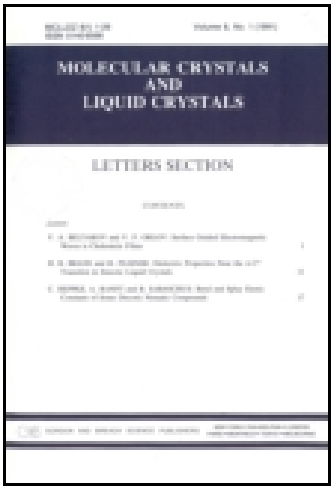

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Effect of Polyacrylamide and Dextran-Polyacrylamide Graft Polymers on Absorption and Fluorescence Spectra of Hematoporphyrin

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Absorption and fluorescence spectra of hematoporphyrin (HP) solutions in distilled water and in the presence of polyacrylamide and three dextran-graft-polyacrylamide branched polymers are studied. HP is shown to interact with the studied polymers leading to the destruction of HP aggregates, which is positive for the use of porphyrin as a photosensitizer in the photodynamic therapy. For the concentrations of the polymers of 0.001%, the studied polymers demonstrated a similar impact on HP.

Keywords Hematoporphyrin aggregation; graft polymers; fluorescence spectra; photodynamic therapy

Introduction

Porphyrins efficiently produce singlet oxygen upon photoexcitation. Due to this property, porphyrin derivatives are used as sensitizers in photodynamic therapy (PDT) for the destruction of cancer cells [1]. At the same time, it was demonstrated that the addition of polymers results in an enhancement of the photodynamic efficiency of photosensitizing molecules [2]. So, the study of the spectral-luminescent properties of porphyrins in the presence of polymers is of high importance for the further development in the field of PDT. Thus, we studied the influence of the polymer polyvinylpyrrolidone on the absorption and the fluorescence spectra of hematoporphyrin (HP, Fig. 1) and showed that the binding to this polymer results in the destruction of HP aggregates [3], which is positive for using HP as a photosensitizer in PDT.

Recently, a series of dextran-graft-polyacrylamide branched polymers obtained by grafting several polyacrylamide (PAA, Fig. 1) chains to dextran was synthesized [4]. Branched polymers are of high interest because of their controlled internal molecular structure. The number of variable parameters in these polymers are overwhelmingly large,

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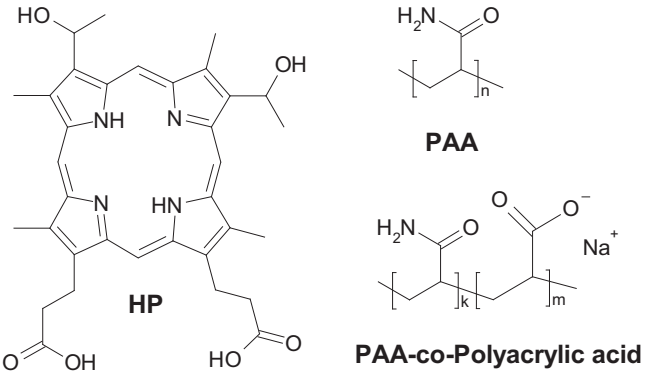

Figure 1. Chemical structure of hematoporphyrin (HP), polyacrylamide (PAA), and PAA-co-Polyacrylic acid.

namely, the initial polymer architecture, average degree of polymerization, solubility properties, distance between grafts, nature and flexibility of a backbone and grafts, etc. The additional factors affecting the internal structure appear for branched polyelectrolytes, e.g., the charge density or pH, nature of the charge distribution (static or dynamic), valence and nature of counterions. Due to the structure peculiarities, the local concentration of functional groups in branched polymers is notably higher than that in linear ones. Therefore, they should be promising materials for nanochemistry and nanotechnology, as well as for biomedical and technological applications [4]. Here, we studied the absorption and the fluorescence spectra of HP solutions in distilled water and in the presence of polyacrylamide, dextran-graft-polyacrylamide branched polymers D70-g-PAA5 and D20-g-PAA15, and polyelectrolyte graft polymer D70-g-PAA5PE, consisting of a dextran core and grafted PAA or PAA-co-Polyacrylic acid (Fig. 1) chains [4]. Since the dextran chain does not make more than 5% of a graft polymer, the co-polymer predominantly consists of PAA or PAA-co-Polyacrylic acid. The structure of graft co-polymers corresponds to the theoretical model of star-like polymer with dextran core and PAA or PAA-co-Polyacrylic acid arms [5].

