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Determination of *n*-octanol/water partition and membrane binding of cationic porphyrins

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Abstract

Porphyrin and their derivatives are being systematically studied as photosensitizers for photodynamic therapy. The ability to predict their membrane partition properties is of key importance to unveil their in vivo activity and applications. Several *n*-octanol/water partition coefficients ($\log P_{OW}$) of porphyrin derivatives have been reported in the literature but large discrepancies have been observed. Reproducible and reliable $\log P_{OW}$ data for a series of 20 cationic *meso*-phenyl(pyridyl)porphyrin derivatives were determined by correlating $\log P_{OW}$ with the partition coefficients measured in a more adequate *n*-butanol/water system. Linear correlations as a function of the number of positively charged groups bound to the periphery of the porphyrin rings were found within each series. A significant effect of the stereochemistry and nature of the positively charged substituents was also observed, but diminished steadily converging to a similar value in the mono-substituted derivatives. Binding constants to liposomes were shown to be proportional to $\log P_{OW}$, except for the *cis*-isomers of doubly charged porphyrins. The *cis*-isomer presented smaller $\log P_{OW}$ and higher membrane affinity. The effect was explained based on the amphiphilic nature of the *cis*-porphyrin.

Keywords: Partition coefficients; *n*-Octanol/water; *n*-Butanol/water; Cationic porphyrins; Amphiplilic porphyrins; Membrane binding

1. Introduction

Photodynamic therapy (PDT) is a promising therapeutic modality for the treatment of various tumors and nonmalignant diseases, based on the combination of a photosensitizer, which should be selectively concentrated in the target tissue, and irradiation with visible light. The method can be applied in selected areas promoting photodamage and subsequent cell death (Merchat et al., 1996; Ochsner, 1997; Reddi et al., 2002; Schmidt-Erfurth and Hasan, 2000; Smijs and Schuitmaker, 2003; Tardivo et al., 2005). The synthesis of new photosensitizers with strong absorptions at wavelengths longer than 650 nm and high quantum efficiency of singlet oxygen ($^1O_2$) generation (Araki et al., 2003; Engelmann et al., 2002, 2004; Hudson et al., 2005; Reddi et al., 2002) continue to be one of the main goals in the area of PDT. However, these photophysical parameters are not the only aspects to be considered. In fact, membrane partition, tissue accumulation and cyto-localization are of uppermost importance to define the success of the PDT treatments (Dummin et al., 1997; Ricchelli et al., 2005).

The interaction of photosensitizers or other drugs with biological systems can be evaluated by the partition coefficients (*P*), i.e. the ratio of the concentration of a compound in a biphasic organic/aqueous system. It has long been known that the *n*-octanol/water system is a good mimic of the water/membrane interface (Collander, 1951), and invariably the so called $\log P_{OW}$ is evaluated by measuring the concentrations of a chosen species in both phases. Accordingly, $\log P_{OW}$ has been extensively utilized to predict the relative tendency of compounds to interact/incorporate in biological membranes (Hansch and Fujita, 1964; Schoenwald and Huang, 1983).

There are two most common methods employed to measure $\log P_{OW}$. The first one is based on measurement of retention times in a reverse phase HPLC column saturated with *n*-octanol, using water as mobile phase. Log P_{OW} can be determined using

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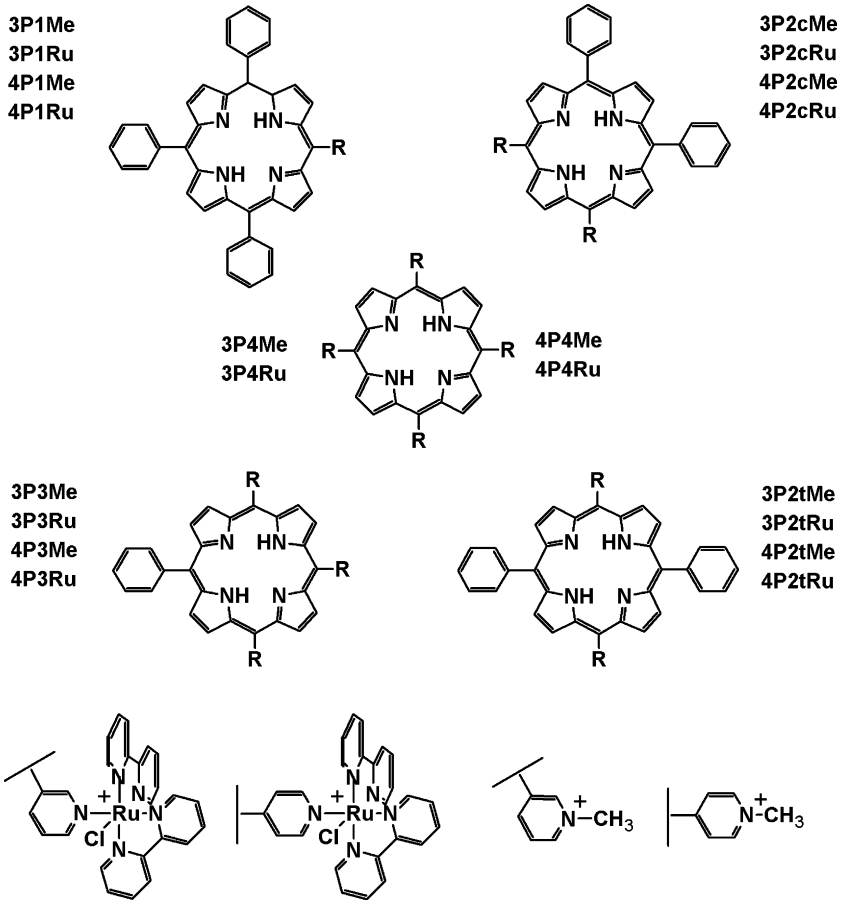
a calibration curve prepared with appropriate standard compounds. This is a precise method but it is limited to relatively lipophilic species, more specifically those with $\log P_{OW}$ in the 0–6 range (Braumann, 1986).

