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ORIGINAL PAPER

Spectroscopic Detection of Tetracationic Porphyrin H-Aggregation on Polyanionic Matrix of Inorganic Polyphosphate

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Abstract Self-assembly of tetracationic porphyrin $TMPyP^{4+}$ onto polyanionic matrix of inorganic polyphosphate (PPS) in aqueous solutions has been studied in a wide range of molar phosphate-to-dye ratios using techniques of polarized fluorescence, absorption, resonance Raman spectroscopy and static light scattering. The binding of $TMPyP^{4+}$ to PPS is characterized by the binding constant of 3×10^5 M^{-1} and the cooperativity parameter of about 150. The fluorescence quenching of the bound $TMPyP^{4+}$ evidences the stacking of the porphyrine chromophores. Under the stoichiometric binding ratio $TMPyP^{4+}$ forms extended continuous face-to-face aggregates (so-called H-aggregates) which manifest themselves by a blue shift (12 nm) and a large hypochromisity (51%) of the Soret absorption band. Each face-to-face $TMPyP^{4+}$ stack is formed with participation of four PPS chains. Formation of such columnar aggregates is promoted by the ability of PPS chains to take a helix conformation where negative charges are arranged along two oppositely situated rows with intercharge distance of 0.36 nm which corresponds to the thickness of the porphyrin π-electronic system. The ability of each PPS strand to be template for formation of two porphyrin stacks results in the integration of the adjacent stacks into higher-order aggregates which dimension was estimated from the fluorescence polarization data.

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Introduction

The phenomenon of molecular aggregation of porphyrins is a subject of considerable attention conditioned by unique electronic and spectroscopic properties as well as the significant role of one-dimensional chromophore self-assemblies in the development of nano-scale molecular devices [1, 2]. Two main types of molecular aggregates formed by porphyrin molecules are known, namely, J- and H-aggregates [3, 4]. In J-aggregates the transition dipoles of the constituent molecules are relatively oriented as "head-to-tail", so that porphyrin molecules are arranged as "offset card deck" (side-by-side arrangement), and they can be disposed linearly or circularly [5]. Unlike this, in H-aggregates the transition dipoles of the individual monomers are aligned parallel to each other and perpendicular to the line joining their centers, in such a way the porphyrin macrocycles are stacked together face-to-face forming columnar structures [6]. The above two aggregation types have different spectroscopic signatures [7]: the Soret absorption band for porphyrin J-aggregates is red shifted relative to its position in the dye monomeric spectrum, while for H-aggregates it is blue shifted.

Earlier aggregation of tetraaryl-substituted porphyrins which exhibit a tendency to self-associate in aqueous solutions was widely investigated [8–13]. At the same time it has been shown [14, 15] that tetracationic *meso*-tetrakis (4-N-methyl-pyridyl)porphine ($TMPyP^{4+}$, Fig. 1a) is incapable to form aggregates or dimers in aqueous solutions

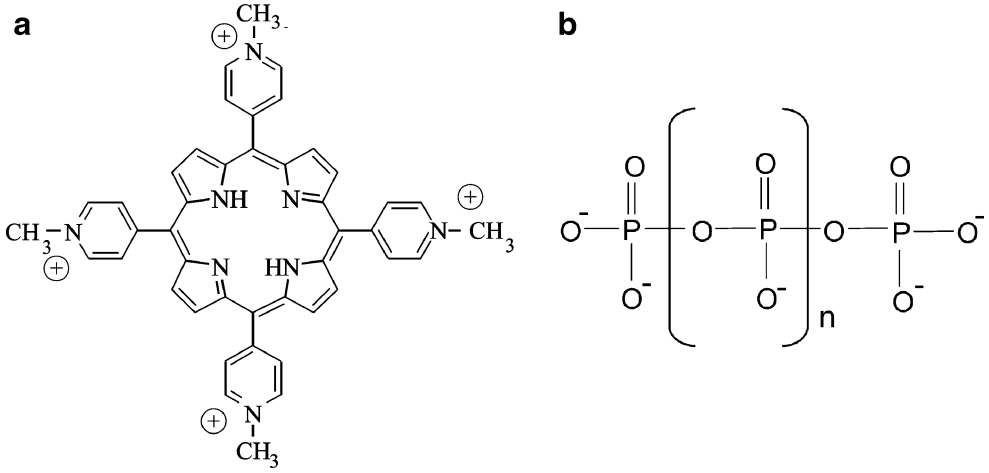

Fig. 1 Structures of tetracationic porphyrin $TMPyP^{4+}$ (**a**) and polyanionic chain of polyphosphate (**b**)

owing to electrostatic repulsion of their molecules. However the aggregation of $TMPyP^{4+}$ occurs when its peripheral positive charges are neutralized under the association with anions. So, it took place in aqueous systems containing sodium borohydride [16] or a calixarene derivative [17], where formation of porphyrin J-aggregates was observed. Extended self-assembly of $TMPyP^{4+}$ on polyanionic backbones of DNA [18, 19] and polynucleotides [20] obviously also belongs to J-type aggregation. At the same time, extended H-assemblies of this porphyrin in aqueous solution have not been obtained, although its H-dimer or H-tetramer in LB films has been reported [21, 22]. The possible mutual disposition of $TMPyP^{4+}$ molecules composing J- and H-aggregates is clearly shown in [17].

