, with lipid bilayers composed of neutral (DPPC) or charged (DMPG) phospholipids were studied by means of scanning differential calorimetry. We found only minute effects exerted by studied compounds on DPPC, while phase transitions of charged DMPG were seriously affected by porphyrins. Analysis of experimental data revealed that due to the electrostatic interactions DMPG bilayers are perturbed not only in the polar head group region. Putative rearrangements of the polar heads packing affects also the acyl chain region of this lipid bilayer. Perturbation of DMPG polar heads induced by porphyrin in complex with manganese atoms is bigger than that induced by other porphyrins.

INTRODUCTION

Porphyrins, quite often in the form of complex with metal atoms, play crucial roles in many biological processes and applications, starting from the photosynthesis up to the photodynamic therapy (PDT) (Pandey *et al.*, 2006). In this last case porphyrins are used as photosensitizing agents responsible for light-induced damage of target cells or tissues. Most of the porphyrins used in PDT exert their photoactivity after association with the target structures – mainly biological membranes (Richelli, 1995). Binding with membranes and aggregation enhance porphyrin photoactivity and improve its efficiency (Richelli *et al.*, 1998). Since singlet oxygen can diffuse on only a very short distance before its decay, it is very important to co-localize the photosensitizer molecules and target objects – lipids or membrane proteins (Brohnstein *et al.*, 2004). Interactions with cellular membranes are also responsible for the increased uptake of the photosensitizer molecules by cancer cells (Hahn *et al.*, 2006).

In present paper, using calorimetry, we investigated the interactions of three cationic metalloporphyrins with different types of lipids (charged or neutral). The chemical structure of studied porphyrins was identical and they differed exclusively in type of metal atom (Fe, Co, Mn) present in the center of porphyrin ring.

MATERIALS AND METHODS

Two synthetic lipids: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphatidylglycerol (DMPG), both purchased from Sigma-Aldrich (Poznań, Poland) were used in microcalorimetric measurements without any further purification. Metalloporphyrins: [Fe{T(4-N-MePy)P}]Cl₅, [Co{T(4-N-MePy)P}]Cl₅ and [Mn{T(4-N-MePy)P}]Cl₅ were purchased from Mid-Century Chemicals (Posen, IL, USA). Chemical structure of these compounds is presented in Fig. 1, hydrophilic character of these compounds was confirmed by their very good solubility in water.

Calorimetric samples were prepared by hydrating 2 mg of lipid using 20 µl of the solution of appropriate metalloporphyrin in 20 mM Tris-HCl buffer (150 mM NaCl, 0.5 mM EDTA, pH = 7.4) buffer. Concentration of metalloporphyrin in solution was chosen to obtain the desired porphyrin/lipid molar ratio. Samples were heated to the temperature 10 °C higher than the transition temperature of the used lipid and vigorously shaken until the homogeneous mixture was obtained. The mixtures were transferred to the aluminum pans and sealed. Samples were scanned immediately after preparation. Calorimetric measurements were performed using Rigaku calorimeter, which was partially rebuilt in our laboratory. For each porphyrin/lipid molar ratio two separate samples were prepared, each sample was scanned four times. Presented data are the average valu-

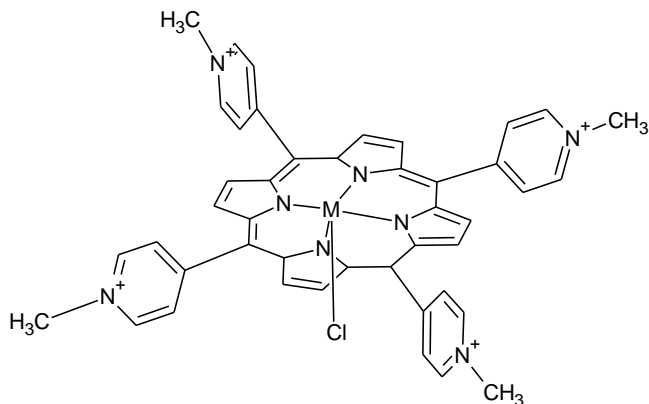


Fig. 1. Chemical structure of dissociated studied metalloporphyrins (M = Fe, Co, Mn).

es of eight measurements. The thermograms were stored on the computer hard disk and analyzed off-line using software developed in our laboratory, scanning rate was 1,25 °C/min. Pure water was used as the reference. Thermogram analysis allowed us to determine transition temperature (T_m), enthalpy change during the transition (ΔH) and transition width at the transition peak half-height ($\Delta T_{1/2}$).