Materials and Methods

Hematoporphyrin was generously provided by M.F. Gamaleia (R.E. Kavetsky Institute for Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine). PAA and dextran-graft-polyacrylamide branched polymers D70-g-PAA5 and D20-g-PAA15, as well as polyelectrolyte graft polymer D70-g-PAA5PE, were synthesized as described in [4]. D70-g-PAA5 and D20-g-PAA15 were obtained by grafting, respectively, 5 and 15 PAA chains to dextran chains of molecular weights equal to 70,000 and 20,000, respectively. D70-g-PAA5PE was obtained from D70-g-PAA5 by conversion of 34% of electrically neutral amide groups to negatively charged carboxylate ones [4]. Dimethylformamide (DMF) and distilled water were used as solvents.

Stock solution of hematoporphyrin (10^{-3} M) was prepared in DMF. The solutions of polymers (0.001%) were prepared in distilled water. To prepare the working solutions, 4 μL aliquot of HP stock solution was added to 2 mL of distilled water or 0.001% polymer

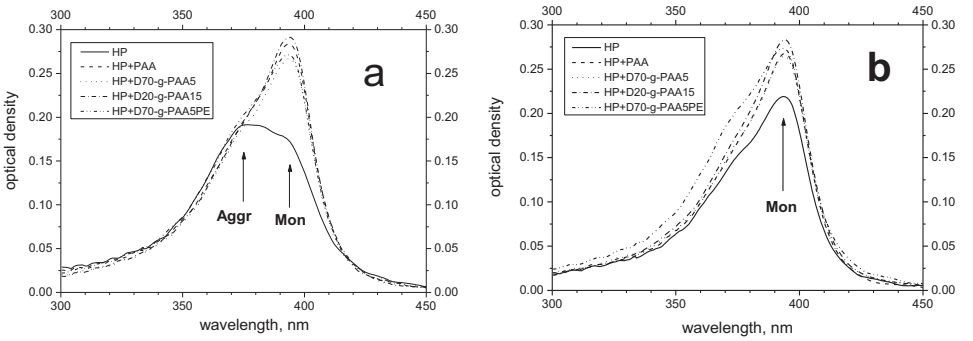

Figure 2. Absorption spectra of hematoporphyrin (2×10^{-6} M) in distilled water and in the presence of 0.001% of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE immediately (a) and in 1 h (b) after the preparation of the sample.

solution. The concentration of HP thus was 2×10^{-6} M. Between measurements, the working solutions were kept in darkness at room temperature.

Absorption spectra were registered with a Specord M-40 spectrophotometer (Carl Zeiss, Germany). Fluorescence spectra were measured with the help of a Cary Eclipse fluorescence spectrophotometer (Varian, Australia). Fluorescence excitation anisotropy spectra were measured as described in [6]. For measurements, the solutions were placed into a 1 cm $\times$ 1 cm quartz cell. All measurements were performed at room temperature.

Results and Discussion

Absorption spectra of HP in water, as well as in the presence of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE, were registered immediately and in 1 and 3 hours after the preparation of a solution. The absorption spectrum of HP, as those of other porphyrins, consists of the short-wavelength intense B (or Soret) band and non-intense long-wavelength Q-bands; all these bands are considered to be a result of the interaction of four electronic transitions between two highest occupied and two lowest unoccupied electronic orbitals [7]. The Soret band of the spectra, where the difference between solutions is manifested, is presented in Fig. 2.

It is seen from Fig. 2 that, immediately after the solution preparation (Fig. 2a), the Soret band of HP in water has maximum at 376 nm that is due to HP aggregates [3, 8]. These aggregates that are known to be mostly HP dimers [8] demonstrate the properties characteristic of the sandwich-type (i.e., H-type) dimers, namely the short-wavelength shift of the absorption maximum with respect to the spectrum of monomers. In 1 h (Fig. 2b), the maximum moves to 393 nm, which corresponds to the destruction of aggregates into separate HP monomers. At the same time, for the HP solutions in the presence of 0.001% of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE, the Soret band has maximum at 393 nm immediately after the solution preparation, and the aggregates manifest themselves near 376 nm as a weak shoulder. In 1 h, this shoulder still decreases (except for the case of D70-g-PAA5PE where it stays the same). This means that, unlike the free HP solution, the presence of the mentioned polymers leads to the immediate destruction of HP aggregates. It might be supposed that such destruction is due to the HP interaction with the studied polymers. It should be added that, for the concentrations studied, no significant difference between polymers in their action on HP was registered.