The shake-flask method does not require standard compounds and is based on the direct determination of equilibrium partition concentrations of a compound in a biphasic system, namely *n*-octanol and water. The determinations can be carried out by any analytical method such as gas chromatography and UV–vis spectroscopy, which allow the convenient measurement of the concentrations in both phases. Another convenient strategy to evaluate $\log P_{OW}$ is by theoretical calculations using specialized programs such as Pallas 3.1. Generally, these programs give results in good qualitative agreement with experimental data obtained by the above-described methods (Benfenati et al., 2003; Kepczynski et al., 2002; Obata et al., 2005).

Porphyrins are aromatic macrocyclic compounds with potential PDT activity and $\log P_{OW}$ values of many of them have already been published. In particular, Meng et al. (1994) measured $\log P_{OW}$ of a series of *meso*-(4-*N*-methylpyridinium)phenylporphyrins (Scheme 1) and found 1.23, 1.11, −0.05 and −0.35 for 4P2cMe, 4P2tMe, 4P3Me and 4P4Me, respectively. The expected tendency of decrease of $\log P_{OW}$ values as the number of methylpyridinium groups increase is observed. However, there are low agreement in between the measurements carried out by different authors. For example, significantly different values have been published for 4P4Me by Meng et al. (1994) (−0.35), Kepczynski et al. (2002) (−2.24) and Ricchelli et al. (2005) (−2.75), all obtained under similar experimental conditions. Such differences should be due to systematic errors in the measurements because porphyrins have a large tendency to aggregate (Dixon and Steullet, 1998) in solution and/or to adsorb on surfaces, including glass and plastic recipient walls.

We found out that it is very difficult to obtain reproducible results by following their experimental procedures. Furthermore, Meng et al. (1994) evaluated the porphyrin concentration only in the aqueous phase assuming that the difference between the initial and equilibrium concentrations was in the *n*-octanol phase. Consequently, the hypothesis of porphyrin being adsorbed onto the flask walls was completely neglected, but this was shown to be one of the main factors responsible for the discrepant results.

In this article, we describe a strategy that can be used for the determination of $\log P_{OW}$ of water-soluble porphyrins in general. Accordingly, $\log P_{OW}$ for twenty cationic *meso*-phenyl/pyridil-porphyrins compounds (Engelmann et al., 2002; Onuki et al., 1996; Ravanat et al., 1998) (Scheme 1) were measured and shown to be consistent within the series and in qualitative agreement with the results obtained using the



Scheme 1. Structures of *meso*-(3-*N*-pyridyl)phenylporphyrins (3P1, 3P2c, 3P2t, 3P3 and 3P4 (series 3)) and *meso*-(4-*N*-pyridyl)phenylporphyrins (4P1, 4P2c, 4P2t, 4P3 and 4P4 (series 4)) bond to 1, 2 (*cis* and *trans*), 3, and 4 methyl (Me) or $[Ru(bipy)_2Cl]^+$ (Ru) groups through the *N*-pyridyl atoms.

Pallas 3.1 program. Linear relationships between log P_{OW} and the number of positively charged groups around the porphyrin ring in each series were found. For the series of *meso*-(3-*N*-methylpyridinium)phenyl porphyrins (series 3 porphyrins, Scheme 1), log P_{OW} values were also correlated with binding constants to liposomes (K_B^V). Interesting differences between the *cis* and *trans* isomers of doubly charged porphyrins were revealed.