In the present paper we have provided evidence of the extended H-type self-assembly of $TMPyP^{4+}$ in aqueous solutions induced by its interaction with polyanionic matrix of inorganic polyphosphate (PPS). PPS represents a linear chain of orthophosphate residues each carrying a monovalent negative charge (Fig. 1b) therefore it can serve as polyanionic scaffold to assemble cationic macromolecules [23]. The rotational flexibility of the P-O-P bonds allows the conformational adjustment of PPS chains to the $TMPyP^{4+}$ stacks. The aim of the work was the spectroscopic and structural characterization of the $TMPyP^{4+}$ H-aggregates revealed.

Experimental

The tetra-*p*-tosylate salt of *meso*-tetrakis(4-N-methyl- pyridyl)porphine was obtained from Aldrich Chemical Co. and sodium polyphosphate with degree of polymerization approximately 75 was purchased from Sigma Chemical Co. The deionized water from Millipore-Q system was used as a solvent for all sample preparations. The porphyrin concentration was determined spectrophotometrically in water using the extinction coefficient of ε_{424}=226000 M^{-1} cm^{-1} at the Soret band maximum [24]. The PPS concentration was estimated from the dissolved substance weight (1% solution corresponds to 98 mM phosphates).

Electronic absorption spectra were measured on a SPECORD M40 spectrophotometer (VEB Carl Zeiss, Jena). Quantitative measurements of both steady-state fluorescence and light scattering characteristics were carried out by the method of photon counting with a laboratory spectrofluorimeter [25]. The exciting light beam was selected by a double monochromator from stabilized radiation of a Philips halogen lamp. To determine the sample absorbance under the excitation wavelength, the intensity of beam was registered after its passing through the optical cell using a photodiode detector. The fluorescence and light scattering intensities were registered at a right angle to the incident beam. The radiation from small solution volume selected in the centre of the cuvette was collected, so that the emission from the porphyrin molecules adsorbed onto cuvette walls did not hit inside the emission monochromator. Ahrens prisms were used to polarize linearly the exciting beam as well as to analyze the fluorescence polarization and scattered radiation. The spectrofluorimeter was equipped with a quartz depolarizing optical wedge to exclude the monochromator polarization-dependent response. Fluorescence spectra were corrected on the spectral sensitivity of the spectrofluorimeter. When measuring the fluorescence or light scattering intensities, the pulses from photomultiplier tube were accumulated during 10 s for each data point and measurements were repeated five times, at that the measurements error was about 0.5%. A cuvette compartment of the spectrofluorimeter was equipped with a telescope to observe visually aggregation processes in solutions.

The binding of $TMPyP^{4+}$ to PPS was followed by changes in parameters of the porphyrin fluorescence under titration experiments. The fluorescence of $TMPyP^{4+}$ was excited at λ_{exc}=500 nm (in the region of the Q_y (0,1) absorption band) and its intensity was registered near the

maximum of uncorrected emission band, at λ=677 nm, (the emission spectral slit was 1 nm). The fluorescence intensity, F, and degree of fluorescence polarization, p, were calculated using formulas [26]

$$F = F_{\parallel} + 2F_{\perp}, \quad p = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}} \tag{1}$$

where $F_{\parallel}$ and $F_{\perp}$ are measured intensities of the emitted light, which are polarized parallel and perpendicular to the polarization direction of the exciting light beam, respectively. The relative quantum yield of the fluorescence was calculated from the equation

$$\frac{Q}{Q_0} = \frac{F_S}{F_{S0}} \cdot \frac{A_0}{A} \tag{2}$$

where F_{S0} and F_S are integral intensities of $TMPyP^{4+}$ fluorescence in the free state and in the mixture with PPS, respectively, which are calculated as areas under the corresponding fluorescence spectra; A_0 and A are the absorbances of these samples under λ_{exc}.

The aggregation process arisen in the solutions was followed by the intensity of steady-state light scattering (SLS) (both the excitation and emission monochromators were set at λ=500 nm). The polarized components of the scattering intensity $I_{\parallel}$ and $I_{\perp}$ were measured by similar way to measurements of $F_{\parallel}$ and $F_{\perp}$, and the depolarization ratio was defined as $\rho_v = I_{\perp}/I_{\parallel}$.

