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Aggregation and Solution Behavior of 5-(1-(4-Carboxybutyl) Pyridinum-4-yl) 10,15,20-tris (1-methylpyridinium-4-yl) Porphyrin: A Resonance Light Scattering, Fluorescence and Absorption Spectroscopic Study

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Aggregation behavior of water soluble porphyrins, 5-(1-(4-carboxybutyl) pyridinum-4-yl) 10,15,20-tris (1-methylpyridinium-4-yl) porphyrin (5-CBPyP) in the presence of various concentrations of calf thymus DNA (ct-DNA) and sodium chloride were studied in comparison with meso-tetrakis (4-N-methylpyridinum) porphyrin (TMPyP), by optical absorption, fluorescence and resonance light scattering (RLS) spectroscopies. Both porphyrins obey Beer's law in extended range of concentration. Optical absorption and RLS measurements demonstrated nonaggregation for both porphyrins under increasing concentration of ct-DNA and NaCl. However, in comparison, 5-CBPyP had less tendency for aggregation that may be taken as an advantage for its probable application in photodynamic therapy of cancer. The trend of changes in absorption spectra of both porphyrins in the presence of ct-DNA indicates the homogeneous intercalation binding mode. The values of $(2.81 \pm 0.28) \times 10^6$ M^{-1} and $(0.95 \pm 0.09) \times 10^6$ M^{-1} were obtained for apparent binding constant of TMPyP and 5-CBPyP from analysis of optical absorption data, respectively. This indicates the less affinity of 5-CBPyP to ct-DNA in comparison with TMPyP. The binding of both porphyrins to ct-DNA quenches fluorescence emission of Ethidium bromide (EB) that is bound to ct-DNA. The quenching process obeys linear Stern-Volmer relationship indicating the displacement of EB from its binding sites by these porphyrins. The results of this technique also represent the intercalation mode of binding for both porphyrins and higher binding affinity of TMPyP compared with 5-CBPyP.

Keywords*:* Porphyrin, DNA, Aggregation, Optical absorption, Resonance light scattering, Fluorescence

INTRODUCTION

Cationic porphyrin macrocycles represent a large and expanding class of compounds which have application in biology, medicine, catalysis and materials [1]. Interaction of porphyrins and metalloporphyrins with DNA is of considerable significance due to their medical applications.

Their special properties: high optical absorption, relatively high quantum yields of triplet state and fluorescence, or paramagnetism of some metal complexes, provide the use of porphyrin in medicine, as active compounds in radiological [2,3] and magnetic resonance imaging [4,5] of cancer detection and as photosensitizers in photodynamic therapy (PDT) of cancer [6,7]. Porphyrin demonstrates the photodynamic activity against psoriasis atheromatous plaque, viral and bacterial infections including HIV [8].

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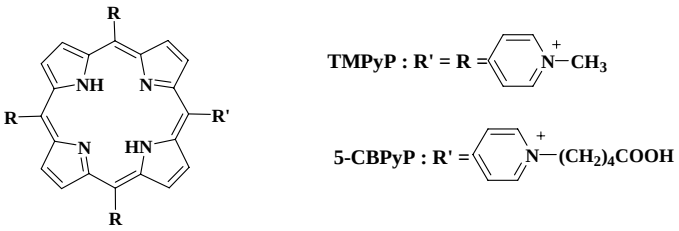
Porphyrins can bind to various structures in the organism and DNA is one of the most important targets. High affinity of cationic porphyrins for DNA is well-known owing to the pioneer works of Fiel [9], Pasternack *et al*. [10,11]. Research in this respect is ever-increasing (see, for instance, Refs. [12-23] and references therein).

Biological effects of porphyrin derivatives depend strongly on their physico-chemical properties, the phenomenon of aggregation of special interest. Aggregation changes the porphyrin absorption spectra [24,25,29-32], fluorescence intensity [24,25,33], paramagnetic properties [30], lifetime and quantum yield of triplet state; hence the production of single molecular oxygen [34-38]. The aggregate formation depends on the structure of the porphyrin molecule: the presence and the type of the central metal atom, the protonation state, structures of substituents, environmental characteristics such as solvent polarity, pH, hydrogen bond formation, ionic strength, *etc.* [24-27], as well as on [DNA]/[Porphyrin] mole ratio [24,25]. The presence of microheterogenous system in the solution can stimulate aggregation of certain heterocyclic compounds, porphyrins in particular, at their binding sites [39]. So, binding to macromolecules, for instance, to DNA or serum proteins, induces aggregation of some water soluble porphyrins in solution [24-27,33,34,40,41]. These qualities should be kept in mind in the course of the biomedical application of such compounds.

The rational functionalization of the porphyrin peripheral substituents allows preparation of porphyrin derivatives of different charge, size and hydrophobicity. The interest in porphyrins bearing polar-lipophilic substituents or substituents with low polarizability stems from the fact that such substituents facilitate the transport of the porphyrin through biological membranes. By the same token, lipophilic substituents cause the porphyrins to aggregate in aqueous solutions [42]. Before any potential biological application of such porphyrins could be considered, one must address the aggregation behavior, which is the subject matter of this article. The aggregation has, therefore, an enormous impact on the ability of porphyrins to effect photosensitization and cleavage of nucleic acids.