RESULTS AND DISCUSSION

The effects of the metalloporphyrin presence in studied mixtures differed depending on the type of the lipid used in experiment. For DPPC we observed only minute changes in the thermal behavior of this lipid when it was mixed with any of the studied metalloporphyrins at

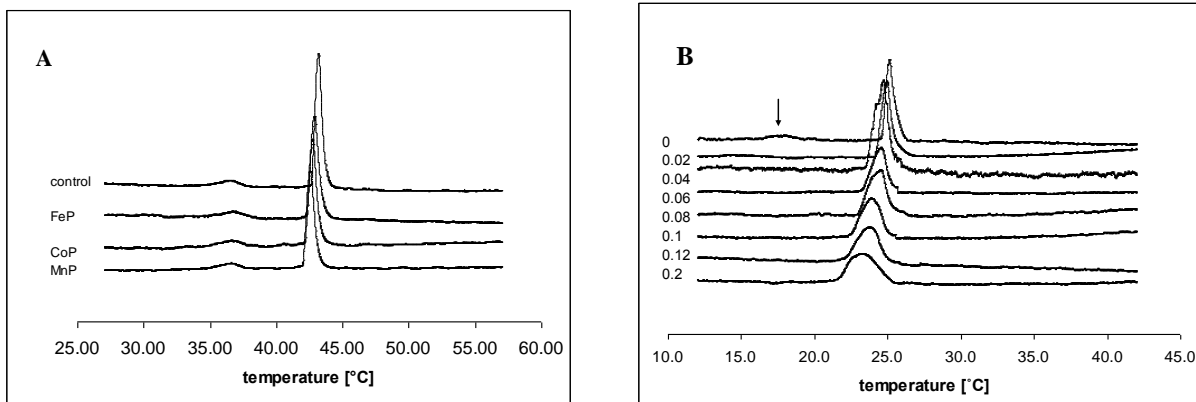


Fig. 2. Example thermograms of: A – DPPC mixed with three studied metalloporphyrins at 0,2 porphyrin/lipid molar ratio, B – DMPG mixed with $[Mn\{T(4-N-MePy)P\}]Cl_5$ at different porphyrin/lipid molar ratios. The top thermogram represents pure lipid. An arrow in panel B points to the DMPG's pretransition (poorly visible due to the scale of figure).

porphyrin/lipid molar ratio 0,2. First of all the DPPC's pretransition was not significantly affected by metalloporphyrins. Due to the broadness of transition peaks precise determination of transition temperature was not possible but slight increase of this parameter in the presence of porphyrins could be seen in Fig. 2A. This transition is sensitive to any bilayer packing perturbations (Heimburg, 2000) and therefore its presence in lipid/porphyrin mixtures can be treated as an indicator for the absence of any important lipid

molecule's packing alterations. Possible increase of pretransition temperature could point to the localization of porphyrins in the polar head region of DPPC bilayers. As can be seen in Fig. 2A metalloporphyrins caused slight decrease of the DPPC's main transition temperature ($[Fe\{T(4-N-MePy)P\}]Cl_5$ – 0,17°C, $[Co\{T(4-N-MePy)P\}]Cl_5$ – 0,39°C and $[Mn\{T(4-N-MePy)P\}]Cl_5$ – 0,57°C) but enthalpy and transition peaks width were not affected. According to Przyczyna *et al.* (2002) this small decrease of main transition