Resonance Raman spectra were recorded on Raman spectrometer DFS-52 (Russia) equipped with a double monochromator (reverse dispersion 3.5 Å/mm) using the excitation by 457.9 nm emission line of an Ar ion laser (power 25 mW). The Raman signal was collected using conventional 90° geometry and detected with a thermoelectric-cooled (till −30°) CCD camera. The comparative peak positions in the spectra were determined with an accuracy of 0.3 cm^{-1}.

In titration experiments the sample of [$TMPyP^{4+}$]= 10 μM was added with increasing amounts of the concentrated PPS solution containing the same porphyrin content, whereupon fluorescence and SLS intensities were measured. The time from 7 to 10 min was required to reach the thermodynamic equilibrium in the system which was verified from approach to the stability in the fluorescence or light scattering signal. The aim of titration experiments was to obtain dependences of the fluorescence and light scattering characteristics on the molar phosphate-to-dye ratio, P/D. The experiments were carried out in deionized water and in 0.14 M aqueous solution of NaCl.

All measurements were carried out in quartz cuvettes at room temperature from 22 to 24 °C.

Results and discussion

Fluorescent titration curves

The results of fluorescence titration of $TMPyP^{4+}$ with PPS are shown in Figs. 2 and 3 as P/D-dependences of the changes in the relative fluorescence intensity, F/F_0 (F_0 is fluorescence intensity of the free porphyrin), and fluorescence polarization degree, p. Both the dye fluorescence quenching and p increase indicate formation of stacking-aggregates by the tetracationic porphyrin molecules on PPS polyanionic matrix. In the case of titration in water without counterions, the fluorescence parameters reach the extreme values: $(F/F_0)_{min}$=0.18 at P/D= 5.6 and p_{max}=0.095 at P/D= 5.2 (Fig. 3). With a further P/D increase the titration curves reflect decomposition of $TMPyP^{4+}$ aggregates since F/F_0

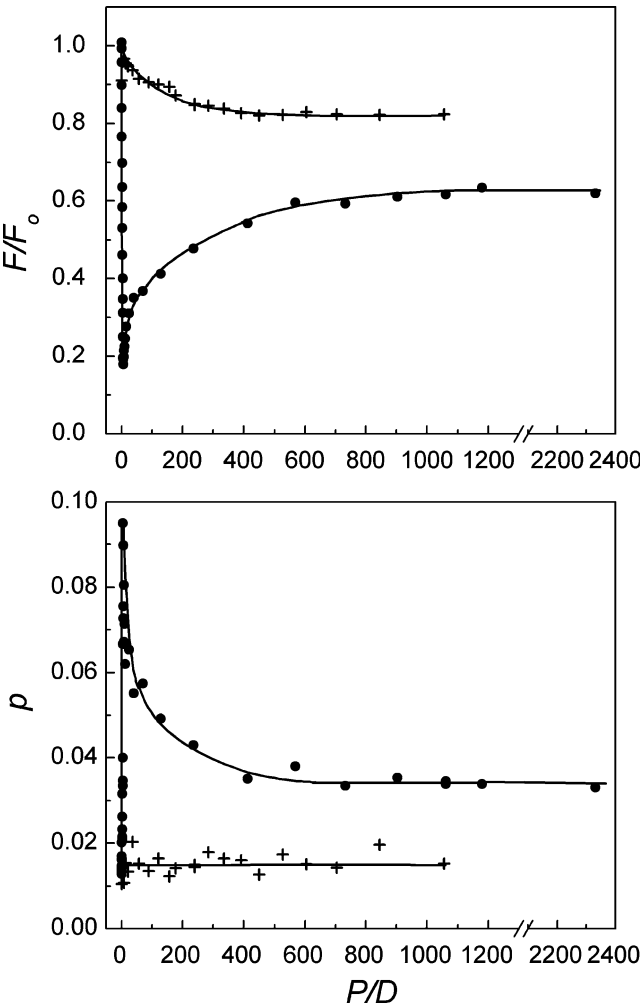

Fig. 2 Dependence of the relative intensity, F/F_0 (F_0 is the fluorescence intensity of the free dye), and polarization degree, p, of $TMPyP^{4+}$ fluorescence on the molar polyphosphate-to-porphyrin ratio, P/D. The data were obtained in deionized water (•) and in water with 0.14 M NaCl (+). The total porphyrin concentration was constant, i.e. [$TMPyP^{4+}$]=10 μM

