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Charged Porphyrin – dopa Melanin Interaction at Varied pH: Fluorescence Lifetime and Photothermal Studies

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The influence of pH on the spectral forms of negatively and positively charged porphyrin dyes: tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$) and tetra(4-*N, N, N, N*-trimethylanilinium) porphyrin (TAP) in the presence of dopa melanin is investigated. The interaction between dyes and dopa melanin is shown to be dependent on the kind of dye spectral forms and on environmental pH. The creation of different forms, deactivation of their electronic excited states, and their interaction with dopa melanin have been monitored by absorption, fluorescence, and photoacoustic spectroscopy in the pH range of 2–9. This article demonstrates that the TAP–dopa melanin complex is destroyed at the lowest pH because of competition between positively charged peripheral groups of TAP and protons, which interact with negative centers of dopa melanin. Otherwise, dopa melanin affects photothermal and fluorescence properties of monomeric dications (TAP and $TPPS_4$) rather weakly. It has also been suggested that the aggregated dicationic of $TPPS_4$ can serve as an acceptor in the energy transfer from dopa melanin. Presented results seem to be applicable to a photodynamic therapy of cancer.

KEY WORDS: Dopa melanin; fluorescence; porphyrins; pH; photoacoustic spectroscopy.

INTRODUCTION

Porphyrins are widely proposed as photosensitizers in photodynamic therapy (PDT). The sensitizers should preferably be located in a tumor tissue, exhibit appropriate uptake, and cause selective photodamage triggered by irradiation with light of a particular wavelength. However, first-generation photosensitizers have demonstrated significant disadvantages—they are not very selective and thus can cause skin photosensitivity. Because they are a mixture containing monomers, dimers, and higher oligomers and/or a complex of variable composition, the perfect active photosensitizer has not yet been isolated and characterized. Thus, no adequate interpretation of the dose-response relationship can be made [1–3]. Second-generation sensitizers have well-established structures and absorb at the long-wavelength region of visible absorption.

Currently, the best photosensitizer is Photofrin® [4], which is widely used in PDT, but other drugs that would be more suitable for photodynamic reactions are sought. For that reason, we focus our attention on other porphyrins with charged peripheral groups because little is known about charged dyes as possible agents in PDT. This article analyzes the interaction of charged porphyrins with dopa melanin at different pH levels.

Spectroscopic research can be useful to separate and characterize various forms of dye. The creation of dye forms depends not only on the molecular structure of dyes, on the charging of the peripheral groups at-

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ABBREVIATIONS: dopa melanin, dihydroxylphenylalanine melanin; ET, energy transfer; PAS, photoacoustic spectra/signal; TAP, tetrakis-(4-N,N,N,N-trimethylanilinium)porphyrin; $TPPS_4$, tera(4-sulphonatophenyl) porphyrin; M, monomer; D, monomeric dication; AD, aggregated dication; C, complex TAP - dopa melanin

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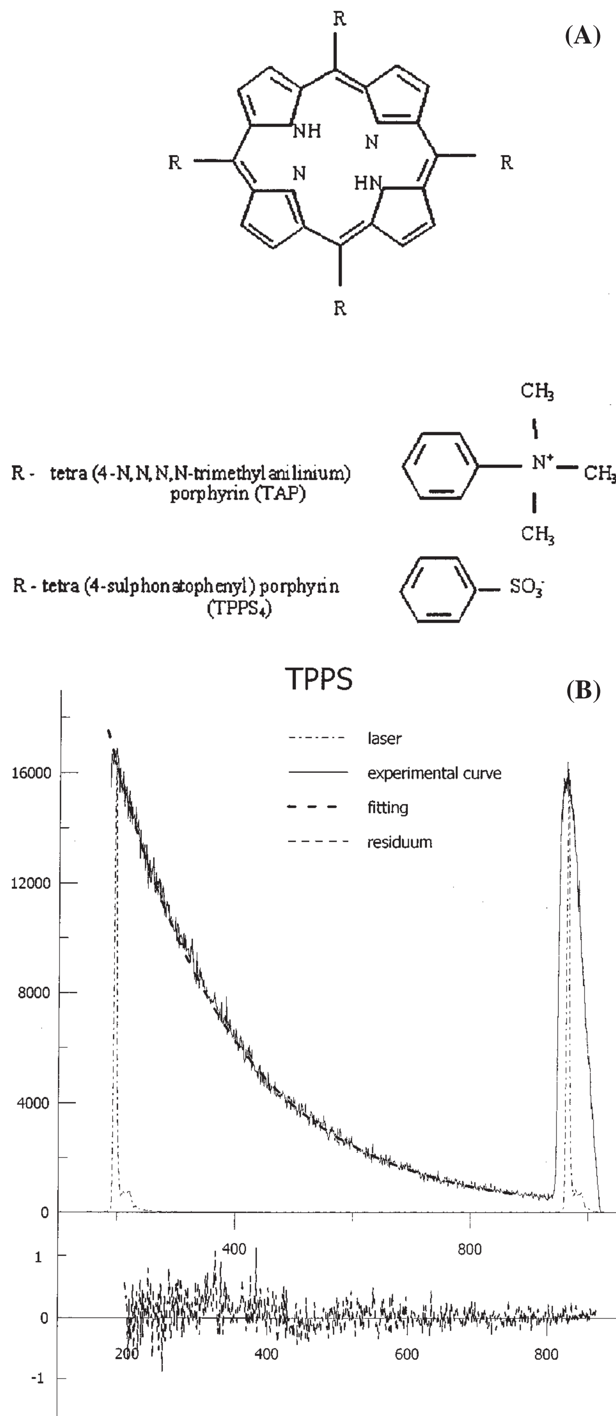

