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On the localization of water-soluble porphyrins in micellar systems evaluated by static and time-resolved frequency-domain fluorescence techniques

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ABSTRACT

Fluorescence quenching of meso-tetrakis-4-sulfonatophenyl ($TPPS_4$) and meso-tetrakis-4-*N*-methylpyridil (TMPyP) porphyrins is studied in aqueous solution and upon addition of micelles of sodium dodecylsulfate (SDS), cetyltrimethylammonium chloride (CTAC), *N*-hexadecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (HPS) and *t*-octylphenoxypolyethoxyethanol (Triton X-100). Potassium iodide (KI) was used as quencher. Steady-state Stern–Volmer plots were best fitted by a quadratic equation, including dynamic (K_D) and static (K_S) quenching. K_S was significantly smaller than K_D. Frequency-domain fluorescence lifetimes allowed estimating bimolecular quenching constants, k_q. At 25 °C, in aqueous solution, TMPyP shows k_q values a factor of 2–3 higher than the diffusional limit. $TPPS_4$ shows collisional quenching with pH dependent k_q values. For TMPyP quenching results are consistent with reported binding constants: a significant reduction of quenching takes place for SDS, a moderate reduction is observed for HPS and almost no change is seen for Triton X-100. Similar data were obtained at 50 °C. For CTAC–$TPPS_4$ system an enhancement of quenching was observed as compared to pure buffer. This is probably associated to accumulation of iodide at the cationic micellar interface. The attraction between CTAC headgroups and I^-, and repulsion between SDS and I^-, enhances and reduces the fluorescence quenching, respectively, of porphyrins located at the micellar interface. The small quenching of $TPPS_4$ in Triton X-100 is consistent with strong binding as reported in the literature.

1. Introduction

Biological membranes are extremely complex structures that require models of much less complexity to investigate different aspects of essential bilayer properties and functions [1]. Micelles are a very simplified model of the membranes, since they do not possess a bilayer structure and are characterized in many cases by an approximate spherical or spheroidal shape possessing significant flexibility. The presence of polar headgroups and hydrophobic chains in micellar structures allows one to study the affinity of a great variety of molecules to membrane systems using the micelles as a very simple model [2–7]. Many biological processes occur at the surface of membranes or within their hydrophobic moiety. The physico-chemical properties of different compounds were the subject of extensive studies involving the interaction with micelles and other model membrane systems [2–13].

In this context, the importance of porphyrins and related compounds as therapeutic drugs and targeting agents has been widely recognized [14–17]. Spectroscopic characterization of porphyrins interacting with different micelles has attracted the interest of many researchers in the last decades [5–10]. Incorporation of porphyrins in micelles dramatically influences their aggregation mode. Porphyrin aggregates may be easily studied by several techniques such as UV/vis absorption spectroscopy by observing spectral shifts in the Soret band from monomers to the red region (J-aggregates) or to the blue (H-aggregates); fluorescence spectroscopy which shows characteristic intensity reduction of the monomer spectrum emission upon aggregation; resonance light scattering (RLS) which displays enhancement of light scattering intensity due to aggregated forms, whereas the monomeric porphyrin does not show RLS effect [5,7,9,10,17–19]. Different parameters, such as porphyrin concentration, pH of environment, and ionic strength, affecting the aggregation process, have been investigated by several authors [20–23]. In recent years a significant effort has been made to elucidate the nature of porphyrin aggregation, as well as the aggregate structure of porphyrin molecules both in the absence and presence of surfactant solutions [5,7,9,24,25]. Despite the existence of all of these studies this problem remains an interesting and yet not completely solved one, especially concerning the detailed molecular structures of the aggregates, which remains unknown.