2. Materials and methods

2.1. Chemicals

The *meso*-phenyl(3-*N*-pyridyl)porphyrins and *meso*-phenyl (4-*N*-pyridyl)porphyrins and the derivatives obtained by coordination of $[Ru(bipy)_2Cl]^+$ complexes to the *N*-pyridyl atoms were prepared and purified as described before (Engelmann et al., 2002). The respective methylated porphyrin derivatives were obtained by refluxing the *meso*-phenyl(pyridyl)porphyrins with an excess of methyl tosylate in *N*,*N*′-dimethylformanide (DMF) for 4 h. The tosylate counter-ion was exchanged by Cl^- to improve their solubility in water. Accordingly, the following compounds (Scheme 1) were employed in the experiments: *meso*-mono(3-*N*-methyl-pyridyl)triphenylporphyrin chloride (3P1Me); *meso*-di-*trans*(3-*N*-methyl-pyridyl)diphenylporphyrin dichloride (3P2tMe); *meso*-di-*cis*(3-*N*-methyl-pyridyl) diphenylporphyrin dichloride (3P2cMe); *meso*-tri(3-*N*-methyl-pyridyl)monophenylporphyrin trichloride (3P3Me); *meso*-tetra (3-*N*-methyl-pyridyl)porphyrin tetrachloride (3P4Me); *meso*-mono(4-*N*-methyl-pyridyl)triphenylporphyrin chloride (4P1-Me); *meso*-di-*trans*(4-*N*-methyl-pyridyl)diphenylporphyrin dichloride (4P2tMe); *meso*-di-*cis*(4-*N*-methyl-pyridyl)diphenylporphyrin dichloride (4P2cMe); *meso*-tri(4-*N*-methyl-pyridyl) monophenylporphyrin trichloride (4P3Me); *meso*-tetra(4-*N*-methyl-pyridyl)porphyrin tetrachloride (4P4Me); μ-{(3-*N*-pyridyl)triphenylporphyrin}-{bis(2,2′-bipyridyl)chlororuthenium(II)} (3P1Ru); μ2-{*trans*-di(3-*N*-pyridyl)diphenilporphyrin}-bis{bis(2,2′-bypiridyl)chlororuthenium(II)} (3P2tRu); μ2-{*cis*-di(3-*N*-pyridyl)diphenilporphyrin}-bis{bis(2,2′-bypiridyl) chlororuthenium(II)} (3P2cRu); μ3-{tri(3-*N*-pyridyl)phenilporphyrin}-tris{bis(2,2′-bypiridyl)chlororuthenium(II)} (3P3Ru); μ4-{tetra(3-*N*-pyridyl)porphyrin}-tetrakis{bis(2,2′-bypiridyl) chlororuthenium(II)} (3P4Ru); μ-{(4-*N*-pyridyl)triphenylporphyrin}-{bis(2,2′-bipyridyl)chlororuthenium(II)} (4P1Ru); μ2-{*trans*-di(4-*N*-pyridyl)diphenilporphyrin}-bis{bis(2,2′-bypiridyl)chlororuthenium(II)} (4P2tRu); μ2-{*cis*-di(4-*N*-pyridyl)diphenilporphyrin}-bis{bis(2,2′-bypiridyl)chlororuthenium(II)} (4P2cRu); μ3-{tri(4-*N*-pyridyl)phenilporphyrin}-tris-{bis(2,2′-bypiridyl)chlororuthenium(II)} (4P3Ru); μ4-{tetra-(4-*N*-pyridyl)porphyrin}-tetrakis{bis(2,2′-bypiridyl)chlororuthenium(II)} (4P4Ru).

2.2. Partition coefficients in n-octanol/water

The *n*-octanol/water partition coefficients were measured using a variation of the shake-flask method. The partition coefficients were obtained in *n*-butanol/water (log P_{BW}), where the porphyrins are more soluble, and then correlated with the corresponding values in *n*-octanol/water system by using a calibration curve prepared with well-known standards (Ran et al., 2002). Distilled water and *n*-butanol (from LabSynth) were mixed vigorously for 24 h at 25 °C, to promote solvent saturation in both phases. The solvents were separated and the porphyrin dissolved in the aqueous phase (absorbance ~ 1 at the Soret band). Then, a volume of the water-saturated *n*-butanol was added in order to get a *n*-butanol/water volume ratio of 0.2 (P), 1.0 (P2c), 1.0 (P2t), 4 (P3) and 10 (P4) and vigorously vortexed for 10 min at 25 °C. The mixture was centrifuged at $3 \times 10^3 \times g$ for 1 min and UV–vis absorption spectra of the porphyrins registered in both phases. The value of log P_{OW} can be easily determined by Eq. (1), considering that molar absorptivities are the same in both solvents (confirmed experimentally) and partition is the only responsible for the changes in absorbance. A normalization factor corresponding to the ratio of water and *n*-octanol phase volumes (Dearden and Bresnen, 1988) is also considered in Eq. (1), where A_W and A_B are the absorbances at the Soret band and V_W and V_B are the volumes of aqueous and *n*-butanol phases, respectively. Standard deviations smaller than 5% were obtained considering four independent measurements in each experimental condition.