It appears that the formation of different types of aggregation could be tuned, to a large extent, by the character of the peripheral substituents. This is an important feature because it may allow for predetermination of the aggregate structure by chemical design and synthesis. In this regard, we present in this article the results of our study describing the aggregation of 5-(1-(4-carboxybutyl)pyridinium-4-yl)10,15,20-tris(1-methylpyridinium-4-yl)porphyrin (5-CBPyP) in comparison with meso-tetrakis (4-N-methylpyridinium) porphyrin (TMPyP) with positively charged pyridinium groups (Scheme 1), in the presence of calf thymus DNA (ct-DNA) at different concentrations and ionic strength effect using optical absorption, fluorescence and resonance light scattering (RLS) spectroscopies, and demonstrate the aggregation behavior of these porphyrins on the basis of their molecular structure and intermolecular forces.



Scheme 1. Chemical structures of TMPyP and 5-CBPyP

EXPERIMENTAL

Porphyrins were prepared and purified according to literature methods [43,44]. 5,10,15,20-Tetrapyridyl porphyrin (TPyP) (Fluka), ethyl 5-bromopentanoate, methyl iodide (Acros Chemicals) were used as received. TMPyP was obtained by methylation of TPyP [10]. For the preparation of 5-CBPyP, TPyP was alkylated with an excess of ethyl 5-bromopentanoate, in $CHCl_3$/EtOH, leading to a mixture of different N-alkylation products, out of which 5-(1-(4-ethoxy carbonyl)butyl)pyridinium-4-yl)-10,15,20-tripyridylporphyrin bromide was separated by chromatography on a short silica gel column and obtained in a reasonable 33% yield. The present preparation afforded the ester in a large scale associated with an easy separation of the expected isomer. The remaining three free pyridine substituents were then quantitatively alkylated by methyl iodide to afford the teracationic porphyrin. The ester function was hydrolyzed with aqueous HCl to the corresponding acid 5-CBPyP in 91% yield.

The green porphyrin was formed upon treatment with 1 M HCl. The mixture was allowed to cool and filtered on a sintered glass filter No. 4. The solvent was evaporated, and water (50 ml) was added and evaporated three times to ensure the complete elimination of HCl. The solution of final products (5-CBPyP and TMPyP) was not green. The precipitated iodide salt of porphyrins were dissolved in 0.1 N HCl and then applied to an ion-exchange column (Dowex 1 × 8 Cl^- form resin, mesh size 200-400 nm, Merck) to replace the counter anion with the chloride ion. To provide a clear picture of the preparation procedure of TMPyP and 5-CBPyP, the synthysis pathway is shown in Scheme 2.

All experiments were run in phosphate buffer (consisting of 2.5 mM NaH_2PO_4 + 5 mM Na_2HPO_4 dissolved in Milli-Q quality water) at pH 7.2. Calf thymus DNA was purchased from Sigma. To prepare DNA stock solution, 2 mg of DNA was dissolved in 1 ml of phosphate buffer the day before the experiment and stored at 4 ºC. The concentration of DNA and porphyrins were determined from their optical absorption using molar absorption coefficients ε (260 nm) = 1.32×10^4 M^{-1} cm^{-1} for DNA [26,27] (*i.e.* reported in molar base pairs) and ε (423 nm) = 2.492×10^5 M^{-1} cm^{-1} , ε (423 nm) = 1.588×10^5 M^{-1} cm^{-1}, for TMPyP and 5-CBPyP, respectively at their Soret bands. The titration was made by addition of the DNA stock solution into a 1 cm cuvette containing the porphyrin solution of appropriate concentration. The effect of ionic strength was investigated by titration of porphyrin solution of appropriate concentration in 1 cm cuvette by NaCl stock solution.

For the optical absorption experiments the porphyrin solutions were prepared in concentrations varying in the range of 3-50.0 μM to observe the Soret band. The absorption spectra were recorded on Cary 500 scan UV-Vis-NIR spectrophotometer.

The scattered-light intensity was monitored using the right-angle in the synchronous scanning regime of the excitation and emission monochromators within the range of 300 to 600 nm. The experimental light-scattering spectra were corrected taking into account the solution optical absorption and the instrument sensitivity dependence on the wavelength as described elsewhere [41]. The light scattering measurements were monitored on a RF-5000 spectrofluorimeter.

Emission spectra of Ethidium bromide (EB) bound to DNA in the absence and presence of the porphyrin were also recorded on a spectrofluorimeter Shimadzu mod. RF-5000. In a typical experiment, titration of a mixed DNA and EB solution with porphyrin in phosphate buffer was performed by stepwise addition of porphyrin solution in the same buffer directly to the cuvette. The concentration of DNA and EB were 1.50×10^{-5} and 5.00×10^{-6} M, respectively. The solutions were excited at 515 nm and the emitted light intensity was measured in the range of 520-800 nm. The UV-Vis and fluorescence spectra were corrected for dilution.