Fig. 1. Molecular structure of porphyrins (A); an example of the decay fluorescence for porphyrin dye ($TPPS_4$) in phosphate buffor (pH 2) (B).

tached to the main molecular core, and on the dye concentration, but also on the pH of the environment. Because it is known that normal and tumorous tissues are characterized by different pH [2], we studied the influence of pH on the interaction between some porphyrins and synthetic melanin. In our previous paper [1], we found that porphyrins can create the different spectroscopic species at various pH. As shown, negatively charged tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$) exists in three spectroscopic forms: monomeric (M), dicationic (D, protonated monomeric), and aggregated dicationic (AD) [1]. Positively charged 4-*N, N, N, N*-trimethylaniliniumporphyrin (TAP) exists in a monomeric form and its protonated species at different pH are also described in the same article [1]. These forms are characterized by different absorption and fluorescence properties. These properties are incorporated into the so-called second-generation PDT agents. In this work, we studied the spectroscopic behavior of charged porphyrins in the presence of dopa melanin in a buffer of various pH. Some photophysical properties of charged porphyrins and their influence on membranes of living organisms were investigated in references [5,6]. Melanin is a class of a biological pigment found in many living organisms and its functions vary; a major function in human skin is solar photoprotection provided via optical absorption. Melanin has persistent free radical centers [7], it can bind multivalent metal ions [8], and participate in an electron exchange reaction. Natural melanin is difficult to characterize spectroscopically, however, synthetic dopa melanin can serve as a reasonable model for studying this obscure pigment [9].

This work, we studied the influence of pH on the interaction between dopa melanin and charged porphyrins ($TPPS_4$ and TAP) by monitoring deactivation of their electronic excited states by fluorescence, fluorescence lifetime, and thermal deexcitation processes.

MATERIALS AND METHODS

Dopa melanin was prepared according to the procedure described elsewhere [10]. Porphyrins (Fig. 1A) were obtained from Midcentury (Posen, IL) and used without further purification. In all experiments the stock solution of dopa melanin (5 mg/mL), porphyrins (1 mM), and porphyrin concentrations (3×10^{-5} M) were the same in all samples. The pH value was established with 5% HCl and changed from 2 to 9.

The absorption spectra were measured with Specord M40 Carl Zeiss Jena (Germany) spectrometer in the range of 300–800 nm. The fluorescence and excitation spectra were obtained using the equipment described previously [11]. The fluorescence emission spectra were detected in the range of 600–750 nm. The excitation spectra were collected in the 350–700 nm range. The fluorescence decays were obtained by the time-correlated single photon counting (TCSPC) method described in [12]. The fluo-

rescence excitation of the samples was provided by a mode-locked Ar^+ laser ILA 120 from VEB Carl Zeiss Jena (excitation wavelength $\lambda_{ex} = 420$ nm, pulse duration 100 ps; repetition rate 123.2 MHz). The experimental device for TCSPC was based on the system SPC 100 from the Central Institute of Optics and Spectroscopy, Academy of Sciences of Germany, Berlin. This is a computer-controlled arrangement containing the electronics for processing and registration of the single photon pulses, control, and data processing [13]. The samples were immersed in a closed cuvette that was excited from below. The fluorescence emission was detected perpendicularly to the excitation beam and focused onto the horizontal slit of a monochromator with high light intensity and a spectral resolution of 10 nm. Observation wavelength was specified for bands λ_{ob} at 645 and 670 nm. Figure 1B illustrates an example of the results of the deconvolution procedure done for $TPPS_4$.

