Spectroscopic studies on the interaction of a water-soluble cationic porphyrin with bovine serum albumin

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Abstract. The interaction of a water-soluble cationic porphyrin, Cobalt(III) 5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl) porphyrin [Co(III)TMPyP], with bovine serum albumin (BSA) has been studied in 1 mM phosphate buffer pH 7.0 containing 5 mM NaCl by UV-vis absorption, resonance light scattering (RLS) and fluorescence spectroscopies at 25°C.

The results of RLS studies represent no aggregate formation of porphyrin in the surface of BSA and low tendency of this porphyrin for aggregate formation.

The binding of porphyrin complex to BSA quenches fluorescence emission of BSA via a dynamic mechanism and the quenching process obeys a linear Stern-Volmer relationship. The values of Stern-Volmer constants, K_{SV}, was determined nearly 10^5 M^{-1}, that depend on BSA concentration. The average aggregation number of BSA calculated from the analysis of fluorescence quenching data indicates that absence of any porphyrin induced aggregation of BSA due to its interaction with porphyrin complex. The binding of Co(III) TMPyP had no obvious effect on the molecular conformation of the protein. Electrostatic force played an important role in the binding due to the opposite charges on porphyrin and the protein.

Keywords: Porphyrin, serum albumin binding, fluorescence quenching, optical absorption

1. Introduction

The research on water-soluble porphyrins has received much interest in recent years [1–6]. Because of their excellent bioactivity water-soluble cationic porphyrins can be used as potential anticancer [7, 8], antibacterial agents [9, 10] and probe for structure and dynamics of DNA [11]. Serum albumin is the natural carrier protein for photosensitizers such as porphyrin [12] and performs a key role in the transport of drugs such as porphyrins *in vivo* [13]. Porphyrin molecules could be carried to tumour region selectively and transported into tumour cells by serum albumin. The binding interaction between porphyrin and protein with one or several binding sites can affect the transportation and metabolism of porphyrin [14]. On the contrary, the binding of porphyrin on protein will change the molecular conformation affecting the physiological function of the protein [14].

Therefore, it is of great significance to study interaction between porphyrin and biomacromolecules such as protein and DNA. The interaction between porphyrin and proteins has been well reported, but this has predominantly involved the deuteroporphyrin [15–18] and hematoporphyrin [19, 20]. The interaction between water-soluble cationic Cobalt(III) 5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl) porphyrin

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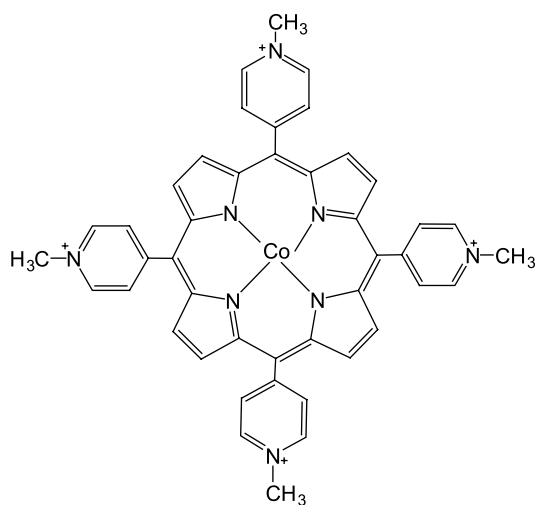
Scheme 1. The Chemical structure of Co(III)TMPyP.

(Co(III)TMPyP) and DNA has been well studied [21] but its binding with protein has not been reported so far as we know.

In the present work, we have reported the interaction of water-soluble cationic porphyrin Co(III)TMPyP (scheme 1) with bovine serum albumin (BSA) a typical globular protein, using absorption, fluorescence and resonance light scattering (RLS) spectroscopy. Fluorescence experiments allowed for determining binding sites, Stern-Volmer constants, BSA aggregation number and quenching rate constants. To examine the possibility of porphyrin aggregate formation in the surface of BSA, the RLS measurements were done.