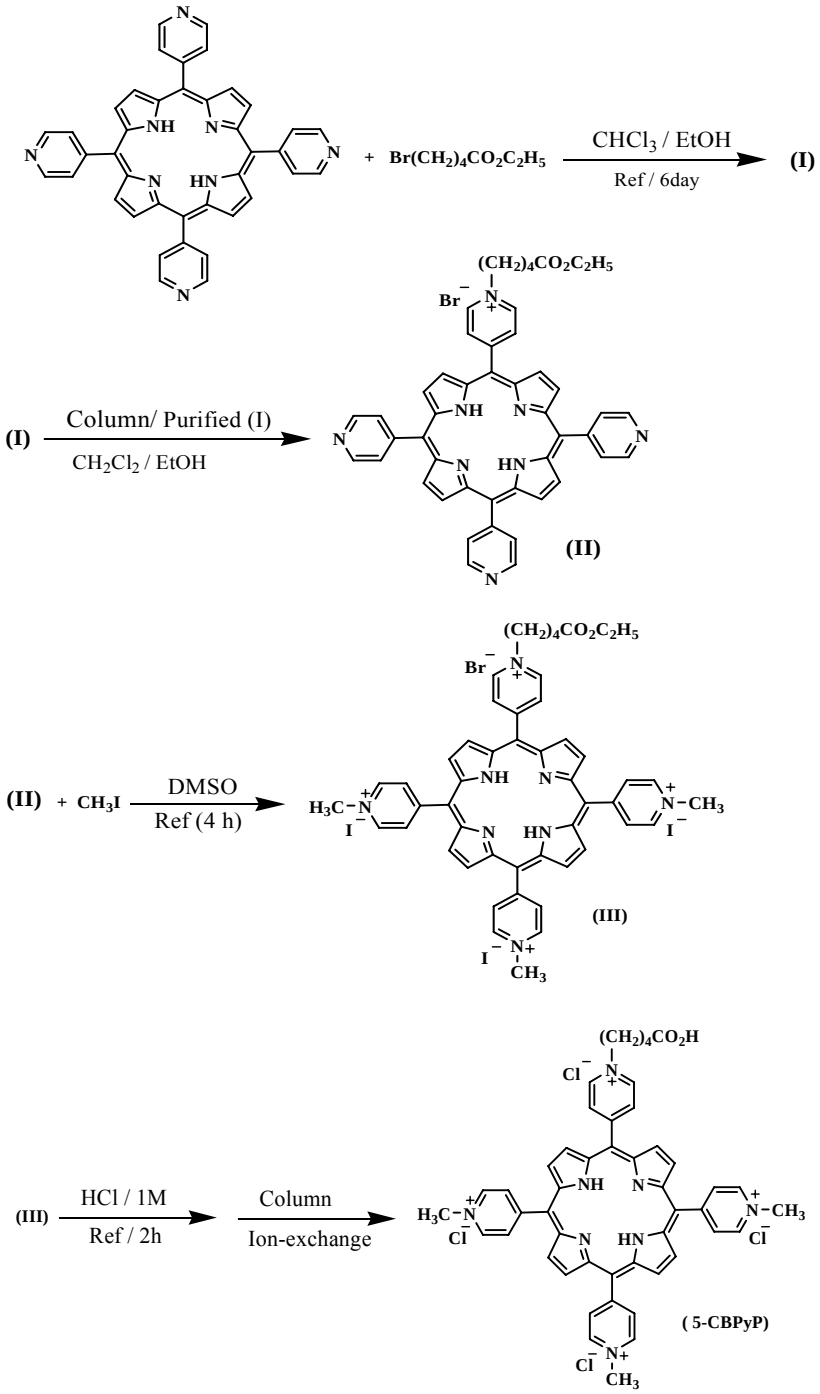
Both UV-Vis spectrophotometer and spectrofluorimeter were well-equipped with the thermostate cell compartment for keeping the temperature constant at 25 ± 1 ºC.

RESULTS AND DISCUSSION

Analysis of the Optical Absorption Spectra

In order to identify the solution properties of TMPyP and 5-CBPyP, we employed UV-Vis and RLS spectroscopies. The optical absorption spectrum of both porphyrins shows four Q-bands and a Soret band feature. Beer's law experiments were carried out for porphyrins in homogeneous aqueous solutions at pH 7.2. A summary of the molar absorptivity of these bands is presented in Table 1. The absorption in the Soret bands obeys Beer's law in the concentration range of 2.8 to 13.8 μM and 3 to 22 μM, for TMPyP and 5-CBPyP, respectively. A negative deviation from Beer's law in the Soret band was observed after this range which may be due to concentration dependent aggregation. Although none of the porphyrins show concentration dependent aggregation in relatively high concentration range, the results indicate the less tendency of 5-CBPyP for concentration dependent aggregation in comparison with TMPyP. This conclusion is drawn with respect to the greater value of upper limit of 5-CBPyP concentration range (22 μM in comparison with 13.8 μM).

The absorption spectrum of the 5-CBPyP is similar to TMPyP, and has a Soret band at 423 nm and four Q-bands at 519, 556, 585 and 638 nm. The smaller values of extinction coefficients at the Soret band and Q-bands for 5-CBPyP in comparison with TMPyP, is expected since the symmetry of the ring is reduced. 5-CBPyP has a similar 18 electron π-system as TMPyP, but the reduced symmetry is expected to induce a splitting of the e_g lowest unoccupied molecular



Scheme 2. The reaction scheme of 5-CBPyP synthesis

orbitals [45]. To a first-order approximation, this change in orbital energy results in lower-energy transitions for some of the Q-bands and a higher-energy transition for the Soret band. However, it seems that this substitution in 5-CBPyP does not have any effect on energy level and only decreases the extinction coefficients that is due to a decrease in the