$$\log P_{BW} = \log\left(\frac{A_B}{A_W}\frac{V_W}{V_B}\right) \quad (1)$$

The same procedure was employed for the standard compounds caffeine, pyridine, cyclohexanone, pyridazine and thiourea. The log P_{BW} measured for these compounds were compared with the respective log P_{OW} found in the literature in order to find the correlation between the log *P* values in *n*-butanol/water and *n*-octanol/water systems. Such correlation

Table 1
Partition coefficients measured in *n*-butanol/water (log P_{BW}) and converted to the *n*-octanol/water system (log P_{OW}) using the equation $\log P_{OW} = 1.55(\log P_{BW}) - 0.54$ (best fitting of the log P_{OW} vs. log P_{BW} of standard compounds)

Compound	Log P_{BW}		Log P_{OW}		Log P_{OW}[a]
	Me	Ru	Me	Ru	Me
3P1	1.95	–	2.49	–	7.31
3P2t	0.69	–	0.53	–	3.31
3P2c	0.42	1.08	0.11	1.13	3.31
3P3	−1.27	−0.28	−2.52	−0.98	−0.68
3P4	−2.49	−1.34	−4.41	−2.63	−4.68
4P1	1.88	–	2.38	–	7.31
4P2t	0.79	1.14	0.68	1.23	3.31
4P2c	0.63	1.06	0.43	1.10	3.31
4P3	−0.56	0.26	−1.41	−0.14	−0.68
4P4	−1.97	−0.63	−3.61	−1.52	−4.68
Caffeine			0.89		0.81[b]
Pyridine			0.76		0.65[b]
Ciclohexanone			0.28		−0.07[b]
Piridazine			−0.11		−0.73[b]
Thiourea			−0.30		−1.02[b]

[a] Calculated using Pallas 3.1 at pH 7.0 and zero ionic strength.
[b] Values found in literature (Ran et al., 2002).

was used to calculate the log P_{OW} (Collander, 1951; Eltayar et al., 1991; Seiler, 1974) from the experimentally determined log P_{BW} for the four series of porphyrins shown in Scheme 1 (Table 1). Log P_{OW} values were also estimated theoretically using the computer program Pallas 3.1, considering pH 7.0 and ionic strength equal to zero.

2.3. Vesicle binding

Multilamelar vesicles with lipid composition of 20% cardiolipin (heart-disodium salt, CL) and 80% 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) were prepared by smooth sonication of lipid films in buffer solutions (5 mM Hepes at pH 7.2). Negatively charged vesicles were used to mimic the negative charges of the majority of biological membranes and in special of the mitochondrial internal membrane (Gabrielli et al., 2004). Lipid concentrations were determined spectrophotometrically (Rouser et al., 1970). Direct measurements of porphyrin partition into liposomes were obtained by centrifugation (the suspensions were centrifuged for 3 min at $1.3 \times 10^4 \times g$ and 25 °C) and separation of bound and free porphyrin species. Titration curves were obtained up to the porphyrin concentrations that saturated all the binding sites of the vesicles. These experiments were performed for the series 3 porphyrins, except for the monocharged species that were not enough soluble in buffer to allow saturation of the vesicle binding sites. Binding constants (K_B^V) were calculated assuming simple *Langmuir* type binding equilibria (Angeli et al., 2000).

3. Results

3.1. Log P_{OW} measurements

The procedure for the determination of log P_{OW} by the classical shake-flask method is straight forward (Dearden and Bresnen, 1988): (i) preparation of a porphyrin solution in *n*-octanol saturated with water or vice-versa; (ii) mixture of a known volume of that solution with a known volume of the other phase and vigorous shaking until partition equilibrium (~10 min, keeping the temperature constant); (iii) separation by centrifugation and measurement of UV–vis spectra of both phases. We exhaustively repeated this procedure using the four series of porphyrins, but unfortunately large experimental errors and irreproducible results were obtained.

The method seems to be very simple and generally is applied without any further consideration by following a procedure similar to that described above. However, problems can arise if the experiments are not carried out diligently. For example, we noticed a decrease in the aqueous phase absorbance of 4P4Me and 3P4Me after addition of *n*-octanol phase, as expected. However, no porphyrin was found in the organic phase even though the molar absorptivity at the Soret band is higher than $2 \times 10^5\ M^{-1}\ cm^{-1}$; and when the flask content was removed and aqueous HCl solution added it became green colored. Consequently, one concluded that the main reason for the low reproducibility is the adsorption or precipitation of variable amounts of porphyrin in the flask walls, made of virtually any available material (glass, silanized glass and plastic were tested). In addition, the problem becomes worse as the number of positively charged *N*-methylpyridinium groups decreased increasing the lipophilicity. A compound should be minimally soluble in both phases in order to get reliable results by that method. However, this does not seem to be the case when the *meso*-phenyl(pyridyl)porphyrin derivatives in *n*-octanol/water biphasic system is considered.