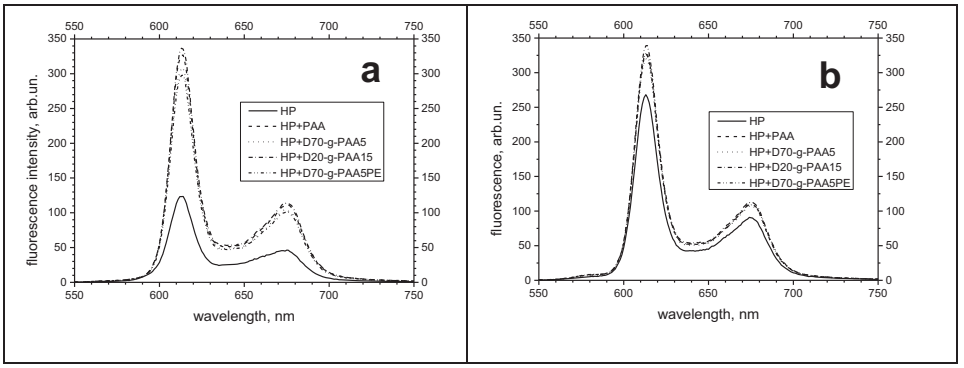

Figure 3. Fluorescence spectra (excitation at 400 nm) of hematoporphyrin (2×10^{-6} M) in distilled water and in the presence of 0.001% of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE immediately (a) and in 1 h (b) after the preparation of the sample.

Fluorescence spectra of HP in water and in the presence of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE are represented in Fig. 3. It is seen that the spectra of all studied solutions immediately after the preparation contain the bands with maxima at 613 nm and 675 nm that correspond to the emission of HP monomers. The difference is that, in the case of a free HP solution, the intensity of the 613-nm band of monomers is 123 a.u. (arbitrary units) (Fig. 3a), growing up to 267 a.u. in 1 h (Fig. 3b). At the same time, for HP solutions in the presence of polymers, the intensity of the mentioned band reached its maximum value immediately after the solution preparation (between 298 and 336 a.u.), slightly growing in 1 h only to 320–339 a.u. Such observation supports the assumption made above about the slow self-dissociation of HP aggregates (and thus increasing the concentration of HP monomers) in water and their immediate destruction in the presence of 0.001% of studied polymers due to their binding with monomer HP molecules. At the same time, the aggregates causing the 376-nm absorption are not manifested in fluorescence spectra. It is possible that namely the high rate of radiationless internal conversion in HP aggregates causes the absence of their fluorescence and is also responsible for a decreased intersystem conversion rate and thus for a decreased photosensibilization in PDT.

To characterize the formed complexes of HP monomers with the polymers in more details, polarization properties of the HP fluorescence were studied. As is seen from the fluorescence excitation anisotropy (r) spectra of HP in water and in the presence of polymers (Fig. 4), the fixation of HP in the formed complexes with polymers is not rigid. While the anisotropy value reaches 0.3 near 600 nm (that is near to the maximum r value) for a HP solution in glycerol (used as a reference), this value is close to zero for HP in the presence of polymers. Thus, the fixation of HP monomers in the polymers studied permits the HP rotation fast enough to lead to the fluorescence depolarization.

One more observation that could be done from the fluorescence spectra of HP is the emission band with maximum at 580 nm, which appears in some time after the solution preparation, both in the case of free HP and HP in the presence of polymers. To understand the nature of the 580-nm band in more details, the fluorescence excitation and emission spectra of HP solution in water in 22 h after the solution preparation are represented in Fig. 5.

