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Effect of surfactant phase transition on the inclusion behaviour of an amphiphilised porphyrin derivative†‡

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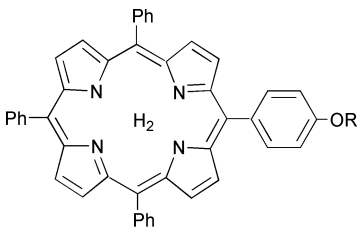
The macroscopic morphology of a micelle biomembrane model strongly affects the aggregation state of an included porphyrin derivative.

The study of the interaction of porphyrins with synthetic or biological membranes are of fundamental importance in the construction of artificial enzymes with Cytochrome P450-like activity.[1] One of the most important features affecting the activity of porphyrins once included in membrane models is, undoubtedly, their aggregation state. This can be influenced by several factors such as the physicochemical properties of the membrane,[2] the bulk pH of the aqueous medium,[3] and the hydrophobicity of the included porphyrin, which is related to the presence of charged or polar groups on the frame of the macrocycle.[4] These issues are of striking interest in the field of Photodynamic Therapy (PDT) of malignant tumors and related diseases,[5] as the sensitiser uptake and, consequently, its photodamaging activity (*i.e.* cytotoxic singlet oxygen formation upon porphyrin photosensitisation) at cellular level, are strongly affected by the aggregation state inside the cells.[6]

Over the course of our studies devoted to the construction of a P450 biomimetic system,[7,8] we have found that porphyrin derivative **1** (Scheme 1), characterised by the presence of a neutral, polar functionality, is included in a non-ionic micelle type (Brij 35) in a monomeric form, whereas it is included in a cationic surfactant aqueous solution (CTAN) with evident degree of aggregation. In this paper we report on the possibility of tuning the aggregation state of **1** in CTAN by varying the macroscopic morphology of the cationic micelle pseudophase.

The inclusion of **1** in a CTAN 0.01 M aqueous solution occurs with a substantial degree of aggregation. UV-vis spectra of **1** in CTAN show a typical red shift and broadening of the relative Soret and Q bands, compared to that featured in an aqueous solution of Brij 35 (0.01 M), a non ionic surfactant (Fig. 1). The aggregation phenomenon is also testified by steady



1: R = $(CH_2CH_2O)_2CH_2CH_2OH$

Scheme 1 Porphyrin derivative **1** employed in the spectroscopic studies.

† Electronic supplementary information (ESI) available: experimental details of spectroscopic studies. See http://www.rsc.org/suppdata/cc/b1/b111692d/

‡ The synthesis of porphyrin **1** is described in ref. 8. Brij 35 and CTAN stand for polyoxyethylene(23)lauryl ether, and cetyltrimethylammonium nitrate, respectively. Critical Micelle Concentration (cmc) of Brij 35 is 0.05 mM (see ref. 11); that of CTAN is 0.8 mM (see ref. 15).

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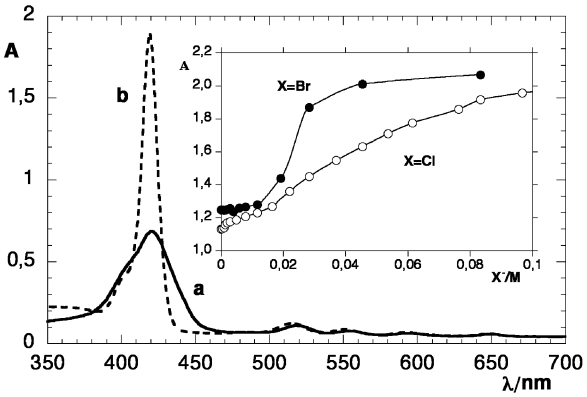

Fig. 1 UV-vis spectra of porphyrin derivative **1** (2.3×10^{-6} M) in CTAB, 0.01 M (a); Brij 35, 0.01 M (b). Inset: plot of absorption maxima of **1** (λ 417 nm, 1.2×10^{-6} M) *vs.* [X⁻] at varying salt concentrations: (●) X = Br, (○) X = Cl.