Accordingly, we tried to determine the log P_{OW} values by liquid chromatography (Poole and Poole, 2003) using a reverse-phase C18 column and methanol as eluent. Unfortunately, the cationic porphyrins apparently have very high affinity for the stationary phase silane matrix and retention times did not follow the same trend of the standard compounds, hampering the use of this method.

The problem was solved by utilizing the approach described by Collander (1951) and confirmed by Seiler (1974) and Eltayar

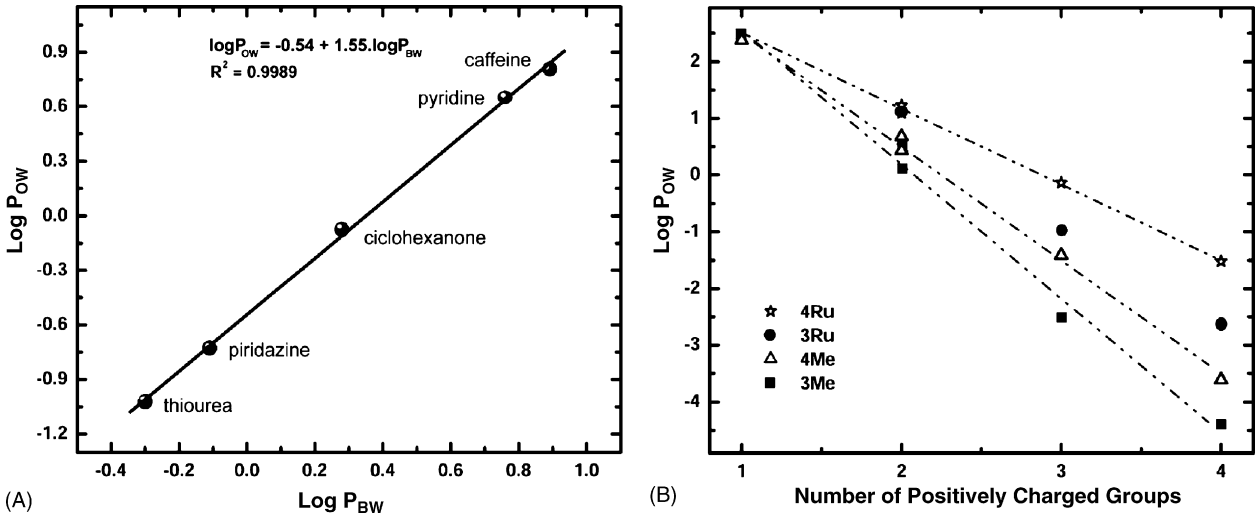


Fig. 1. (A) *n*-Octanol/water partition coefficients (log P_{OW}) as a function of the respective *n*-butanol/water partition coefficients (log P_{BW}) of selected standard compounds (Ran et al., 2002). (B) Log P_{OW} as a function of the number of positively charged substituents for 20 cationic *meso*-phenyl(pyridyl)porphyrin derivatives.

et al. (1991). They have shown that the partition coefficient data obtained in similar solvent/water systems can be correlated by a linear relationship $\log P_{OW} = a \log P_{sol/W} + b$, where $\log P_{sol/W}$ is the $\log P$ measured in a biphasic solvent mixture different from *n*-octanol/water. Accordingly, partition coefficients of the standard compounds caffeine, pyridine, cyclohexanone, pyridazine and thiourea were determined in *n*-butanol/water and the results plotted as a function of corresponding values reported in *n*-octanol. In this way, the relationship $\log P_{OW} = 1.55(\log P_{BW}) - 0.54$ (Fig. 1) was obtained and used to convert the $\log P_{BW}$ measured for the cationic *meso*-phenyl(pyridyl)porphyrin derivatives into $\log P_{OW}$ values (Table 1, Fig. 1). These data and those calculated by using Pallas 3.1 software show significant differences except for the tetra-methylated species 4P4Me and 3P4Me. In spite of such differences, the general trends are similar. The $\log P_{OW}$ for 4P4Me was measured by several authors, but our results are the closest ones to theoretically predicted values (−3.61 compared with −4.68). However, the program has limitations such that caution should be exercised. For example, the Pallas 3.1 software cannot distinguish the influence of stereochemical effects, for example the *meta* and *para* or *cis* and *trans* isomers.

3.2. Vesicle binding

It is shown in Fig. 2 the plot of $\log P_{OW}$ (left axis expressed as points and a straight line) and binding constant to vesicles (right axis expressed as bars) as a function of the number of positively charged groups around the porphyrin ring. As shown in Fig. 1, $\log P_{OW}$ increases proportionally with the decrease in the number of charges in the porphyrin ring. Similar tendency is observed for the K_B^V values of the 3P4, 3P3, 3P2t porphyrins. Note that for these porphyrins K_B^V increases proportionally with $\log P_{OW}$ (Fig. 2). However, in the case of 3P2c that is amphiphilic, i.e., both charges are at the same side of the molecule, K_B^V is clearly much larger than it would be expected based on $\log P_{OW}$ measurements. This difference must be related with the perfect structural matching of the *cis*-isomer for inclusion into membranes, maximizing the hydrophobic and hydrophylic interactions and consequently the equilibrium constant.