2. Experimental

2. Materials and methods

2.1. Chemicals and preparations

Co(III)TMPyP was synthesized and purified by the method described previously [22]. BSA (free fatty acid, fraction V) was obtained from Sigma Chemical Co. and purified as follow: About 500 mg of albumin sample was dissolved in ∼10 mL of 0.010 M 1, 10-phenanthroline (pH 6.0), then dialyzed against several charges of the 1, 10- phenantroline buffer over several days at 4°C for removing copper ions, followed against several charges of 0.1 M NaClO$_4$ in 1 mM phosphate buffer (pH 7. 0) to remove protein bound phenanthroline, and finally against several charges of 1 mM phosphate buffer (pH 7. 0) to remove perchlorate anions The solution was kept in fridge for about 6 hour and then transferred to freezer drier for lyophilizing. A white-yellow powder was obtained after three days storing in refrigerator (about 4°C) and used for preparation of fresh protein solution before any measurement. Other materials were purchased from Aldrich and Merck companies. All experiments were carried out in triple distilled water in 1 mM phosphate buffer, pH 7.0, containing 5 mM NaCl at 25°C. All solutions were prepared immediately prior to use.

2.2. Optical absorption

For the optical absorption experiments the porphyrin solutions were prepared in concentrations varying in the range from 0 to 12.31 μM for measurements in the sort-band region. The absorption spectra were recorded on Cary 500 scan UV-vis-NIR spectrophotometer.

2.3. Fluorescence measurements

Porphyrin complex do not show any fluorescence emission spectra but emission of BSA is quenched due to its interaction with porphyrin complex. Emission spectra were recorded on a spectrofluorimeter Shimadzu model RF- 5000. In a typical experiment, titration of 22 μL of a BSA solution (6.32×10^{-7} M) in 26 mM phosphate buffer, 5 mM NaCl, pH 7.0 with porphyrin was performed by stepwise addition of porphyrin solution (6.25×10^{-6} M) in the same buffer directly to the cuvette. The solutions were excited at 290 nm and the emitted light intensity was measured at 342 nm, respectively. The observed fluorescence intensities were also corrected for dilution and temperature was kept constant at 25°C during titration experiments. The competition experiment with phenyl butazone was done using spectrofluoremeter. In this regard, 2.5 mL of BSA (6.32×10^{-7} M) containing 3 μL of phenyl butazone (1.06×10^{-5} M) was titrated by porphyrin solution (6.25×10^{-6} M).

2.4. Resonance light scattering

The light scattering measurements were done on a Shimadzu model RF-5000 spectrofluorimeter. The scattered-light intensity was monitored using the

right-angle in the synchronous scanning regime of the excitation and emission monochromators in the region from 300 to 600 nm. The experimental light-scattering spectra were corrected taking into account the solution optical absorption and instrument sensitivity dependence on the wavelength as described elsewhere [23]. All experimental data are the averaged values of at least five independent experiments.

3. Results and discussion

3.1. Fluorescence measurements

Fluorescence spectroscopy was used to monitor perturbation in the tertiary structure of BSA induced by porphyrin binding. These interactions can, in principle, produce changes in the position or orientation of the tryptophan residues altering their exposure to solvent, and leading to alteration the relative quantum yield of fluorescence. The stepwise addition of Co(III)TMPyP to BSA in 1 mM phosphate buffer pH 7.0 resulted in a progressive quenching of the intensity at all wavelengths, as typically shown in Fig. 1.

BSA has two major binding sites for drugs: (i) the direct contact site from tryptophan to porphyrin-like drugs involves site I and (ii) from tyrosine involves site II [24]. 290 nm light excites tryptophan residues, while 280 nm light excites both tryptophan and tyrosine residues [25]. A comparison of quenching effects when BSA was excited at 290 and 280 nm reveals that only the tryptophan of BSA interacts with porphyrin complex. Therefore, it can be inferred that the most probable binding site for porphyrin compound is site I of BSA, which is placed in sub-domain IIA where tryptophan 214 is located. A large hydrophobic cavity is present in the IIA sub-domain [26–28]. Therefore, interaction between porphyrin compound and BSA should be predominantly hydrophobic. However, it can not ignord the minor role of electrostatic interaction that causes the binding affinity. Binding of porphyrin and porphyrin-like compounds to site II of BSA correlates well with PDT efficiency *in vivo* [24]. This can be due to decreasing of molar absorbtivity of bound porphyrin in its Q-band as a consequence, this porphyrin compound, which bind only to site I of BSA might be inactive as photosensitizer in PDT. However, the binding is an equilibrium and porphyrin can be active after dissociating from BSA.
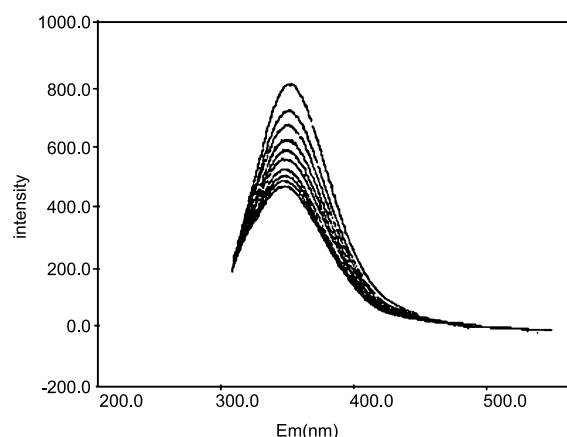