Table 1. Summary of UV-Vis Absorption Bands and Extinction Coefficients for TMPyP and 5-CBPyP in 7.5 mM Phosphate Buffer, pH 7.2 and at 25 ºC

	TMPyP	5-CBPyP
λ_{Soret} (nm)	424	423
λ_{Q1} (nm)	519	519
λ_{Q2} (nm)	556	556
λ_{Q3} (nm)	585	585
λ_{Q4} (nm)	639	638
ε_{Soret} (M^{-1} cm^{-1})	2.49×10^5	1.59×10^5
ε_{Q1} (M^{-1} cm^{-1})	2.13×10^4	1.05×10^4
ε_{Q2} (M^{-1} cm^{-1})	8200	4800
ε_{Q3} (M^{-1} cm^{-1})	8900	5000
ε_{Q4} (M^{-1} cm^{-1})	1400	1200

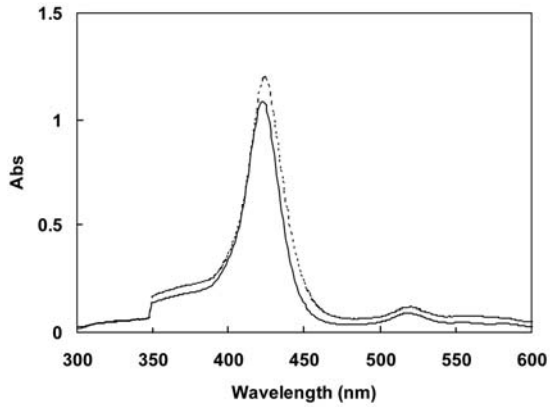


Fig. 1. UV-Vis spectra of TMPyP at constant concentration of 3 μM in 7.5 mM phosphate buffer (pH 7.2) in the absence (—) and presence of 2 M NaCl (----).

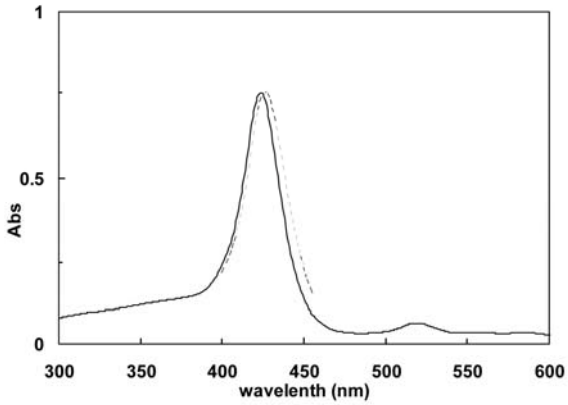


Fig. 2. UV-Vis spectra of 5-CBPyP at constant concentration of 5.3 μM in 7.5 mM phosphate buffer (pH 7.2) in the absence (—) and presence of 2 M NaCl (----).

probability of transition. The decrease in the relative extinction coefficients of the Soret band and Q-bands can be attributed to the decrease in symmetry observed in 5-CBPyP relative to TMPyP. The lower symmetry of the 5-CBPyP is due not only to the lower symmetry of the carboxylate group, but, most probably, to the intrinsically reduced symmetry of going from a tetra-substituted derivative to a tri-substituted one.

The effect of NaCl on the absorption spectrum of the porphyrins in water solution is shown in Fig. 1 and Fig. 2. As the concentration of NaCl increases, band width at half height, $W_{1/2}$ and wavelength of maximum absorption spectrum of the porphyrins, λ_{max}, (Soret band) do not show significant changes. The absorption spectrum of porphyrins show no significant electrolyte effect; in fact, no new band appears even in high concentrations of salt. These results indicate that TMPyP and 5-CBPyP do not form aggregates even in high concentrations of salt.

Porphyrins TMPyP (2.8 μM) and 5-CBPyP (5.3 μM) were titrated with a solution of ct-DNA. The fixed amount of cationic porphyrin in phosphate buffer was titrated with a stock solution of DNA. The absorbance change in the Soret region is shown in Fig. 3 and Fig. 4. These figures show that the intensity of the Soret band at 424 nm decreases with the earlier addition of DNA. The observed red shift of the Soret band during the titration suggests the binding of the porphyrin to ct-DNA. Cationic porphyrin TMPyP exhibited 62% hypochromism and 15 nm red shift of the Soret band; however, in the case of cationic porphyrin, 5-CBPyP changes with 37% hypochromism and 11 nm red shift of the Soret band in UV-Vis spectra was observed. The intercalative binding porphyrins to a DNA helix has been characterized [10] by: (i) large red shift ($\Delta\lambda \geq 15$ nm) and hypochromic ($H \geq 35\%$) shifts of their Soret maxima, (ii) negative (-) induced GC-rich DNA sequences. In contrast, outside binders displayed: (i) much smaller red shifts ($\Delta\lambda \leq 8$ nm) and little hypochromicity ($H \leq 10\%$), and sometimes hyperchromicity)

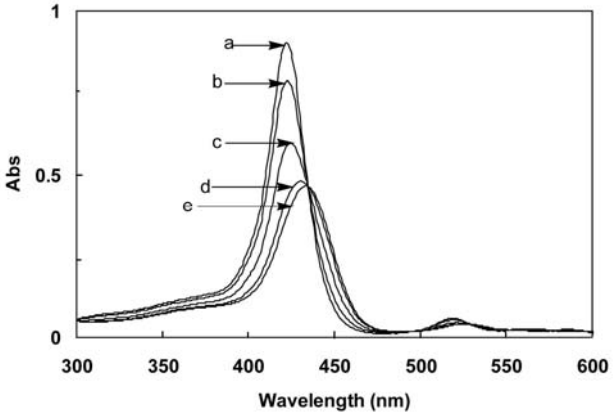


Fig. 3. UV-Vis spectra of 3 μM TMPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of varying concentrations of calf thymus-DNA: 1.3 μM (b), 3.8 μM (c), 6.2 μM (d) and 7.5 μM (e).