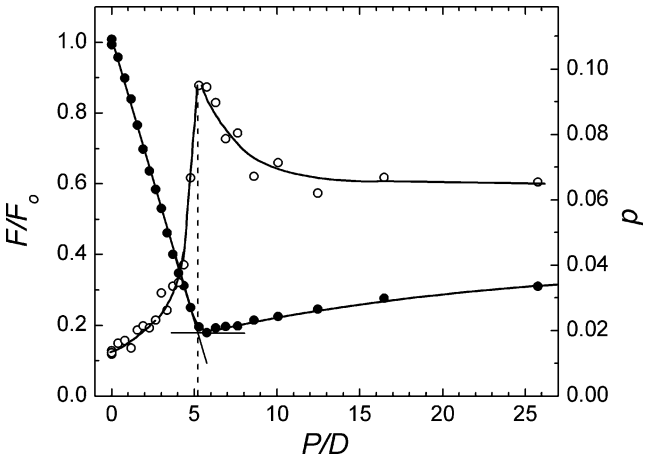

Fig. 3 Initial sections of fluorescence titration curves for PPS-$TMPyP^{4+}$ system in deionized water which are shown in Fig. 2. Here (●) symbols correspond to the relative fluorescence intensity data, F/F_0, and (○) ones indicate the fluorescence polarization degree data, p

rises and p goes down. Under $P/D \geq 1{,}000$ these parameters have reached steady levels of $F/F_0=0.63$ and $p=0.034$ (Fig. 2). The last value is twice as large as that for the free monomeric dye, $p_0 \approx 0.015$. The increased p value evidences that after the decomposition porphyrin molecules remain bound to the polymer. The presence of 0.14 M NaCl in the solution strongly reduces the binding of $TMPyP^{4+}$ to PPS due to the competitive binding of Na^+ ions to the polyanionic matrix. It is evident from the weak fluorescence quenching and small change of p observed for the last sample (Fig. 2). The P/D-dependencies of F/F_0 and p were found to be reproduced when reverse titration experiments were carried out, that is under P/D decrease from highest to zero values. It means that a precipitation of the complexes has not occurred.

Absorption and fluorescence spectra

The visible absorption and fluorescent spectra of the free $TMPyP^{4+}$ and one bound to PPS are shown in Fig. 4. As can be seen, upon the porphyrin aggregation its absorption spectrum undergoes transformations depending on P/D value. Thus, at $P/D=5.6$ corresponding to the maximal fluorescence quenching, the absorbance maximum of the Soret band is decreased by 51% and blue-shifted by 12 nm (from 422 to 410 nm) whereas the $Q_y(0,1)$ band is red-shifted by 5 nm (from 518 to 523 nm) with respect to those of the free $TMPyP^{4+}$. The blue shift and large hypochromisity observed for the Soret band clearly indicate formation of H-aggregates [7]. The wide fluorescence spectrum of the free porphyrin with maximum at ~712 nm becomes narrowed and splits into distinct Q(0,0) and Q(0,1) fluorescence bands which peaks at ~667 and ~727 nm have approximately equal intensities. Such transformation of the fluorescence spectrum can be explained by the reduction of mixing of the porphyrin singlet excited state and CT one (intramolecular charge transfer between π-system of porphyrin macrocycle and pyridinium groups) for the assembled $TMPyP^{4+}$ molecules, caused by the (1) restriction of the pyridinium groups rotation toward coplanar geometry with the porphine ring or (2) destabilization of the CT state induced by the leaving of the molecules from the water environment [14].

The decrease in the fluorescence intensity evidences that the porphyrin chromophores come into stacking interaction. However, it should be mentioned that in the case of H-type porphyrins aggregation the fluorescence quenching is substantially less then for J-type ones. So, for our system the fluorescence quantum yield is about five times diminished ($Q/Q_0=0.23$). The same effect ($Q/Q_0=0.22$) was also observed earlier for H-aggregates formed by dianionic porphyrin H_4TPPS^{2-} [5]. While in the cases of J-type porphyrin aggregation the fluorescence was quenched almost completely, for instance, for H_2TPPS^{4-} ($Q/Q_0=0.008$) [5] and $TMPyP^{4+}$ (the exact Q/Q_0 ratio was not established) [16].

At the very high excess of PPS ($P/D \geq 1{,}000$) the H-aggregates dissociate into monomer molecules bound to PPS. However, their absorbance has not revert to the shape of the free dye spectrum showing the hypochromicity of ~23% and ~2 nm blue shift of the Soret band maximum. The fluorescence spectrum also differs from that for the free dye displaying a decreased intensity. Thus, at $P/D=1{,}200$ we have registered $Q/Q_0 \approx 0.8$. Such changes in spectroscopic properties of the bound monomeric porphyrin are presumably caused by the effect of neutralization of the positive charges at the N-methylpyridyl moieties under their interaction with the PO^- groups of PPS. Such an effect was earlier observed under binding of $TMPyP^{4+}$ to poly(L-glutamic acid) [27].