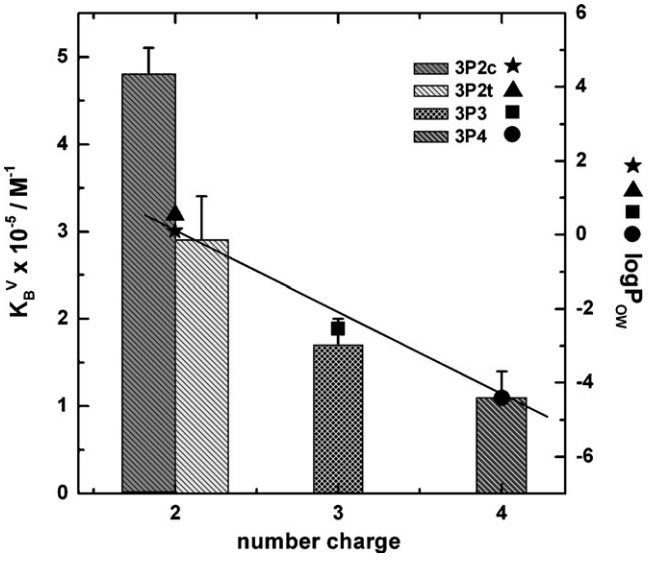


Fig. 2. Log P_{OW} (right axis: expressed as points and fitted line by linear regression) and binding constants to vesicles (K_B^V) (left axis: expressed as bars) as a function of the number of charges in series three porphyrins. Log P_{OW} were extracted from Table 1.

4. Discussion

In general, the $\log P_{OW}$ of cationic *meso*-phenyl(pyridyl) porphyrin derivatives increased as the number of positively charged groups bond to the ring periphery decreased following the order: P4 < P3 < P2c < P2t < P1 (Fig. 1B, Table 1). Clearly, the *meta*-isomers (series 3) exhibit lower $\log P_{OW}$ values than the corresponding *para*-isomers (series 4), as expected for the higher dipole moment induced by the out of plane arrangement of positively charged peripheral groups. This result is reflected in the much higher solubility of series 3 as compared with series 4 cationic porphyrins in water and polar organic solvents.

The nature of peripheral substituents should also influence the partition coefficients. In fact, the substitution of methyl groups by ruthenium complexes increased the lipophilicity because a small methyl group was substituted by two large and hydrophobic bipyridine molecules while keeping the +1 total charge. Consequently, the $\log P_{OW}$ for the corresponding *meso*-phenyl(pyridyl)porphyrin derivatives increased in the following order for analogous species: 3PMe < 4PMe < 3PRu < 4PRu (Table 1). Nevertheless, the differences in $\log P_{OW}$ values decrease steadily as the number of positively charged peripheral groups diminish, tending to converge in the porphyrin derivatives with only one positive charge, no matter the stereochemistry or the nature of the substituents. This result suggests that variations in chemical composition and spatial distribution of peripheral groups tend to be less important in the case of porphyrin derivatives with a relatively large hydrophobic portion and only one hydrophilic head.

In molecular terms $\log P_{OW}$ can be decomposed into a volume term that express mainly solvent cavitation and an intermolecular interaction term that would include solute–solvent intermolecular interactions (Tayer et al., 1992). The smaller effect of substituint in the monocharged species is probably related with the fact that the solvent cavitation term is the only term relevant in these porphyrins. With the increase in the number of charged substituints, there is a stronger effect of the solute–solvent interactions. In general, membrane binding constants follow $\log P_{OW}$ (Fig. 2).

Note also that in the case of di-substituted porphyrins, there is a small (∼7%) but significant difference in the $\log P_{OW}$ values for the *cis* and *trans*-isomers. The *trans* derivatives have larger $\log P_{OW}$ values indicating that they are more soluble in *n*-octanol than the *cis* isomers. However, when membrane binding is considered (Fig. 2) the *cis* isomer binds to the vesicles with affinity 60% larger. We hypothesized that the assymetric distribution of the charges donates an amphiphilic character to the *cis*-porphyrin allowing better packing into the membrane and

maximizing intermolecular interactions with the aqueous and lipid phases.

Besides log P_{OW}, two other properties are important in terms of membrane binding and permeation, i.e., molecular size and hydrogen bonding capacity (Van de Waterbeemd et al., 2001). Both these properties are equal/similar for the *cis* and *trans* isomers, which have different membrane binding affinities (Fig. 2). Therefore, molecular assimetry or amphiphilicity should also be considered by medicinal chemists when designing new drugs.