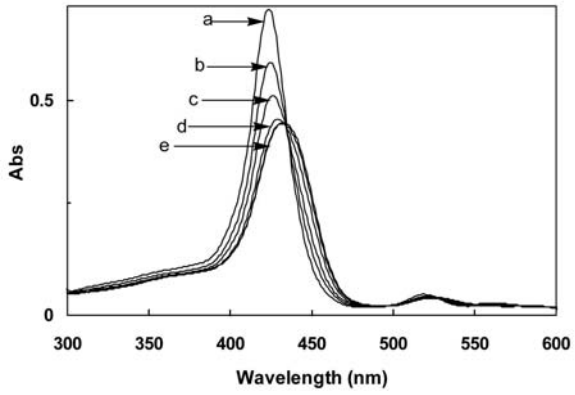


Fig. 4. UV-Vis spectra of 5.3 μM 5-CBPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of varying concentrations of calf thymus-DNA: 1.98 μM (b), 13.9 μM (c), 15.9 μM (d), 17.9 μM (e).

CD activity in the Soret region, and (iii) high selectivity for of their Soret maxima, (ii) positive (+) induced CD bands in the Soret region, and (iii) a distinct preference for AT-rich minor groove segments. Nevertheless, our study shows that the porphyrins bind to DNA through intercalation. The presence of isosbestic points in their UV-Vis titration spectra also confirms the homogeneous binding mode (see Fig. 3 and Fig. 4).

Binding constants for the interaction of cationic porphyrins with DNA were determined by the analysis of titrations absorption spectra at 25 ºC, using familiar procedure as follows [46-48]:

The changes in absorbance of the Soret band upon addition of DNA were monitored at the maximum of the Soret band. The apparent binding constant, K_{app} of cationic porphyrins to DNA was calculated using Eq. (1):

$$\frac{[DNA]_{total}}{(|\varepsilon_{app} - \varepsilon_f|)} = \frac{[DNA]_{total}}{(|\varepsilon_b - \varepsilon_f|)} + \frac{1}{K_{app}(|\varepsilon_b - \varepsilon_f|)} \quad (1)$$

where ε_{app}, ε_f and ε_b correspond to $A_{obsorved}$/[porphyrin], the extinction coefficient for the free porphyrin and the extinction coefficient for the porphyrin in the fully bound form, respectively. In the plot of $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs*. $[DNA]_{total}$, K_{app} is given by the ratio of the slope to the intercept. The value for apparent binding constants of TMPyP and 5-CBPyP at our experimental conditions were calculated to be (2.81 ± 0.28) × 10^6 M^{-1} and (0.95 ± 0.09) × 10^6 M^{-1}, respectively (Sigma Plot software was used for data analysis). It indicates that the substitution of $(CH_2)_4COOH$ in 5-CBPyP reduces its affinity to DNA relative to TMPyP. A convincing interpretation of such observation can be related to the introduction of a negative charge on the periphery of the 5-CBPyP (the -$(CH_2)_4COOH$ moiety is fully deporotonated at pH 7.2). The presence of such a negative charge should hamper the interaction with the negatively charged phosphate groups of the nucleic acid. This can be also the origin of the unanticipated result of diffusion into the double helix from the opposite site of substitution.

In order to examine the role of electrostatic interaction in the binding process, the effect of NaCl on the absorption spectrum of Porphyrin-DNA was studied. In this regard, the NaCl stock solution was added stepwise to the mixture of DNA-Porphyrin solution. The results for TMPyP and 5-CBPyP are shown in Figs. 5 and 6, respectively. As shown, the concentration of NaCl increases, adsorption increases at Soret band. This effect reflects the dissociation of the porphyrin-DNA, and increase free concentration of porphyrin,

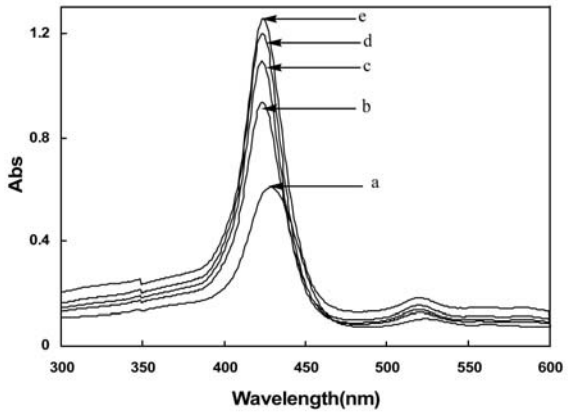


Fig. 5. UV-Vis spectra of DNA/TMPyP solution (at a molar ratio of 2.28) 5.3 μM 5-CBPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of varying concentrations of NaCl: 0.28 μM (b), 0.75 μM (c), 1.12 μM (d) and 1.98 μM (e).