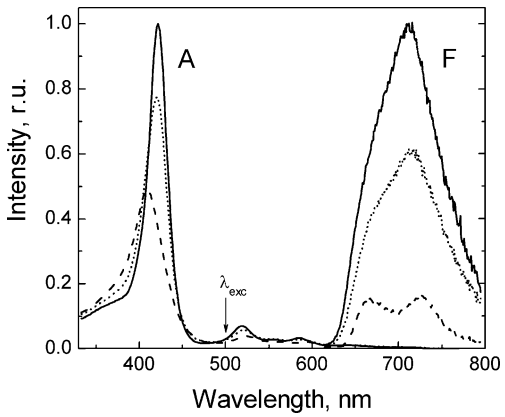

Fig. 4 Normalized absorption (A) and fluorescence (F) spectra of free $TMPyP^{4+}$ (——) and bound to polyphosphate one at $P/D=5.6$ (- - - - - - -) and $P/D=1200$ (·····), measured in deionized water. $[TMPyP^{4+}]=10$ μM; $\lambda_{exc}=500$ nm is the wavelength of fluorescence excitation

Light scattering

The aggregation process arising under binding $TMPyP^{4+}$ to PPS in water solutions without NaCl is readily observable from SLS measurements since it induces significant light scattering effect which can be clearly seen visually via a telescope. As shown in Fig. 5, in the initial part of titration curve (under low P/D values) the SLS intensity increases proportionally to *P/D*, reaching a maximal value at $P/D=5.3\pm0.1$ where the extreme fluorescence parameters $(F/F_0)_{min}$ and p_{max} are observed. Then it gradually went down with *P/D*. At $P/D=2{,}300$ SLS intensity amounts to only twice as much scattering signal from the porphyrin solution without PPS. Also we watched via a telescope the change in the sample viscosity during the titration experiments, namely from the damping of a solution rotational movement after the sample stirring. The viscosity was found to be not essentially increased indicating that the aggregates have not formed a network in solution. The SLS in the solution with 0.14 M NaCl is not substantially increased due to the competitive binding of Na^+ ions which prevent $TMPyP^{4+}$ binding to PPS as it was established by the fluorescent measurements.

The SLS depolarization ratio for the aggregates is found to be of $\rho_V(90)=0.033\pm0.002$ (see inset in Fig. 5). For comparison, for $TMPyP^{4+}$ alone the value $\rho_V(90)=0.003\pm0.001$ has been obtained. The very small value of the depolarization ratio for monomers shows that the porphyrin molecules are optically highly isotropic. The tenfold increase of $\rho_V(90)$ for the PPS-$TMPyP^{4+}$ complexes signifies increase of the molecular anisotropy under H-stacks formation.

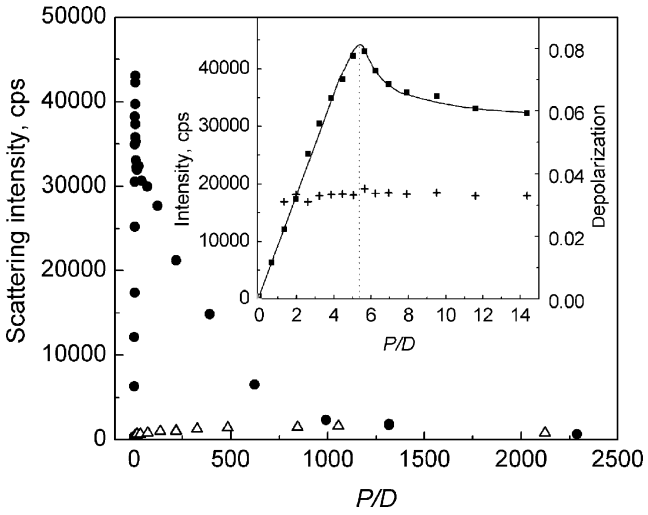

Fig. 5 *P/D* dependences of the light scattering intensity in the PPS-$TMPyP^{4+}$ system measured at λ=500 nm in deionized water (●) and in water with 0.14 M NaCl (Δ). The inset shows the initial part of the SLS intensity curve (■) and the depolarization, $\rho_V(90)$, (+) obtained in deionized water. $[TMPyP^{4+}]=10$ μM is constant

Resonance Raman spectra

Resonance Raman (RR) spectra of free $TMPyP^{4+}$ and its complex with PPS under $P/D=5.6$ (where the H-aggregates are formed) were obtained in the range of 750–1,300 cm^{-1} (see Fig. 6) where vibrations of pyrrol and pyridilic rings become apparent. The small changes in the pattern of RR spectra upon the the porphyrin aggregation were found. The stretching vibrations of the porphyrin macrocycle near 970 and 1,005 cm^{-1} assigned to $\nu(C_a–C_b)$ and $\nu(C_a–C_m)$, respectively [28] are shifted by 2 cm^{-1} to lower frequencies. Changes in vibrations attributed to the N-methylpyridyl group are also revealed. The frequencies of stretching vibration of bonds N^+-CH_3 near 795 cm^{-1} and C_m-pyr near 1,250 cm^{-1} are decreased by 1–2 cm^{-1}. The downward shifts of RR bands attributed to the porphyrin N-methylpyridyl ring suggest the existence of an electrostatic interaction between their N^+-CH_3 groups and PPS PO^- groups in the aggregate formed. Such slight changes in the vibration frequencies (1–2 cm^{-1}) are typical for the Coulomb interaction [29].