Fig. 1. Corrected emission spectra of BSA, (0.2 mg/ml solution) plus added Co(III)TMPyP (4.25×10^{-6} M) solution in 1 mM phosphate buffer, 5 mM NaCl, pH 7.0. The emission intensity is decreased due to the increase of porphyrin concentration.
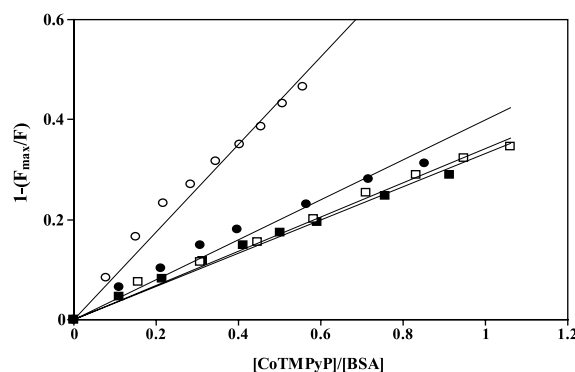

Fig. 2. The Stern-Volmer plots for BSA quenching by porphyrin complex. $\lambda_{ex} = 290$ nm, $\lambda_{em} = 342.4$ nm and spectral band width are 5 nm for both excitation and emission slits, at the different [BSA](mg/l): 0.1 (■), 0.2 (□), 0.3 (●), and 0.4 (○).

Figure 2 shows the titration of BSA solution as a function of Co(III)TMPyP concentration. The intrinsic fluorescence was quenched gradually and small blue shifts of fluorescence spectra were observed on increasing of the Co(III)TMPyP concentration.

Addition of porphyrin complex to BSA does not significantly influence the optical absorption of BSA. Therefore, it can be concluded that the mechanism of quenching is dynamic. For dynamic fluorescence quenching of BSA, a Stern-Volmer linear relationship can be applied:

$$\frac{F}{F_{max}} = 1 + K_{SV}[Q] \qquad (1)$$

Table 1

The values of Stern-Volmer quenching constants (K_{SV}) and average aggregation number of BSA molecule $< J >$, for interaction of porphyrin complex with BSA in 1 mM phosphate buffer, pH 7.0, 5 mM NaCl

[BSA](mg/mL)	$K_{SV}(M^{-1}) \times 10^{-5}$	$< J >$	R^2
0.1	5.97	0.291	0.9961
0.2	2.75	0.334	0.9903
0.3	2.30	0.685	0.9949
0.4	1.71	0.591	0.9934

where F and F_{max} are the luminescence intensities in the presence and the absence of quencher, respectively, [Q] is the quencher concentration and K_{SV}, the Stern-Volmer constant. The linear Stern-Volmer plots shown in Fig. 2 indicate that Equation 1 is applicable for the present system. The quenching plots illustrate that quenching of BSA by porphyrin is in good agreement with the linear Stern–Volmer equation, which also proves that the porphyrin bind to the protein. The values of K_{SV} listed in Table 1 represent the relative affinity of porphyrin complex for BSA. The following binding affinity sequence deduced from K_{SV} values Co(III)TMPyP is in agreement with UV-vis results.