state and time resolved fluorescence spectroscopy studies (Table 1). The porphyrin **1** included in CTAN micelles features, concomitantly to an evident quenching of the overall fluorescence emission, bi-exponential fluorescence decay times (τ) (λ_{exc} = 515 nm, λ_{em} = 650 nm) of 11 and 2 ns, which are typical of monomeric and dimeric forms, respectively.[9]

The addition of NaBr to a **1**/CTAN solution causes a macroscopic colour change, from light yellow to reddish-pink. This undoubtedly indicates the occurrence of a de-aggregation process on the included fluorophore, which is also testified by an evident hyperchromic effect and sharpening of the porphyrin Soret band (Fig. 1, inset). This phenomenon can be ascribed to a sphere-to-rod transition of the CTAN micelle,[10] promoted by addition of the soft Br^- halide ion.§ The onset of micellar growth has been reported as typically occurring at a salt concentration of between 0.035 and 0.1 M by several experimental techniques.[11,12]

Remarkably, the structural transition of CTAN micelles strongly influences the photophysical properties of the included porphyrin, as indicated by the characteristic sigmoidal plot of both the fluorescence quantum yield and anisotropy coefficient

Table 1 Fluorescence time decays (τ), anisotropy coefficients (r), and rotational correlation times (Φ) of **1** in CTAN and Brij 35 micelles at different NaBr concentrations[a]

Sample	τ[b]/ns	r	Φ/ns
1/Brij	12.7	0.042 ± 0.001	
1/Brij[c]	12.3	0.042 ± 0.001	
1/CTAN	2.1 (α_1 = 0.60) 11.3 (α_2 = 0.40)	0.022 ± 0.002	3.2
1/CTAN[d]	11.0	0.038 ± 0.001	6.7
1/CTAN[e]	10.4	0.044 ± 0.001	8.0

[a] [**1**] = 1.2×10^{-6} M, [Surfactant] = 0.01 M, T = 25 °C. [b] α_i represent the pre-exponential coefficients in the fitting function $I(t) = \Sigma_i \alpha_i \exp(-t/\tau_i)$. [c] [$Br^-$] = 0.1 M. [d] [$Br^-$] = 0.017 M. [e] [$Br^-$] = 0.082 M.

changes of **1** (Fig. 2). An evident increase in the fluorescence intensity is observed upon increasing the Br^- concentration (Fig. 2, inset). Moreover, only the longer lifetime (τ = 11 ns) can be detected at high bromide concentrations ([Br^-] = 0.02 M), confirming the complete de-aggregation of **1**. Further addition of Br^- had no longer any effect on the fluorescence behaviour of the chromophore. It is interesting to note that the emission intensity of **1** in CTAN, in the presence of an excess of Br^-, is scarcely quenched, compared to that observed in Brij micelle. This indicates that the fluorophore is probably located in the hydrophobic region of the micelle.[9]

The effect of the structural transition on the inclusion behaviour of **1** can be a consequence of tighter packing, which accompanies the micelle aggregate growth. The structural reorganisation occurs with the expulsion of molecules of water included in the micelle clefts, resulting in the formation of a less polar microenvironment biasing a more favourable porphyrin–surfactant interaction (*e.g.* van der Waals forces between macrocycles and the surfactant hydrocarbon chains) which overwhelms the π–π stacking forces.

Parallel studies of fluorescence anisotropy[13] (λ_{exc} = 515 nm, λ_{em} = 640 nm) confirm this hypothesis. The rotational correlation times (Φ), associated with the measured anisotropy coefficients through a simple Einstein–Smoluchovski modelling of the fluorophore dynamics,[14] more than double on going from [Br^-] = 0 to 0.082 M (Table 1) indicating that the fluorophore is included in a more densely packed environment.