Parameters of the cooperative binding

The curve of F/F_0 dependence on *P/D* under the titration in deionized water (Fig. 3) has the shape typical for cooperative binding of cationic dyes to linear polyanions [30–32]. The straight line part of this curve corresponds to stoichiometric binding conditions. Being extrapolated to the level line of $(F/F_0)_{min}$ it produces the intersection point positioned at $P/D=5.2$ which determines the value of stoichiometric ratio of the complex formation. Of course, it is valid in the case if all porphyrin is bound. Our measurements performed under five times reduced porphyrin concentration, 2 μM, have not revealed an increase in $(F/F_0)_{min}$ within experimental error (not shown) that would be in the case of not enough efficient binding. Thus it is allowable to neglect the fraction of unbound dye.

Using results of fluorescent titration experiment in the presence of 0.14 M Na^+ ions we have calculated the apparent binding constant for the interaction of $TMPyP^{4+}$ with PPS in deionized water, *K*, using the known binding constant for Na^+ ($K_1=1{,}100$ M^{-1}) [33]. For competitive binding of two different ligands interacting with the same linear lattice McGhee and von Hippel [34] have derived an equation, which in our case of Na^+ ion as the competitor occupying one lattice site, i.e. one phosphate group, is [35]

$$\frac{r}{C_f}=\frac{K(1-2nr)^n}{2(1+K_1C_1)^n[1-2(n-1)r]^{n-1}} \quad (3)$$

where *r* is the "binding density" and *n* is the number of PPS phosphates per binding site; C_f and C_1 are concentrations of

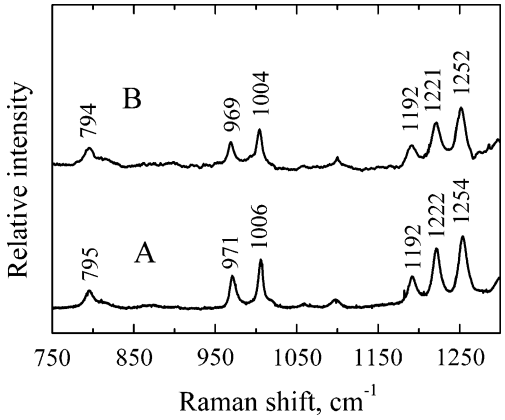

Fig. 6 Fragments of Resonance Raman spectra of free $TMPyP^{4+}$ (trace A) and its complex with PPS under P/D=5.6 where the H-aggregates are formed (trace B), [$TMPyP^{4+}$]=50 μM

the free porphyrin and Na^+ respectively. Under used Na^+ concentration of 0.14 M this kind of ions is in a huge excess in comparison with the polymer content. Since the fraction of bound Na^+ ions is negligibly small in comparison with its total content, we can accepts C_1 value to be equal to 0.14 M. It was supposed that each of four positively charged pyridyl group of the porphyrin interacts with one phosphate group of different PPS chain, thus n=1. The value of apparent binding constant $K=3\times10^5$ M^{-1} was calculated from the Eq. 3 using the experimental data at P/D=5.2. Here the fraction of bound porphyrin in the solution with 0.14 M Na^+ was determined as γ_b=0.05, and the number of bound ligands per matrix unit was $r=\gamma_b D/P\approx 0.01$. K is the cooperative binding constant equal to $K_m q$, where K_m is the monomeric binding constant and q is the binding cooperativity parameter. Taking into account the value of $K_m\approx$2,000 M^{-1} for the electrostatic binding of monocationic dyes to PPS [33], we have obtained $q\approx$150.

Model and size of aggregates

The conformation analysis of the PPS shows that the polymer chain can take a helix shape where negatively charged PO_2^- groups are arranged along two oppositely situated rows with intercharge distance of 0.36 nm. The possible structure of such helix is represented in Fig. 7, where P-O bond length of 0.156 nm taken from X-ray diffraction data [36] as well as valence angle between P-O bonds of 109.5° [37] were used. Indicated above intercharge distance corresponds both to the thickness of the porphyrin π-electronic system and to interplanar distance between adjacent porphyrin molecules involved in the π–π stacking-interaction (0.34–0.36 nm) [38]. Therefore under the binding of $TMPyP^{4+}$ to PPS matrix where positively charged N-methylpyridyl groups of the porphyrin electrostatically interact with negatively charged oxygen atoms of PPS, the spatial arrangement of the polymer negative charges promotes formation of continuous porphyrin stacking-aggregates (two stacks per one PPS molecule). Since the porphyrin molecule is highly symmetric (D_{2h} symmetry) and its four positive charges are oppositely situated, it is supposed that four PPS chains involved in formation of each one-dimensional face-to-face $TMPyP^{4+}$ stack. It is logically possible to suppose that planes of two consecutive molecules in the stack are twisted alternately in the opposite direction (Fig. 8) to prevent the steric hindrance between the methylpyridyl groups of adjacent porphyrins. It can occur since distance between two oxygen atoms belonging to the O-P-O group is approximately 0.2 nm (see the cross-section of the helix in Fig. 7b) and each of them can form ion pair with the methylpyridyl moiety.