It is seen from Fig. 5 that the Soret band in the excitation spectrum corresponding to the 580-nm emission (at 406 nm) is shifted to the long-wavelength region and is more

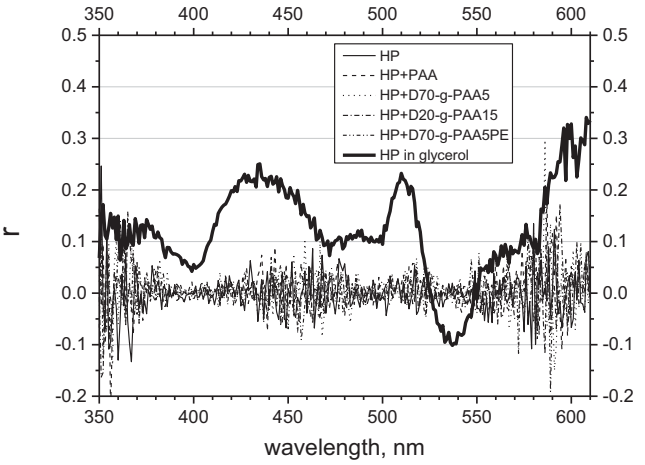

Figure 4. Fluorescence excitation anisotropy spectra of hematoporphyrin (2×10^{-6} M) in distilled water and in the presence of 0.001% of PAA, D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE in 3 h after the preparation of the sample. Fluorescence excitation anisotropy spectrum of HP in glycerol is added for reference. Emission wavelength was 620 nm.

narrow as compared to the Soret band corresponding to the "ordinary" 615-nm emission of HP monomers (at 395 nm). In addition, instead of four Q-bands corresponding to the 615-nm emission (502 nm, 537 nm, 559 nm, the fourth one is overlapped by scattering near 610 nm), only two Q-bands correspond to the 580-nm emission (at 537 nm; the second one is overlapped by scattering near 580 nm). Such decrease of the number of Q-bands from four to two is known to be related to the transition to the higher symmetry of the porphyrin ring [7]. As for the emission spectra, besides the 580-nm maximum, the shoulder near

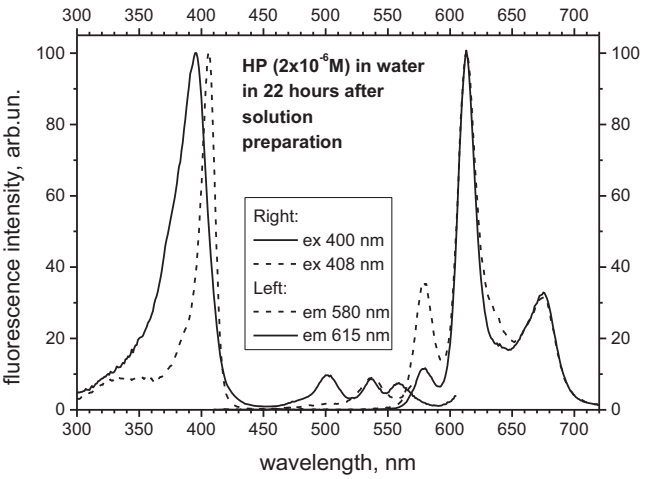

Figure 5. Fluorescence excitation (left) and emission (right) spectra of HP (2×10^{-6} M) in distilled water in 22 h after the preparation of the sample. For emission spectra, fluorescence was excited at 400 nm (solid line) and 408 nm (dashed line). For excitation spectra, the emission wavelength was 615 nm (solid line) and 580 nm (dashed line). Spectra were normalized.

630 nm that appears upon the excitation at 408 nm can be observed. Thus, the emission spectrum of the HP form appearing in some time after the solution preparation contains two bands, as well as the spectrum of "ordinary" HP monomers, the former being shifted to the short-wavelength region as compared to the latter. Such spectra appearing after some time in an HP solution were described in [8] and attributed to HP fluorescent aggregates with the packing of monomers leading to the enhancement of the aggregate symmetry as compared to that of monomers. It should be noted that these aggregates appear both in a free HP solution and in HP solutions in the presence of polymers.

Conclusions

In a water solution, HP interacts with PAA and graft polymers D70-g-PAA5, D20-g-PAA15, and D70-g-PAA5PE leading to the destruction of HP aggregates that is positive for the use of porphyrin as a photosensitizer in PDT. At a concentration of 0.001%, the studied polymers demonstrated a similar impact on HP. The fixation of HP molecules in complexes with the studied polymers is not rigid and permits HP molecules to rotate rapidly enough to cause the depolarization of a fluorescence.

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