The porphyrin de-aggregation phenomenon is dependent on the specificity of the interacting anion, rather than on a mere increase of ionic strength of the medium. In a parallel experiment carried out with a harder (Coulombic) chloride anion, a poorly defined transition point is in fact evidenced (Fig. 1, inset).¶

Straight evidence of the micelle size variation was obtained by measuring the micelle average aggregation number by time resolved fluorescence techniques. A method based on the pyrene/cetylpyridinium chloride (CPyCl) fluorophore-quencher pair was followed.[15] Results are reported in Table 2. The average aggregation number (N_{Av}) steadily increases, from

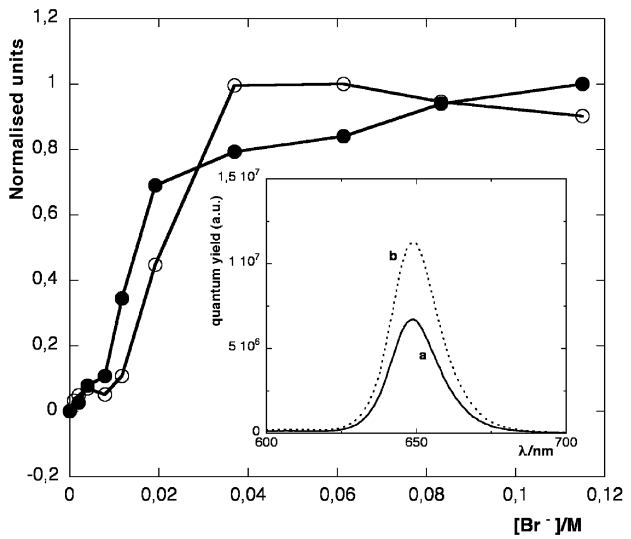

Fig. 2 Relative changes of fluorescence anisotropy (●) and quantum yield (○) of **1** (1.2×10^{-6} M) in CTAN 0.01 M, at varying [Br^-] concentrations. Experimental data are normalised in the 0–1 range for comparison. Inset: fluorescence emission spectra of **1** (1.2×10^{-6} M) in CTAN 0.01 M: (a) [Br^-] = 0 and (b) [Br^-] = 0.12 M.

Table 2 Average aggregation number (N_{Av}), pyrene time decays (τ_0), and quenching rate constants (k_q) for pyrene/CPyCl in CTAN 0.01 M at varying NaBr concentrations[a]

[Br^-]/M	τ_0/ns	N_{Av}	$10^{-7}k_q/s^{-1}$
0	117.3	64	1.0
0.003	106.2	83	1.1
0.009	110.4	85	1.1
0.03	98.2	87	1.2
0.06	95.5	98	1.1

[a] [Pyrene] = 5.0×10^{-6} M, [CPyCl] = 5.0×10^{-4} M, T = 25 °C.

64 at [Br^-] = 0, to 98 at [Br^-] = 0.06 M, in fair agreement with earlier literature reports.[15] Moreover, the invariance of k_q (*i.e.* pyrene/CPyCl quenching constant values) with NaBr concentrations [k_q = 1.1 (±0.1) $\times 10^{-7}$ s^{-1}] indicates the absence of diffusional effects.

In conclusion, these studies may be of great interest as they would open the possibility of the construction of switchable biomimetic machinery, in which, for example, a heme-based light–energy conversion device may be turned on by an external (electrostatic) impulse. Moreover, the described system may result in important applications in ion switching and biological semiochemistry.[16]

Notes and references

§ The halide addition results in a saturation of the surface electrostatic charges. This lowers the repulsive interactions between the cation head groups giving a rod-like, tighter packed structure of the micelle aggregates.

¶ A soft cationic micelle strongly interacts with a soft anion (I^-), rather than with a harder, Coulombic Cl^-. See, for example, ref. 11.

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