Thermal properties were studied with a homemade single-beam photoacoustic spectrometer equipped with a sensitive microphone as described elsewhere [14]. A thin layer of a sample was placed in a special photoacoustic cell (model 300, MTEC Photoacoustics, Inc., Ames, Iowa, USA) filled with helium. Periodic heat generated within the sample by the optical absorption process diffused to the sample–gas interface, and expanded the thin boundary layer of the gas which created an acoustic signal working as a piston on the rest of the gas in the photoacoustic cell [15]. The photoacoustic signal was detected by a microphone, amplified, and collected by a computer. The black body was used as a reference sample to monitor the device response. The light modulation frequency of 10 Hz at the phase shift $\Phi = -90°$ was used.

RESULTS

Absorption

The absorption spectra of $TPPS_4$ with dopa melanin in a phosphate buffer presented in Fig. 2 show distinct changes depending on the pH value. Our previous spectroscopic study showed that $TPPS_4$ at neutral pH does not create a complex with dopa melanin when the dye is in the ground state [16]. At pH 9 the sample absorption spectrum has the main Soret band at 413 nm, which is characteristic for the M spectral form of $TPPS_4$, and this result is in accordance with the observation obtained in our previous work [1]. The decrease in pH from 5 to 2 causes the appearance of the spectral species with absorption bands with maxima at 434/646 nm and 490/706 nm, which have been respectively assigned to the protonated D and AD [1]. The characteristic $TPPS_4$ bands are

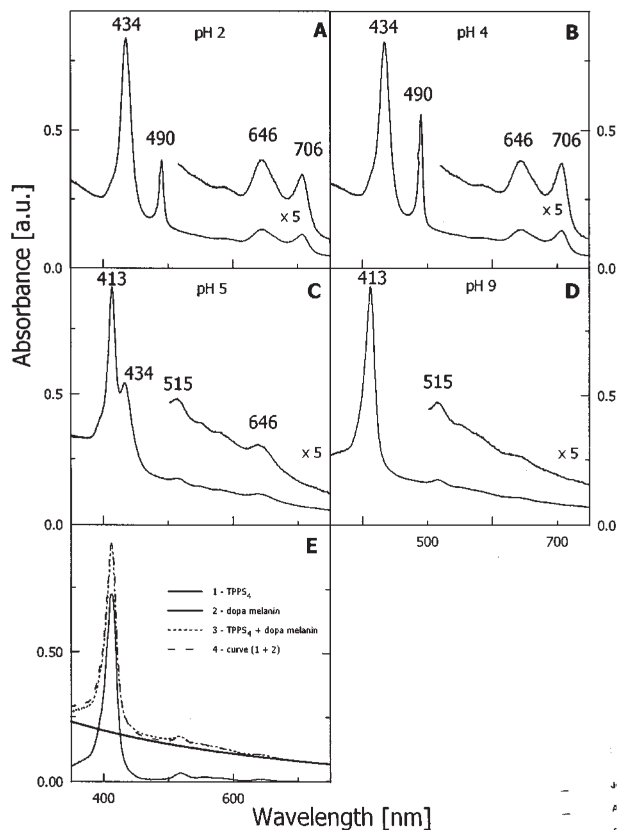

Fig. 2. Absorption spectra of $TPPS_4$ in the presence of dopa melanin at different pH (A–D). (E) shows the experimental absorption spectra of $TPPS_4$ (curve 1), dopa melanin (curve 2), $TPPS_4$ + dopa melanin mixture (curve 3), the computed result (curve 4).

seen on the background of the monotonically decreasing absorbance of dopa melanin [16]. Fig. 2E demonstrates an example of the decomposition of the individual experimental absorption spectra of the dopa melanin–porphyrin mixture (for pH 9). It evidently shows that absorption of this system is a simple superposition of the $TPPS_4$ absorption and of the absorption spectra of melanin. This confirms the result that $TPPS_4$ does not create any spectroscopic complex with dopa melanin when it is not in the M form, in a D form, or in AD forms [16]. All $TPPS_4$ spectroscopic forms observed at different pH in the absence of dopa melanin [1] are preserved in the dopa melanin presence, and pH only slightly changes the intensity of the absorption bands. The results indicate rather weak influence of dopa melanin on $TPPS_4$ in the wide range of pH.