The appearance of the strong light scattering by the sample evidences the formation of macroclusters in the solution studied. Here we propose the possible model of this process. An ability of each PPS strand to be a template for formation of two porphyrin stacks (Fig. 9a) induces an integration of adjacent stacks into higher-order columnar aggregates (Fig. 9b), where each stack is displaced relative to another one by half of porphyrin interplanar distance. For PPS with polymerization degree of 75 the length of the column under the stoichiometric binding is $L\approx 0.36\times 75/2 = 13.5$ nm. Supposing that each column represents rodlike particle we

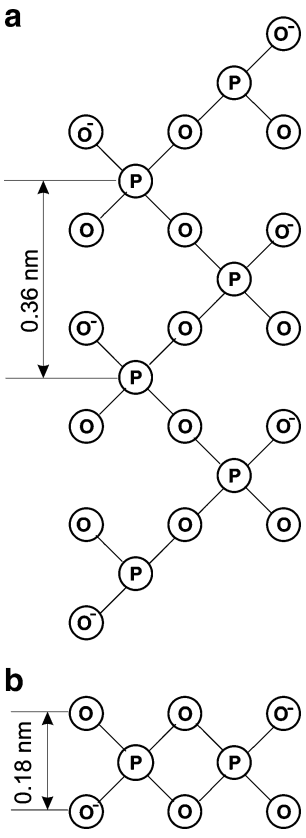

Fig. 7 Side plane projection (a) and cross-section view (b) of PPS helix conformation in which PO_2^- groups are drawn up into two diametrically oppositely situated linear rows

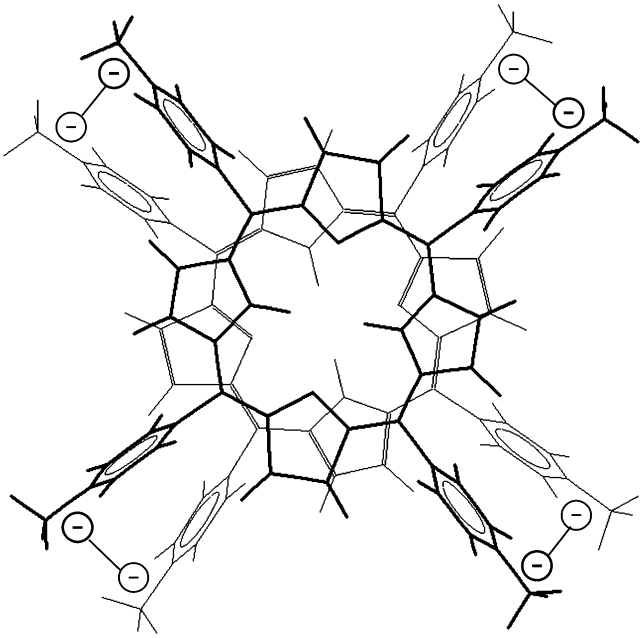

Fig. 8 Cross-section view of two consecutive $TMPyP^{4+}$ molecules belonging to H-stack which interact with four PPS strands. The negative charges of PPS are indicated

have obtained its radius, R, using Tirado and Garcia de la Torre's relation for rotation of rigid circular cylinder around its axis [39]

$$\frac{k_B T}{3.84\pi\eta L R^2 \Theta} \approx 1 \tag{4}$$

where T and η are the temperature and viscosity of medium (water at 22 °C), and the end-effect correction was neglected. A rotation diffusion coefficient θ was calculated from the relation

$$2\theta = 1/\rho \tag{5}$$

Here ρ is the rotational relaxation time which have been obtained from fluorescence polarization data using Perrin equation [26]

$$\frac{p_1}{p} = 1 + \frac{(3 - p_1)\tau}{\rho} \tag{6}$$

where τ is the fluorescence lifetime; p and p_1 are the fluorescence polarization degree with and without the molecular rotation correspondingly. The value p_1= 0.125 determined for $TMPyP^{4+}$ in glycerol solution at −12 °C under λ_{exc}=500 nm was used. Since up to now fluorescence lifetime value for H-aggregates formed by $TMPyP^{4+}$ is not determined experimentally, and at the same time it is known [5] that lifetime of H-aggregate formed by H_4TPPS^{2-} porphyrin is approximately twice as much as its monomer value, we have used τ~10 ns which is equal to a doubled lifetime value for monomeric $TMPyP^{4+}$ determined experimentally as 5.1 ns [14]. From Eq. 6 using $p=p_{max}$=0.095 we have calculated ρ=91 ns, which corresponds to a rotational diffusion coefficient θ=0.0055 ns^{-1}. Using this θ value and Eq. 4, LR^2≈80 nm^3 was found. In such a way, for L≈13.5 nm a diameter of the column is equal to approximately 5 nm. Assuming the distance between two oppositely situated positively charged nitrogen atoms belonging to methylpyridyl groups of $TMPyP^{4+}$ molecule as 1.6 nm, it was estimated that 3 porphyrin molecules are arranged across the diameter of the column and correspondingly 9 ones are disposed in the its cross-section (Fig. 9b). At the same time the porphyrin molecules belonging to the adjacent stacks are mutually displaced in vertical direction by half of their interplanar distance (Fig. 9a). In such a way, as it is seen from Fig. 9b, 9 porphyrin molecules share 48 PPS residues resulted in the phosphate/$TMPyP^{4+}$ ratio of 5.3, that is in good conformity with the stoichiometric binding ratio 5.2

