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Short Communication

Features of interaction of tetraiodide meso-tetra(*N*-methyl-3-pyridyl) porphyrin with bovine serum albumin

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ABSTRACT

The interaction processes spectral study of tetraiodide meso-tetra(*N*-methyl-3-pyridyl)porphyrin with blood serum albumin has been carried out. The results obtained are considered in comparison with similar data for tetraphenylporphyrin and tetraiodide meso-tetra(*N*-methyl-4-pyridyl)porphyrin. It is established that interaction of albumin with the porphyrins under study leads to a change in the secondary structure of the protein accompanied by a decrease in the proportion of disordered fragments of the protein and an increase in β-structuring within the complexes. NCH_3^+ group isomerism position in the porphyrin compound significantly affects binding to albumin. Adding of the substituent in meta-position of the phenyl ring contributes to the increased affinity of protein to the cationic porphyrin, compared to the pair-substituted counterpart.

1. Introduction

Photochemical processes involving porphyrins and metalloporphyrins have been extensively researched during the latest decades. Such great interest of the researchers is conditioned primarily by the application of porphyrins in the development of new photochemical methods and approaches to the treatment and diagnosis of various diseases. Compounds of the porphyrin class are considered as biomarkers and sensors, photosensitizers [1], medicines for cancer treatment [2, 3], medicinal substances possessing antiviral and antibacterial activity [4]. As a result of numerous researches it has been shown that, even with water-soluble compounds, it is advisable to use transport systems for their delivery. Selective delivery systems of porphyrins are monoclonal antibodies, capsules, low density lipoproteins and albumins [5]. Intravenous administration of cationic porphyrins preferably is transported by serum albumin [6]. Therefore, nowadays the study of the porphyrins binding with serum albumin is an important task for developing potential drugs [7, 8].

Earlier when studying DNA complexation with tetracationic porphyrins, differing in the position of NCH3+ group in the porphyrin phenyl ring, the differences not only in the affinity of the DNA with reference to the porphyrins, but also their different impact on the DNA when photoirradiated, were found. It is established that the irradiation of the porphyrins intercalates with DNA leads to DNA fragmentation, fragments of DNA of different sizes being formed in the case of tetraiodide meso-tetra(*N*-methyl-4-pyridyl)porphyrin (TMPyP4). According to earlier data obtained for TMPyP4 interacting with albumin [9, 10] specifically, the protein mentioned is able to act as a transport agent. The object of this work was the interaction processes spectral study of TMPyP3 with blood serum albumin as well as revealing the position of the NCH_3^+ group effect in the phenyl ring of the porphyrin on the protein binding, as similar data for tetraiodide meso-tetra(*N*-methyl-3-pyridyl)porphyrin (TMPyP3) are absent.

2. Experiments

The porphyrins studied were synthesized and purified by the method of column chromatography according to known techniques [11]. The purity was controlled spectrally, the extinction coefficients are consistent with the data known [11]. Bovine serum albumin, fraction V (BSA) was purchased at Acros Organics. NaCl (Reakhim, Russia) >99% was recrystallized before application. To prepare solutions bidistilled water was used. DMF distillation under vacuum was performed before its application. pH of all studied solutions was 7.0 ± 0.1.

Absorption and fluorescence spectra were recorded with the spectrometer AvaSpec-2048 (Avantes BV, Apeldoorn, Netherlands) at 25 °C. AvaLight DHS was used as a light source for absorption spectra. To register the fluorescence spectra of BSA UV diode UVTOP-295 (Sensor Electronic Technology, Inc. USA) was used as a light source, and Monochromatic LEDs LED395-02V, VL425-5-15 (Roitner Lasertechnik Gmbh., Germany) was used for recording porphyrins fluorescence spectra. pH

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was measured with Checker pH Tester HI 98103 (Hanna Instruments, USA).

IR spectra were recorded with IR-Fourier spectrometer Avatar 360 (Thermo Nicolet).

The Scatchard constants were calculated as published earlier [12]. Porphyrin absorption effect on fluorescence spectrum was considered according to the recommendations by Lacowicz [13]. Fluorescence correction was made in terms of the equation:

$$F_{corr} = F_{obs} \cdot 10^{\left(\frac{A_{ex}+A_{em}}{2}\right)}$$

where F_{corr} – corrected fluorescence, F_{obs}-observed fluorescence, A_{ex} is the optical density of the solution at the excitation wavelength and A_{em} is the optical density at the emission wavelength. Quantum-chemical calculations were performed in Gaussian 09w Rev. A. 02 [14] by B3LYP level with 3–21 basis.

3. Results and discussion

According to the spectrophotometric titration, the interaction of albumin with TMPyP3 leads to fluorescence quenching of the protein due to irradiation by light at a wavelength of 295 nm (Fig. 1). The fluorescence of the protein under given radiation conditions is provided by the presence of fluorophores in a protein globule, the former being tryptophan amino acid residues which occupy 135 and 214 position in the polypeptide chain and which are localized in the IB and IIA subdomains.