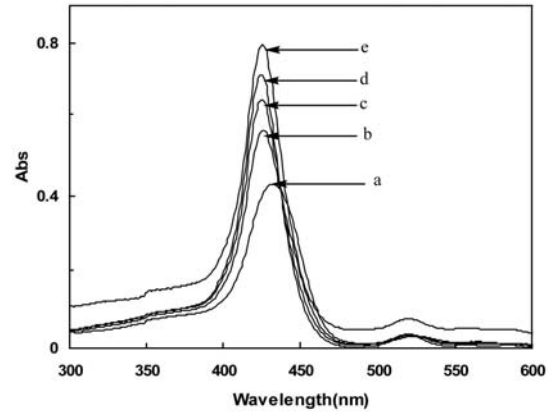


Fig. 6. UV-Vis spectra of DNA/5-CBPyP solution (at a molar ratio of 2.36 in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of varying concentrations of NaCl: 0.21 μM (b), 0.67 μM (c), 1.03 μM (d) and 2.01 μM (e).

due to increase in ionic strength. In fact, the binding of counter ions (sodium cation) to the phosphate groups at the surface of DNA reduces the electrostatic interaction between cationic porphyrin and DNA. Hence, it can be concluded that an increase in ionic strength reduces the electrostatic interaction which in turn reduces the binding affinity of the porphyrins.

Fluorescence Spectroscopic Studies

Ethidium bromide (EB) emits intense fluorescence light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. It was previously reported that the enhanced fluorescence could be quenched by the addition of a second molecule [49,50]. The quenching extent of fluorescence of EB bound to DNA was used to determine the extent of binding between the second molecule and DNA. The emission spectra of EB bound to DNA in the absence and the presence of TMPyP and 5-CBPyP are given in Figs. 7 and 8, respectively. In this case, the reduction in emission intensity could be due to displacement of EB from the interaction sites by the porphyrins, indicating that the binding constants of these porphyrins to ct-DNA are comparable to the binding constant of EB to DNA which is in the order of 10^6 M^{-1}[49]. According to the classical Stern-Volmer equation [50]:

$$\frac{I_\circ}{I} = 1 + K_{sv} r \qquad (2)$$

where I_0 and I are the fluorescence intensities in the absence and the presence of porphyrin, respectively, K_{sv} is a linear Stern-Volmer quenching constant, r is the ratio of total concentration of porphyrin to that of DNA. The fluorescence quenching curves of EB bound to DNA by the porphyrins are shown in Fig. 9. The quenching plots illustrate that the quenching of EB bound to DNA by the porphyrins are in good agreement with the linear Stern-Volmer equation, which additionally proves that the porphyrins bind to DNA. In the plot of I_0/I *vs*. [porphyrin]/[DNA], K_{sv} is given by the ratio of the slope to the intercept (Fig. 9). The values of 45.62 and 14.14 were estimated for K_{sv} of TMPyP and 5-CBPyP, respectively. Such values of quenching constant suggest that the interaction of these porphyrins with DNA is of a strange intercalation [49,51], and the smaller affinity of 5-CBPyP with respect to TMPyP is concordant with optical absorption results.

Light Scattering Studies

The scattered-light intensity (SLI) of a solution in the absence of optical absorption depends on the wavelength as

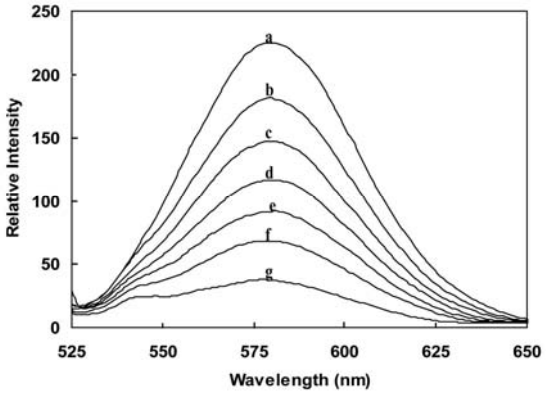


Fig. 7. Emission spectra of EB (5 μM) bound to DNA (12 μM) in 7.5 mM phosphate buffer (pH 7.2) at λ_{ex} =510 nm in the absence (a) and presence of varying concentrations of TMPyP: 0.15 μM (b), 0.3 μM (c), 0.45 μM (d), 0.60 μM (e), 0.75 μM (f) and 0.90 μM (g).

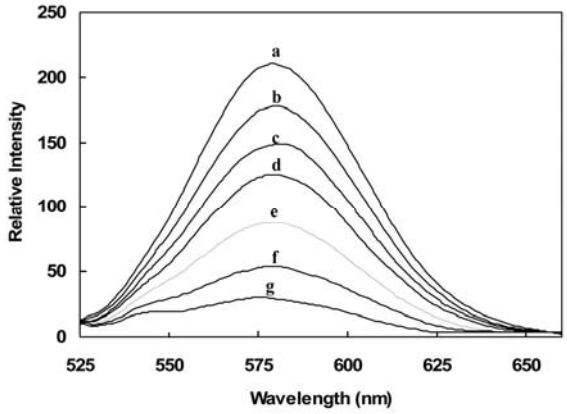


Fig. 8. Emission spectra of EB (5 μM) bound to DNA (12 μM) in 7.5 mM phosphate buffer (pH 7.2) at λ_{ex} =510 nm in the absence (a) and presence of varying concentrations of 5-CBPyP: 0.31 μM (b), 0.62 μM, (c), 0.94 μM (d), 1.25 μM (e), 1.56 μM (f) and 1.88 μM (g).