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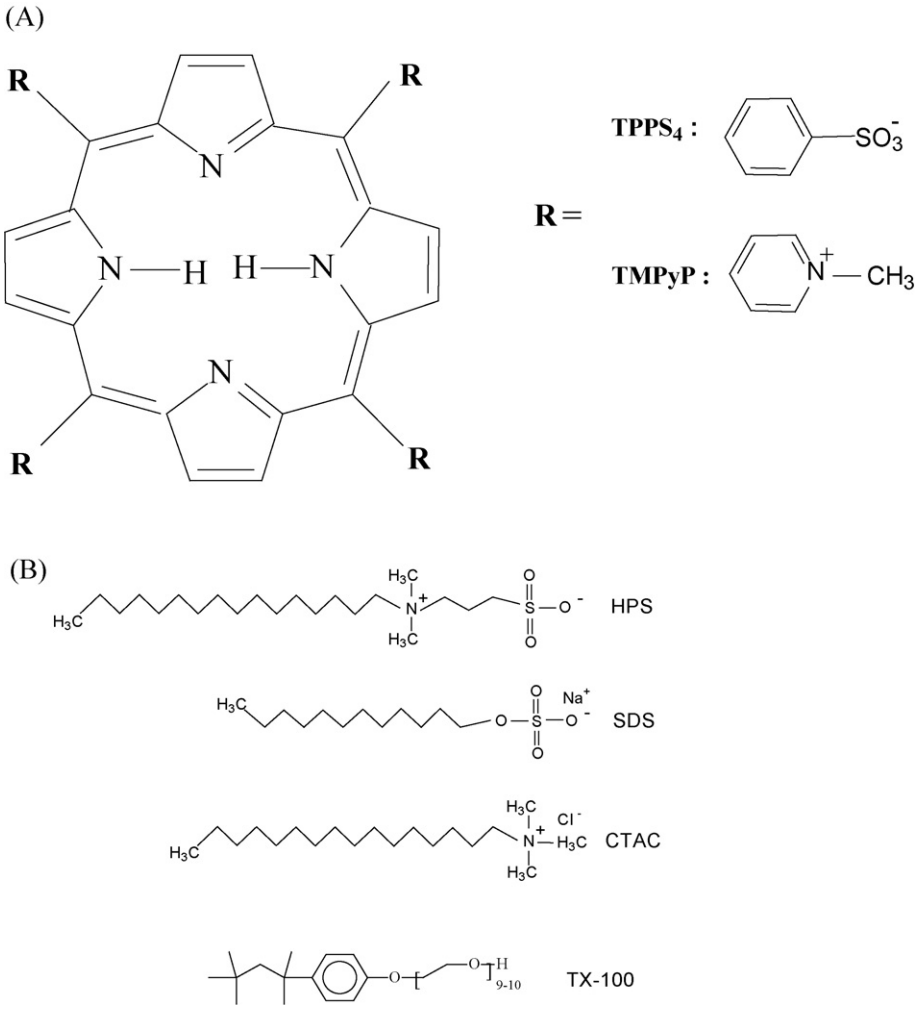

Fig. 1. Structures of porphyrins (A) and surfactants (B) used in this study.

Maiti et al. [7] have reported that porphyrins interact with surfactants leading to stable structures of porphyrin–surfactant aggregates. The kinetics of the formation of the aggregates and their structure depend on the attraction between the ionic surfactant and the opposite charge on the porphyrin. More importantly, the structure of the porphyrin–surfactant aggregate depends upon the concentration of the surfactant. In our previous work, we have studied the interaction of porphyrins and metalloporphyrins with different surfactants [5,6]. It was shown that the mechanisms of porphyrin interaction imply both electrostatic and hydrophobic contributions. Our results have shown, in agreement with literature data [7,9,10], that these interactions depend on the surfactant headgroups and the pH of the medium. In the presence of low concentrations of ionic surfactants with oppositely charges to the porphyrin, premicellar (or non-micellar) porphyrin–surfactant aggregates can be formed. Above cmc, micelles are usually considered only as a means to re-solubilize the premicellar (non-micellar) aggregates of porphyrin derivatives into monomers bound to micelles [5–7,26,27]. Binding constants (K_b) between porphyrins and different micelles were estimated. K_b values were of the order of $10^4\ M^{-1}$ for cetyltrimethylammonium chloride (CTAC)–$TPPS_4$ [5], a factor of 10 higher than K_b for sodium dodecylsulfate (SDS)–TMPyP system [28]. In the presence of zwitterionic HPS or neutral Triton X-100 micelles the premicellar (or non-micellar) aggregates were not observed. For HPS–$TPPS_4$, K_b has a value which is very similar to that obtained with CTAC, whereas for Triton X-100–$TPPS_4$ system K_b was of the order of $10^3\ M^{-1}$. For HPS–TMPyP system the value of K_b was much lower ($\approx 10^2\ M^{-1}$). The differences between the K_b values of cationic and anionic porphyrins are due to the fact that TMPyP has its tetra-positive charge delocalized over the entire macrocycle ring leading to a reduction of the hydrophobic character of the positively charged TMPyP, while $TPPS_4$ has its tetra-negative charge localized at the peripheral substituents of the macrocycle leading to a higher hydrophobicity of this porphyrin [20]. ^1H NMR data indicate that the porphyrins were incorporated into the micelles near the terminal part of their hydrocarbon chains, as evidenced by a strong upfield shift of the corresponding peaks belonging to the terminal methyl and methylenes of the micelles. Therefore, the solubilization of porphyrins within hydrophobic regions of micelles is determined, in general, by non-specific hydrophobic interactions, yet it is significantly modulated by electrostatic factors [5]. This binding of porphyrins-micelles is related to the important problem concerning the aggregation process as well as their micelle localization. Scolaro et al. studied the micellar aggregates of platinum(II) complexes containing porphyrins in anionic SDS and neutral Triton X-100 micelles by using $K_4[Fe(CN)_6]$ as ionic quencher [8]. In SDS the absence of fluorescence quenching indicated that the dissolved porphyrin was not accessible to the anionic quencher, whereas in Triton X-100 two different populations of porphyrins, one accessible to the quencher and another one confined in an internal site of the micelles, were observed.