Membrane binding is a key point for the photodynamic activity and a systematic study is under way involving the correlation of structure, log P_{OW} and degree of membrane interaction with the photodynamic efficiency of those series of cationic porphyrins. Direct correlations between photodynamic efficiency and partition coefficients in mithocondria and erithrocytes were found, except for the amphiphilic *cis*-isomers that systematically showed much higher activity (Engelmann et al., submitted for publication).

Concluding, a modified shake-flask method can be used to obtain reproducible and reliable log P_{OW} values of cationic porphyrin derivatives by performing the measurements in more convenient *n*-butanol/water mixture and using a correlation curve. Data for 20 *meso*-phenyl(pyridyl)porphyrins were obtained and each series showed a linear correlation with the number of positively charged groups bond to the porphyrin ring periphery. A significant effect of the stereochemistry and chemical nature of the positively charged substituents were observed. Such effects tended to diminish as a inverse function of the number of positively charged substituents, such that the partition coefficients tended to converge in the mono-substituted derivatives. A stereochemical effect that greatly enhances the membrane interaction coefficient but is not well accounted by log P_{OW} was observed in the case of the *cis* amphiphilic isomers.

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References

Angeli, N.G., Lagorio, M.G., San Román, E.A., Dicelio, E., 2000. *Meso*-substituted cationic porphyrins of biological interest. Photophysical and physicochemical properties in solution and bound to liposomes. Photochem. Photobiol. 72, 49–56.

Araki, K., Engelmann, F.M., Mayer, I., Toma, H.E., Baptista, M.S., Maeda, H., Osuka, A., Furuta, H., 2003. Doubly *N*-confused porphyrins as efficient sensitizers for singlet oxygen generation. Chem. Lett. 32, 244–245.

Benfenati, E., Gini, G., Piclin, N., Roncaglioni, A., Vari, M.R., 2003. Predicting log *P* of pesticides using different software. Chemosphere 53, 1155–1164.

Braumann, T., 1986. Determination of hydrophobic parameters by reversed-phase liquid-chromatography—theory, experimental-techniques, and application in studies on quantitative structure–activity–relationships. J. Chromatogr. 373, 191–225.

Collander, R., 1951. The partition of organic compounds between higher alcohols and water. Acta Chem. Scand. 5, 774–780.

Dearden, J.C., Bresnen, G.M., 1988. The measurement of partition-coefficients. Quant. Struct.-Activity Relat. 7, 133–144.

Dixon, D.W., Steullet, V., 1998. Dimerization of tetracationic porphyrins: ionic strength dependence. J. Inorg. Biochem. 69, 25–32.

Dummin, H., Cernay, T., Zimmermann, H.W., 1997. Selective photosensitization of mitochondria in HeLa cells by cationic Zn(II) phthalocyanines with lipophilic side-chains. J. Photochem. Photobiol. B-Biol. 37, 219–229.

Eltayar, N., Tsai, R.S., Testa, B., Carrupt, P.A., Leo, A., 1991. Partitioning of solutes in different solvent systems—the contribution of hydrogen-bonding capacity and polarity. J. Pharm. Sci. 80, 590–598.

Engelmann, F.M., Losco, P., Winnischofer, H., Araki, K., Toma, H.E., 2002. Synthesis, electrochemistry, spectroscopy and photophysical properties of a series of *meso*-phenylpyridylporphyrins with one to four pyridyl rings coordinated to Ru(bipy)(2)Cl (+) groups. J. Porphyrins Phthalocyanines 6, 33–42.

Engelmann, F.M., Mayer, I., Araki, K., Toma, H.E., Baptista, M.S., Maeda, H., Osuka, A., Furuta, H., 2004. Photochemistry of doubly *N*-confused porphyrin bonded to non-conventional high oxidation state Ag(III) and Cu(III) ions. J. Photochem. Photobiol. A Chem. 163, 403–411.

Engelmann F.M., Mayer I., Gabrielli D.S., Toma H.E., Kowaltowski A., Araki K., Baptista M.S. Interaction of cationic *meso*-porphyrins with lipossomes, mitochondria and erythrocytes. Biophys. J., submitted for publication.

Gabrielli, D., Belisle, E., Severino, D., Kowaltowski, A.J., Baptista, M.S., 2004. Binding, aggregation and photochemical properties of methylene blue in mitochondrial suspensions. Photochem. Photobiol. 79, 227–232.

Hansch, C., Fujita, T., 1964. Rho-sigma-Pi analysis. Method for correlation of biological activity + chemical structure. J. Am. Chem. Soc. 86, 1616–1626.

Hudson, R., Savoie, H., Boyle, R.W., 2005. Lipophilic cationic porphyrins as photodynamic sensitizers—synthesis and structure–activity relationships. Photodiag. Photodyn. Ther. 2, 193–196.