$1/\lambda^4$ (Rayleigh law). The buffer, NaCl and DNA solutions in the absence of porphyrin were not absorbed in the spectral region studied. Thus, the SLI spectra of solutions at different NaCl and DNA concentrations (I_{NaCl}, I_{DNA}) were described by

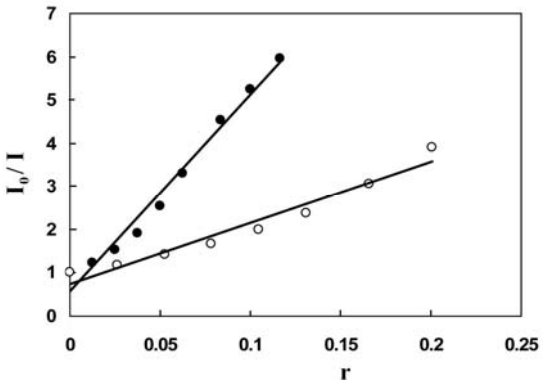


Fig. 9. Fluorescence quenching curve of EB (5μM) bound to DNA (12 μM) by TMPyP (●) and 5-CBPyP (○) at λ_{ex} = 510 nm as a function of [DNA]/[Porphyrin] mole ratio (r).

Rayleigh law.

The reason why this technique of resonance light-scattering is considerably useful for aggregation experiments is that absorption and scattering depend on the size of the aggregate in very different ways. Imagine the case in which a fixed concentration of material is under study. The absorption due to each sphere is proportional to the volume of the sphere, but the number of spheres per unit volume is inversely related to the volume of the sphere. The amount of absorption is therefore independent of the size of spheres. This is implied by the Beer-Lambert law since the absorption for a fixed path length should depend on the concentration of the material in the sample and nothing else. On the other hand, the scattering due to each sphere is proportional to the square of the volume. Since the number of density of spheres depends inversely on the volume, the amount of scattering is directly proportional to the volume of each sphere. Thus, the larger the aggregate, the greater the scattering.

Figures 10 and 11 demonstrate the SLI spectra of TMPyP and 5-CBPyP solutions in the presence of different NaCl concentrations, respectively. The SLI of the porphyrins does not even slightly enhance with [NaCl] increase. The scattering profiles shown in these figures are corrected taking into account both spectroflurimeter sensitivity on λ and NaCl effect.

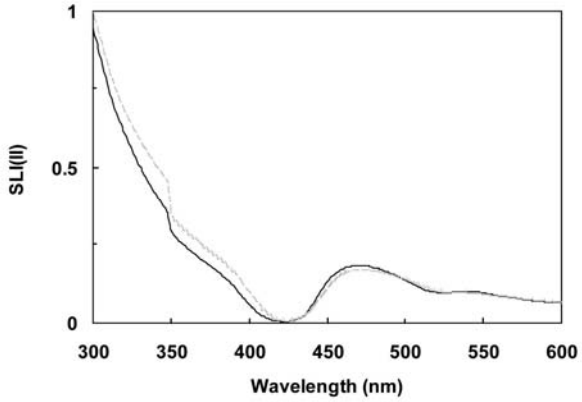


Fig. 10. Light scattering profiles of 3μM of TMPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (—) and presence of 2 M of NaCl (----).

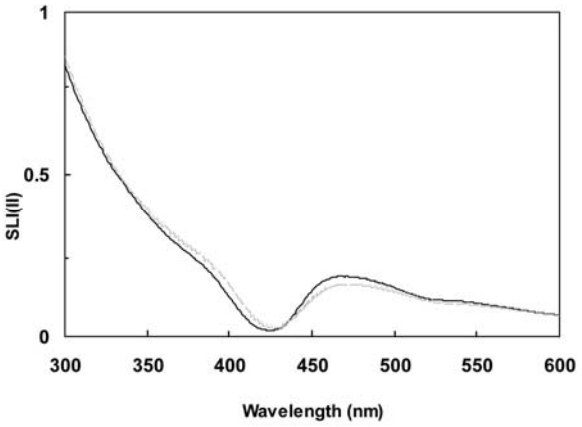


Fig. 11. Light scattering profiles of 5.3 μM of 5-CBPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (—) and presence of 2 M of NaCl (----).

Figures 12 and 13 show corrected SLI spectra of TMPyP and 5-CBPyP solution under addition of DNA concentration. These figures also represent the SLI of the porphyrins which does not enhance with [DNA] increase. Figure 14 shows the intensity of resonance light scattering for both porphyrins at λ = 470 nm. It also shows no enhancement in SLI of porphyrins due to interaction with ct-DNA at 470 nm. Hence, no aggregation is induced in these porphyrins by DNA binding and increasing salt concentration.