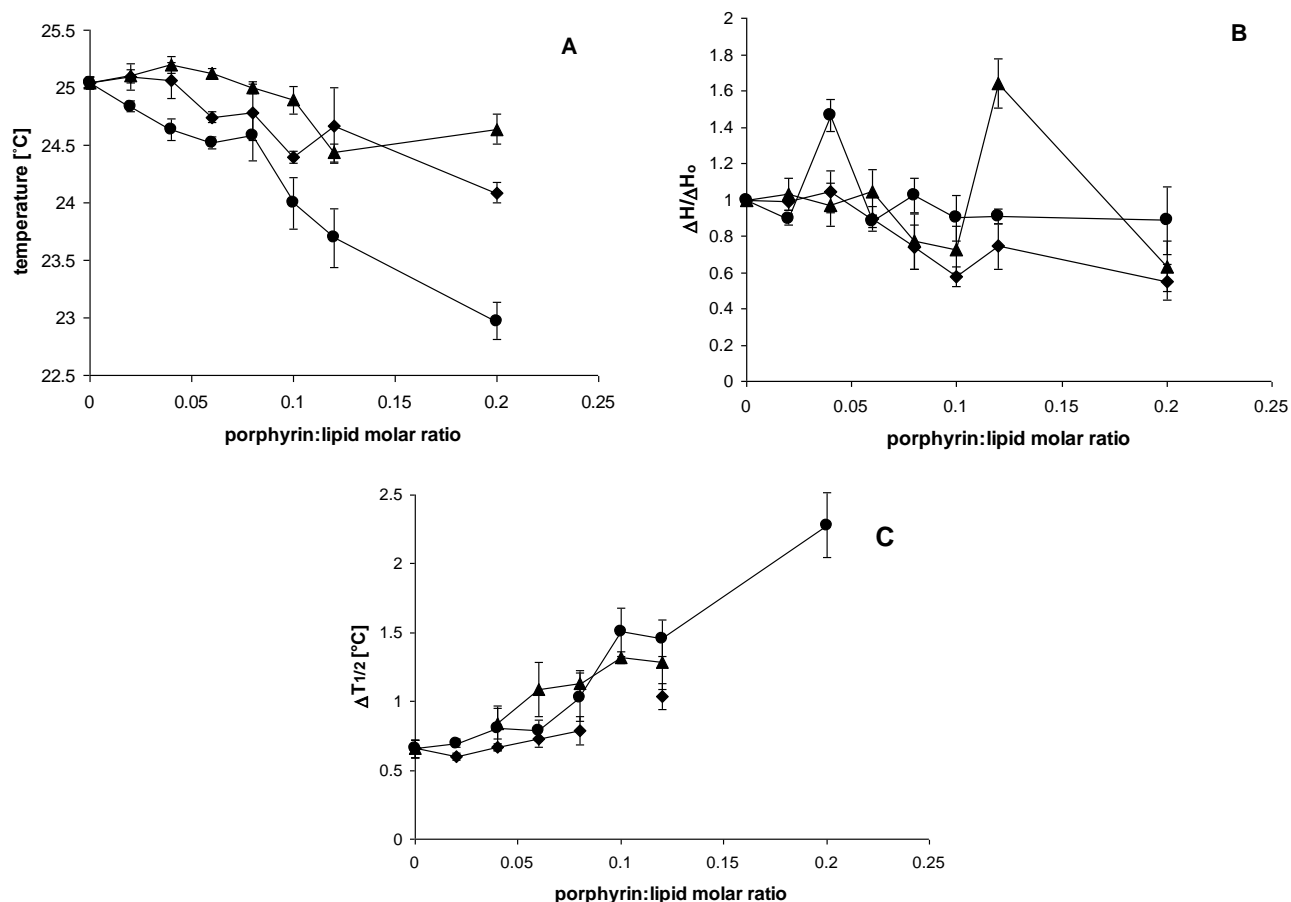


Fig. 3. Alteration of DMPG main phase transition parameters induced by the presence of metalloporphyrins: ● – [Mn{T(4-N-MePy)P}]Cl₅, ◆ – [Co{T(4-N-MePy)P}]Cl₅, ▲ – [Fe{T(4-N-MePy)P}]Cl₅. A – transition temperature, B – relative transition enthalpy, C – transition width at peak half-height. Determination of $\Delta T_{1/2}$ for porphyrin:lipid molar ratio 0,1 of [Co{T(4-N-MePy)P}]Cl₅ as well as for 0,2 of [Co{T(4-N-MePy)P}]Cl₅ and [Fe{T(4-N-MePy)P}]Cl₅ was not possible due to the bad shapes of thermograms.

temperature could be rather related to the bilayer hydration changes induced by the presence of dissociated Cl⁻ ions than to the interactions between positive charges carried by porphyrin molecules and lipid headgroups. Cations usually affect the transition temperature to much bigger extent and they increase the main transition temperature of DPPC (Koyanova & Caffrey, 1998).