The average aggregation number, $< J >$, of BSA potentially induced by porphyrin complex can be determined by quenching data analysis [29]. It has been previously shown that Equation 2 holds.

$$1 - \frac{F}{F_{max}} = \langle J \rangle \frac{[Porphyrin]_o}{[BSA]_o} \qquad (2)$$

Figure 3 shows the variation of $1 - \frac{F}{F_{max}}$ vs $\frac{[Porphyrin]_o}{[BSA]_o}$ molar ratio. The linear portions have just been presented. Deviation from Equation 2 at high porphyrin concentration is due to the presence of free porphyrin molecules in the solution which do not fulfill the assumptions in the corresponding theory [29]. The $< J >$ values, calculated from the slope of the lines in Fig. 3 listed in Table 1, indicate that porphyrin binding does not induce any aggregation in BSA molecules and consequently confirm the 1:1 stoichiometry for porphyrin:BSA adducts, in complete agreement with UV-vis results.

3.2. Optical absorption

The electronic absorption spectral features of Co(III)TMPyP shows a Q-band at 550 nm and a Soret band at 436 nm. Beer's law experiment was carried out for porphyrin in homogeneous aqueous solution at
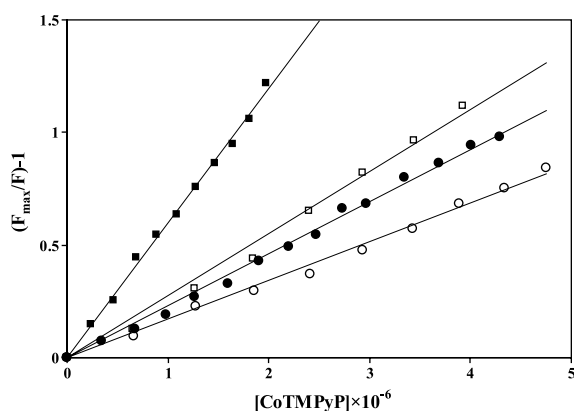
Fig. 3. Determination of the average aggregation number of BSA ($< J >$) in the presence of porphyrin complex. $\lambda_{ex} = 290$ nm, $\lambda_{em} = 342.4$ nm and spectral band width are 5 nm for both excitation and emission slits, at the different [BSA](mg/l): 0.1 (■), 0.2 (□), 0.3 (●), and 0.4 (○).
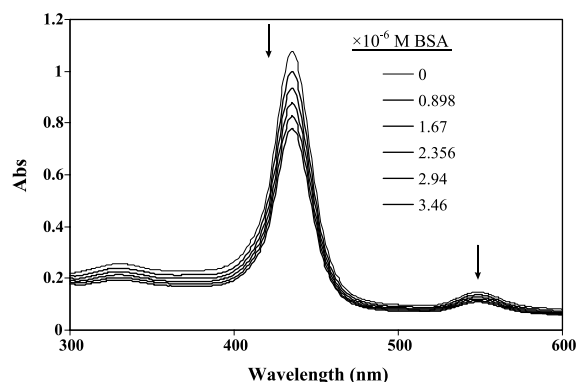
Fig. 4. Absorption spectra of Co(III)TMPyP (4.32×10^{-6} M) upon titration with BSA in solution in 1 mM phosphate buffer, 5 mM NaCl, pH 7.0, at 25°C. The spectra were recorded about 5 min after each addition (30 μL) of BSA stock solution. The arrows indicate the direction of spectra changes due to increasing of BSA concentration.

pH 7. The absorption in the Soret band obeys from Beer's law in the concentration range of 0 to 8.41 μM. These findings suggest that the complex do not aggregate in the experimental concentration range.

UV absorption spectrum is an important method and application to explore the structural change and to know the complex formation [30]. In order to confirm the probable quenching mechanism of fluorescence of BSA by porphyrin is initiated by ground-state complex formation, the UV absorption spectra of porphyrin solution at fixed concentration and varying BSA concentration in phosphate buffer at low ionic strength were measured. Figure 4 shows a representative

spectral change of Co(III)TMPyP upon increasing addition of BSA at 25°C. The addition of protein, BSA changes the intensity of the soret and Q-band in the absorption spectra of Co(III)TMPyP, which indicate that the fluorescence quenching of BSA was mainly caused by complex formation between Co(III)TMPyP and BSA.