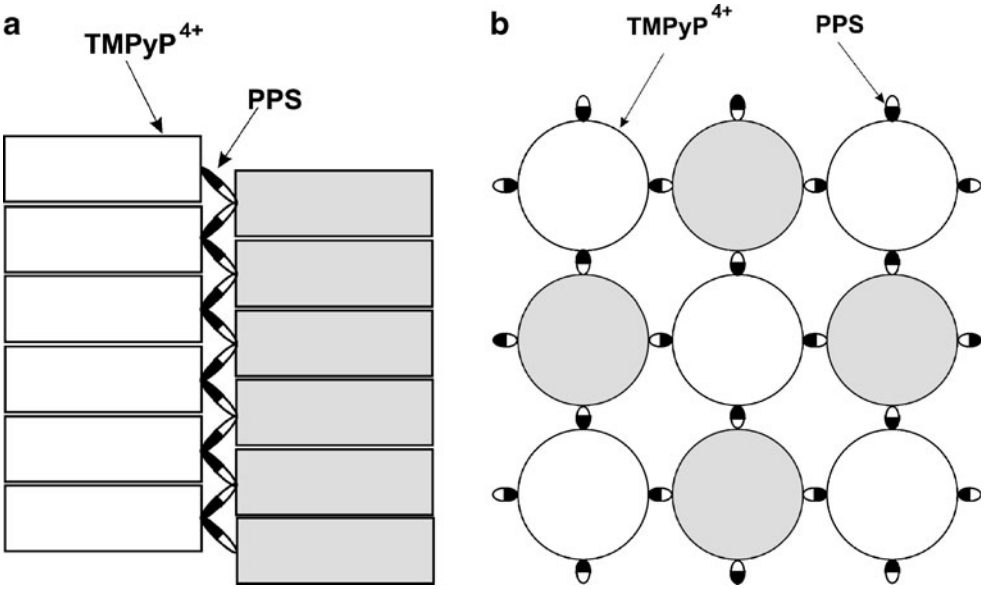

Fig. 9 Plausible scheme of $TMPyP^{4+}$ assembly to H-aggregate: (**a**) Integration of two one-dimensional $TMPyP^{4+}$ stacks by one PPS chain; (**b**) Combining of the one-dimensional stacks into a macrocluster. Displaced layers of porphyrin molecules are colored by white and grey

obtained from the fluorescent titration curve. Thus, it is easy to calculate that 9 porphyrin stacks are combined into the columnar macrocluster consisting of $9L/0.36 \approx 340$ monomeric units.

Conclusions

In the present study a formation of extended face-to-face $TMPyP^{4+}$ aggregates (H-aggregates) in water is revealed under the porphyrin stoichiometric binding to inorganic polyphosphate. The process is characterized by the binding constant of 3×10^5 M^{-1} and the cooperativity parameter of about 150. Formation of such columnar aggregates is induced by the ability of PPS chains to take a helix conformation where negative charges are arranged along two oppositely situated rows with intercharge distance of 0.36 nm, which corresponds to the interplanar distance between adjacent porphyrin molecules involved in the π–π stacking-interaction. This aggregation results in the near five-fold quenching of $TMPyP^{4+}$ fluorescence. An ability of each PPS strand to be a template for formation of two porphyrin stacks results in the integration of the adjacent stacks into higher-order columnar macroclusters which dimension depends on the length of PPS chain. In our case of PPS with polymerization degree of 75 the length of the column and its diameter were estimated to be equal to 13.5 nm and 5 nm correspondingly. The plausible model of H-type $TMPyP^{4+}$ aggregates on the polyanionic PPS template is presented. According this model, 9 one-dimensional porphyrin stacks joined by 24 PPS strands are combined into the columnar macrocluster consisting of approximately 340 porphyrin monomeric units. The phosphate-to-porphyrin ratio calculated for such columnar structure is equal to 5.3. It is in good conformity with the stoichiometric binding ratio of 5.2 obtained from the fluorescent titration curve. The presence of NaCl in the solution reduces the efficiency of $TMPyP^{4+}$ aggregation due to the competitive binding of Na^+ ions to PPS.

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