Contrary to neutral DPPC the thermal behavior of negatively charged DMPG was significantly perturbed by the presence of metalloporphyrins (see the example thermograms in Fig. 2B). As can be seen in this figure the DMPG's pretransition was abolished by the lowest of tested in experiments [Mn{T(4-N-MePy)P}]Cl₅/lipid molar ratios (0,02). The same result was recorded also for other two studied metalloporphyrins. The effects exerted by porphyrins on the parameters of DMPG main

phase transition are presented in Fig. 3. The temperature of DMPG main transition was remarkably decreased in a concentration-dependent manner by all studied compounds. The effects of Co- and Fe-substituted porphyrins were less pronounced than that of Mn-substituted one, which at the highest of studied molar ratios to lipid (0,2) decreased T_m by almost 2°C (Fig. 3A). The concentration-dependent increase of DMPG's transition width was observed also for all studied compounds. Despite that determination of $\Delta T_{1/2}$ was not possible for the highest molar ratios of Co- and Fe-substituted porphyrins, analyzing Fig. 3C one can conclude that [Mn{T(4-N-MePy)P}]Cl₅ broadened the transition peaks most effectively. Also the enthalpy of main transition was affected by the presence of metalloporphyrins in studied mixtures. Neglecting two points shown in Fig. 3B (molar ratios 0,04 for Mn-

substituted and 1,2 for Fe-substituted porphyrins), which might be the result of cumulating experimental errors, the gradual decrease of ΔH can be seen in this figure. Opposite to T_m and $\Delta T_{1/2}$ the transition enthalpy was less significantly decreased by $[\text{Mn}\{\text{T}(4\text{-N-MePy})\text{P}\}]\text{Cl}_5$. In our opinion the described above effects can be attributed to the electrostatic attraction between the positive charges carried by metalloporphyrins and negative charges of DMPG polar head groups. Due to their charges porphyrin molecules can not penetrate the hydrophobic core of bilayer and they stay close to the polar region. Such localization was postulated by Richelli *et al.* (1990, 1991) in the study on the interactions of hematoporphyrin and DPPC liposomes. Similar conclusions were drawn also by Rich *et al.* (1992) for hematoporphyrin derivative-phosphatidylcholine systems (monolayer and bilayer) at neutral pH. pH-dependent depth of membrane penetration by metalloporphyrins was also reported by Bronshtein *et al.*, (2005). The rigid structure of porphyrin ring presumably forces lipid head groups to adopt the spatial arrangement in which they fit to the distribution of charges in porphyrins. Alteration of lipid head groups packing affects also the hydrophobic region and weakens the interactions between lipid molecules. In calorimetric measurements such perturbation is observed as decrease of main phase transition temperature and transition enthalpy accompanied by the peak broadening (decrease of transition's cooperativity). Membrane perturbation induced by different porphyrins was observed in several laboratories. For example glycosylated tetraphenyl-porphyrins, depending on their structure, can penetrate lipid bilayer to the different depth and induce loosening of membrane structure (Voszka *et al.*, 2007). The strong membrane destabilization induced by small amounts of chlorophyll a was recently found by Vladkova *et al.*, (2010).

The type of metal present in complex with porphyrin molecule seems to possess also some importance. Presence of manganese seems to affect to greater extent the main phase transition parameters related with the properties of polar bilayer region (T_m and $\Delta T_{1/2}$) (Jain & Wu, 1977). Relatively smaller changes induced by this metal in transition enthalpy might suggest that lipid acyl chains are not profoundly affected. Iron and cobalt present in complexes perturb polar region of lipid bilayer less effectively but their impact on acyl chains seems to somehow bigger than that of Mn.

Summarizing the presented above results we can conclude that interactions between charged metalloporphyrins and lipids are governed by the electric nature of involved molecules. Neutral, not charged lipids are not affected by the presence of porphyrins. Contrary the charged lipids attract metalloporphyrin molecules and as a result of interaction the polar as well as hydrophobic parts of lipid bilayer

are perturbed. The type of metal atom present in complex with porphyrin molecule is also important.

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