3.3. Resonance light scattering

The scattered-light intensity (SLI) of a solution in the absence of optical absorption depends on the wavelength as $1/\lambda^4$ (Rayleigh law). The buffer and BSA solutions in the absence of porphyrin do not absorb in the spectral region studied, and thus the SLI spectra of solutions at different BSA concentrations are described by Rayleigh law. In the spectral region where a solution has optical absorption an increased SLI can be observed due to the increase of refractive index of the scattering medium in this region (the RLS effect) [31, 32] and references therein. Usually this increase is masked by the absorption. However, when aggregates are formed this effect can be strong enough since the RLS intensity is proportional to the square of the scattering particle volume. Thus, the RLS effect can be used as a test of aggregate formation [31, 32]. Hence, we employed RLS measuring for monitoring any probable aggregate formation of porphyrin. The reason that this technique of resonance light-scattering is so useful for aggregation experiment is that absorption and scattering depend on the size of the aggregate in very different ways. Imagine the case in which affixed 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 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 produces the greater the scattering. Figure 5 shows corrected SLI spectra of porphyrin complex solution under addition of BSA concentration. This profile represents the
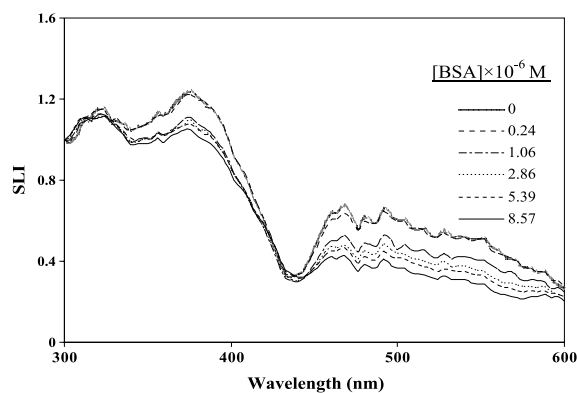
Fig. 5. Spectra of relative scattered light of Co(III)TMPyP solution in 1 mM phosphate buffer, 5 mM NaCl, pH 7.0, upon addition of BSA stock solution at 25°C.
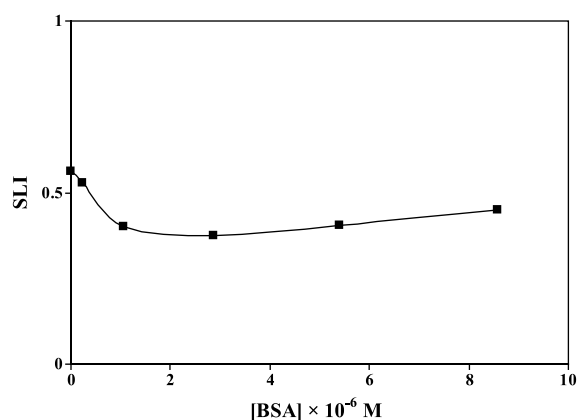
Fig. 6. The intensity of resonance light scattering of Co(III)TMPyP at wavelength of 436 nm, respectively, as a function of BSA concentration.

SLI of the porphyrin complex solution slightly do not enhance with [BSA] increase. Figure 6 shows the intensity of resonance light scattering for Co(III)TMPyP at wavelength of 436 nm as a function of BSA concentration. This also shows no enhancement in SLI of porphyin complex due to interaction with BSA. Hence, no aggregation is induced in porphyrine complex by BSA binding.

4. Conclusion

The results described in this paper showed that Co(III)TMPyP bound effectively to BSA. The aggregation of protein was not considered in the present

study and it could be reasonable for the low concentration of the protein and the electrostatic repulsion due to the binding of cationic porphyrin.

Binding of porphyrin complex to BSA quenches fluorescence emission of BSA through a dynamic mechanism. A linear Stern-Volmer relationship has been successfully applied for analysis of fluorescence quenching data. The values of K_{sv} confirm the UV-vis results on the sequence of affinity of porphyrin complex.

It has been also shown that porphyrin compound bind to site I of BSA located in sub-domain IIA by a competitive experiment with phenyl butazone as well-known site I marker. The results of RLS measurements are also in concords with these results and represents no- stack formation of studied porphyrin on BSA.

Acknowledgments

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