Kepczynski, M., Pandian, R.P., Smith, K.M., Ehrenberg, B., 2002. Do liposome-binding constants of porphyrins correlate with their measured and predicted partitioning between octanol and water? Photochem. Photobiol. 76, 127–134.

Meng, G.G., James, B.R., Skov, K.A., Korbelik, M., 1994. Porphyrin chemistry pertaining to the design of anticancer drugs 2. The synthesis and in vitro tests of water-soluble porphyrins containing, in the *meso* positions, the functional-groups—4-methylpyridinium, or 4-sulfonatophenyl, in combination with phenyl, 4-pyridyl, 4-nitrophenyl, or 4-aminophenyl. Can. J. Chem.-Rev. Can. Chim. 72, 2447–2457.

Merchat, M., Spikes, J.D., Bertoloni, G., Jori, G., 1996. Studies on the mechanism of bacteria photosensitization by *meso*-substituted cationic porphyrins. J. Photochem. Photobiol. B-Biol. 35, 149–157.

Obata, K., Sugano, K., Saitoh, R., Higashida, A., Nabuchi, Y., Machida, M., Aso, Y., 2005. Prediction of oral drug absorption in humans by theoretical passive absorption model. Int. J. Pharm. 293, 183–192.

Ochsner, M., 1997. Photophysical and photobiological processes in the photodynamic therapy of tumours. J. Photochem. Photobiol. B: Biol. 39, 1–18.

Onuki, J., Ribas, A.V., Medeiros, M.H.G., Araki, K., Toma, H.E., Catalani, L.H., DiMascio, P., 1996. Supramolecular cationic tetraruthenated porphyrin induces single-strand breaks and 8-oxo-7,8-dihydro-2′-deoxyguanosine formation in DNA in the presence of light. Photochem. Photobiol. 63, 272–277.

Poole, S.K., Poole, C.F., 2003. Separation methods for estimating octanol–water partition coefficients. J. Chromatogr. B-Anal. Technol. Biomed. Life Sci. 797, 3–19.

Ran, Y.Q., He, Y., Yang, G., Johnson, J.L.H., Yalkowsky, S.H., 2002. Estimation of aqueous solubility of organic compounds by using the general solubility equation. Chemosphere 48, 487–509.

Ravanat, J.L., Cadet, J., Araki, K., Toma, H.E., Medeiros, M.H.G., Di Mascio, P., 1998. Supramolecular cationic tetraruthenated porphyrin and light-induced decomposition of 2′-deoxyguanosine predominantly via a singlet oxygen-mediated mechanism. Photochem. Photobiol. 68, 698–702.

Reddi, E., Ceccon, M., Valduga, G., Jori, G., Bommer, J.C., Elisei, F., Latterini, L., Mazzucato, U., 2002. Photophysical properties and antibacterial activity of *meso*-substituted cationic porphyrins. Photochem. Photobiol. 75, 462–470.

Ricchelli, F., Franchi, L., Miotto, G., Borsetto, L., Gobbo, S., Nikolov, P., Bommer, J.C., Reddi, E., 2005. *Meso*-substituted tetra-cationic porphyrins photosensitize the death of human fibrosarcoma cells via lysosomal targeting. Int. J. Biochem. Cell Biol. 37, 306–319.

Rouser, G., Fleische, S., Yamamoto, A., 1970. 2 Dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids 5, 494.

Schmidt-Erfurth, U., Hasan, T., 2000. Mechanisms of action of photodynamic therapy with verteporphyrin for the treatment of age-related macular degeneration. Survey Ophthalmol. 45, 195–214.

Schoenwald, R.D., Huang, H.S., 1983. Corneal penetration behavior of beta-blocking-agents. 1. Physicochemical factors. J. Pharm. Sci. 72, 1266–1272.

Seiler, P., 1974. Interconversion of lipophilicities from hydrocarbon–water systems into octanol–water system. Eur. J. Med. Chem. 9, 473–479.

Smijs, T.G.M., Schuitmaker, H.J., 2003. Photodynamic inactivation of the dermatophyte trichophyton rubrum. Photochem. Photobiol. 77, 556–560.

Tardivo, J.P., Del Giglio, A., Oliveira, C.S., Gabrielli, D.S., Junqueira, H.C., Tada, D.B., Severino, D., Turchiello, R., Baptista, M.S., 2005. Methylene blue in photodynamic therapy: from basic mechanisms to clinical applications. Photodiag. Photodyn. Ther. 2/3, 175–191.

Tayer, N.E., Testa, B., Carrupt, P.A., 1992. Polar intermolecular interactions encoded in partition coefficients: an indirect estimation of hydrogen-bond parameters of polyfunctional solutes. J. Phys. Chem. 96, 1455–1459.

Van de Waterbeemd, H., Smith, D.A., Beaumont, K., Walker, D.K., 2001. Property-based design: optimization of drug adsorption and pharmacokinetics. J. Med. Chem. 44, 1313–1333.