It should be noted that the fluorescence quenching of protein with TMPyP3 was not complete unlike TMPyP4. The fluorescence quenching of BSA with TMPyP4 at the same concentrations was carried out much more efficiently [10]. The fluorescence quenching of porphyrin may be due to the action of the porphyrin compounds as the quenchers (according to static or dynamic mechanisms of quenching). In this case, the porphyrin needs to be localized in subdomains IB and IIA or porphyrin can bind with the protein according to other binding sites, thus causing a conformational changes in a protein globule in which the quenching of the Trp214 fluorescence and Trp135 is carried out by adjacent amino acid residues of the polypeptide chain. In the latter case, the native conformation of the protein needs to be broken. The conformational state of the protein was controlled by IR spectra (Table 1). In the BSA IR spectra some amide regions were discerned (amide I region 1580–1720 cm^{-1}, amide II region 1400–1580 cm^{-1} and amide III region 1330–1220 cm^{-1}), and the position of the bands in them allows to define the conformational state of a protein. The data obtained are presented in Table 1, along with previously unpublished data for TMPyP4 and TPP.

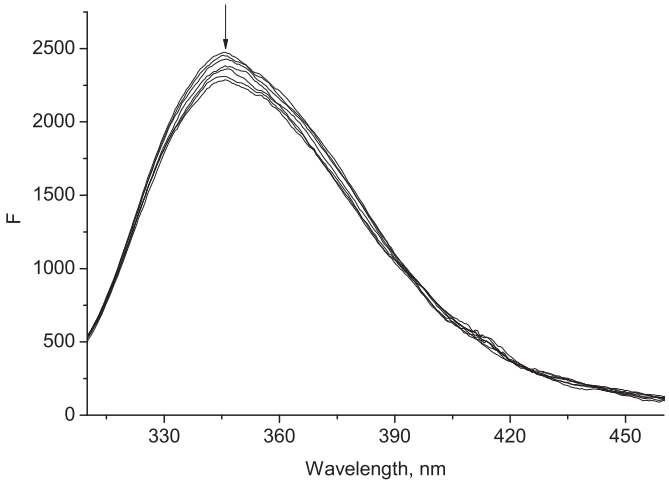

Fig. 1. The corrected fluorescence spectra of BSA (0.08% wt.) during the titration TMPyP3 (0–3·10^{-5} M) in aqueous solution NaCl (0.05 M).

Table 1
The experimental frequency ν (cm^{-1}) in the IR spectra of albumin and its complexes in I-III amide regions.

System	Amide I region, ν, cm^{-1}	Amide II region, ν, cm^{-1}	Amide III region, ν, cm^{-1}
BSA	1654	1537	1304
		1446	1262
			1245
BSA-TMPyP3	1642	1536	1310
		1515[b]	1260
		1451	
		1462[b]	
BSA-TMPyP4	1638	1533	1305
		1512[b]	1277
		1438	1235
		1455[b]	
[a]BSA-TPP	1649	1532	1312
		1512[b]	1260
		1445	
		1460[b]	

[a] Isolated from the solution H_2O–NaCl (0.05 M)-DMF (0.19 M).
[b] The vibrational frequencies recorded in the complex of BSA-porphyrin and individual porphyrin.

IR spectra of BSA are typical for albumin in its native conformation [15–17], where the main type of secondary structure of albumin are α-spirals comprising no <67%, the remaining 23% percent falling on an outstretched, extended, disordered structure and, to a much lesser extent, on β-folding. The complexation of BSA with all the porphyrins results in a shift of the absorption maximum in the I amide range in the low frequency region in 5, 12 and 16 cm^{-1} for TPP, TMPyP3 and TMPyP4-TPP, respectively. This shift is interpreted in the scientific papers literature [18] as the changes in the secondary protein structure associated with the transition from α-spirals to β-folding. The main bands typical for BSA in the native conformation in the amide II region when binding to porphyrins undergo a slight shift. The band in 1538 cm^{-1} region is shifted to low frequency region from 1 to 5 cm^{-1} depending on the porphyrin (Table 1). It is problematic to interpret reliably the shift in the 1446 cm^{-1} region, as all the studied porphyrins have an intense vibration bands in the spectral range, the maxima of which is given in Table 1. The amide III region for the binding porphyrins with protein is also changing (Table 1). Three bands falling into the intervals which are characteristic for the three types of protein secondary structure: α-spiral (1330–1290 cm^{-1}); β-folding (1290–1260 cm^{-1}), random spiral (1250–1220 cm^{-1}) [19] were found in the case of the complexes of TMPyP4 with BSA and free BSA. According to the obtained data (Table 1), there is only α- and β-structure and the signal indicating the presence of disordered segments of the protein in the complexes of BSA with TMPyP3 and TPP is not recorded.

Thus, having made a comparative analysis of IR spectral development of BSA complexation with the porphyrins, one can conclude that the protein binding of porphyrins leads to the changes in protein secondary structure, as well as to the increase to β-folding extent but the native conformation of the protein retains. Therefore, the detected fluorescence quenching of albumin during the TMPyP3 titration (Fig. 1) is due to the binding of the porphyrin at sites IB and IIA proteins.

To quantify the binding parameters of the protein globule of porphyrins the technique of Scatchard was used, the obtained parameters are

Table 2
Scatchard constants and number of binding sites of BSA with the porphyrins.

	Scatchard constants	Number of binding sites
TMPyP3-BSA	1.01×10^5	1.4
	8.4×10^4	2.8
TMPyP4-BSA [10]	2.94×10^5	2.2
TPP-BSA [20]	5.70×10^4	0.83