There exist three possibilities of porphyrin complex formation with DNA [53,54]:

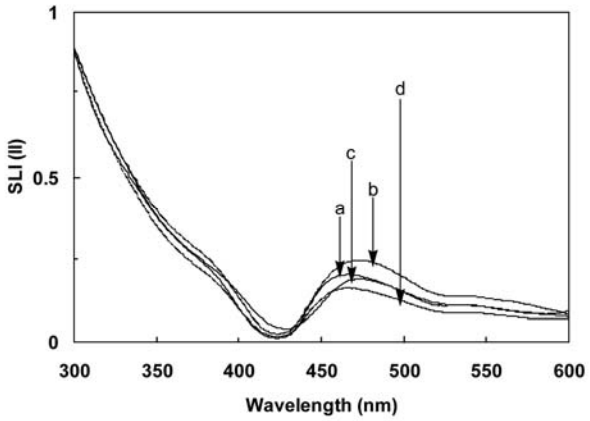


Fig. 12. Light scattering spectra of 3 μM of TMPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of various concentrations of ct-DND: 1.3 μM (b), 3.8 μM (c) and 7.5 μM (d) of DNA.

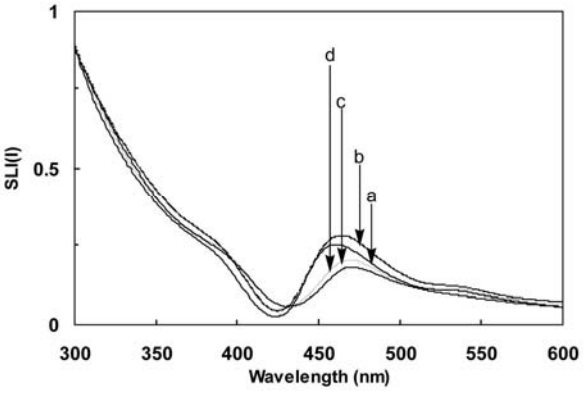


Fig. 13. Light scattering spectra of 5.3 μM of 5-CBPyP in 7.5 mM phosphate buffer (pH 7.2) in the absence (a) and presence of various concentrations of ct-DNA: 1.98 μM (b), 13.9μM (c) and 15.9μM (d).

1. binding of porphyrin monomers on the surface of DNA molecule (external binding),
2. intercalation of porphyrins into the interior of DNA molecules,
3. binding at the DNA molecule surface along with aggregation (self stacking).

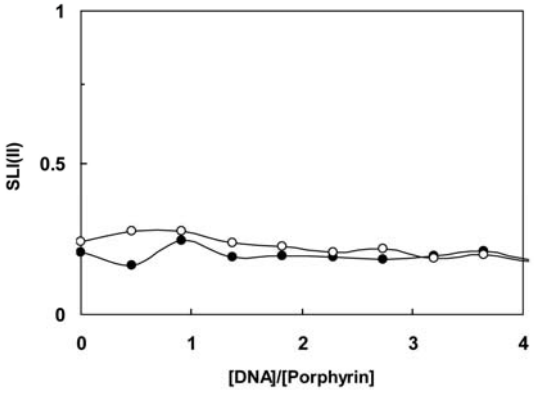
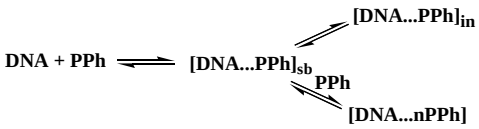


Fig. 14. The intensity of resonance light scattering of 3 μM of TMPyP (●) and 5.3 μM of 5-CBPyP (○) at λ = 470 nm as a function of [DNA]/[Porphyrin] mole ratio.

These porohyrins exist, in general, in equilibrium with each other and with free porphyrin molecules in solution.

The external monomer binding is the first step in the aggregation process. On the other hand, it is reasonable to suppose that this binding is also a necessary step preceding intercalation, as it has been demonstrated for acridine dyes at their intercalation with DNA [55].

In this case the system of equilibriums is written as follows:



where $[DNA…PPh]_{sb}$, $[DNA…PPh]_{in}$ and [DNA…nPPh] represent the superficially bound, the intercalation binding, and aggregation (self stacking) porphyrin molecules, respectively.

Thus, there exists a competition between intercalation binding and aggregation which reduces the degree of aggregation. Intercalation binding is observed for cationic porphyrins such as TMPyP [9-11]. The competition, therefore, exists for both porphyrins studied here, and intercalation binding mode causes no aggregation for the porphyrins at high concentration of ct-DNA and NaCl. On the other hand, the aggregation number depends on type, portonation state, and even on mode binding DNA-porphyrin.

CONCLUSIONS

Optical absorption and RLS measurement demonstrated no aggregation for the two porphyrins under study at high concentration of ct-DNA and NaCl. This is a major quality of both porphyrins in connection with their interaction with DNA.

The results of optical absorption of both porphyrins represent less tendency of 5-CBPyP for aggregation in comparison with TMPyP that can be taken as an advantage for 5-CBPyP to be used in PDT. However, the less values of extinction coefficients of 5-CBPyP that can be due to symmetry reduction, may reduce its efficiency in the production of single oxygen.

Absorption spectra of TMPyP and 5-CBPyP at the presence of ct-DNA results in large changes in the absorbance, and appreciable shift in wavelength, that indicates the intercalation binding mode for both porphyrins. This is in agreement with a recent investigation of pophyrin-nucleic acid interactions that indicates the homogeneous intercalation binding mode for TMPyP [10]. This means that the meso substitution of methyl group by $-(CH_2)_4COOH$ in 5-CBPyP does not change the binding mode. However, the probable steric effect of $(CH_2)_4COO^-$ group and its negative charge reduces the affinity of 5-CBPyP to DNA in comparison with TMPyP.

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