The absorption results for TAP are in contrast to those presented for $TPPS_4$, when dopa melanin is added to the buffer solution. The results are shown in Fig. 3. In the presence of dopa melanin the TAP-dopa melanin complex is created from the coulombic interaction between the positively charged TAP molecules and the neg-

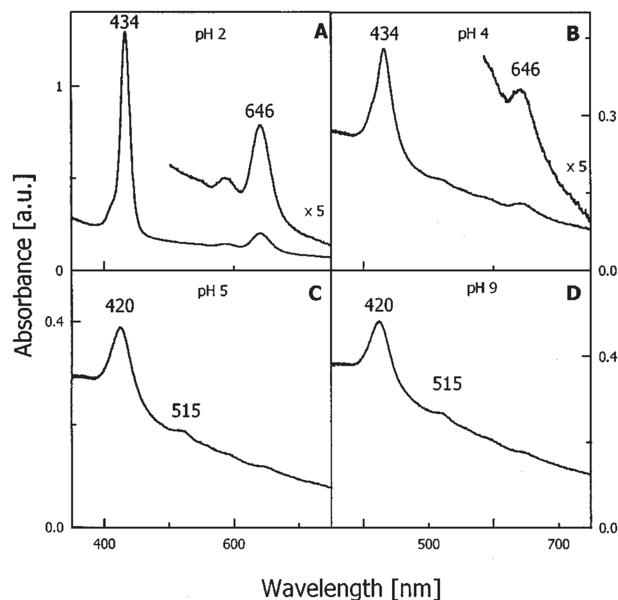

Fig. 3. Absorption spectra of TAP in the presence of dopa melanin at different pH.

ative functional groups of the melanin polymer at high pH [16,17], which is observed as a band with its maximum at 420 nm. For this sample at lower pH (≤ 4), the Soret band is shifted from 420 nm (Fig. 3C,D) to 434 nm (Fig. 3A,B) and a new band at 646 nm is observed. The appearance of the new form is seen clearly only in the sample at low pH. This spectral species is assigned to the D species of TAP, as observed in the acid solution in the absence of dopa melanin [1]. The results presented for TAP in the presence of dopa melanin indicate that the TAP–dopa melanin complex is destroyed in acidic conditions because the absorption bands with the maxima at 434/646 nm are typical for Ds existing even without dopa melanin [1].

Steady-State Fluorescence

In our previous work, we have shown that all spectral forms (M, D, and AD) in the absence of dopa melanin, exhibit fluorescence in different spectral regions depending on the kind of species: 654/706 nm and 645/706 nm ($TPPS_4$-M and TAP-M, respectively), 670 nm and 665 nm ($TPPS_4$-D and TAP-D, respectively), and 714 nm ($TPPS_4$-AD) [1]. In this article, we show the variations in their lifetimes. The fluorescence spectra of $TPPS_4$ and TAP are shown in the presence of dopa melanin under different excitation wavelengths and pH conditions (Figs. 4 and 5). As illustrated in Fig. 4, dopa melanin changes the fluorescence properties of all $TPPS_4$

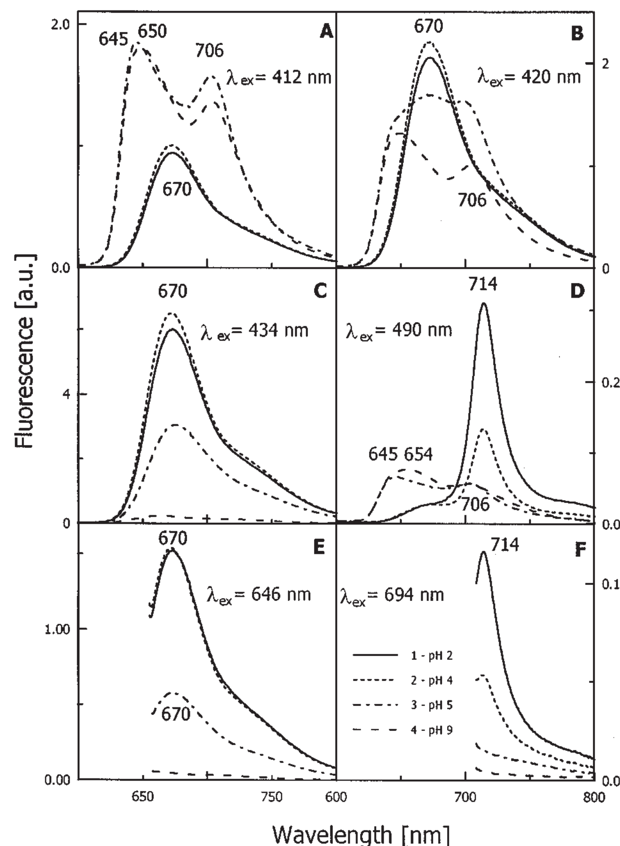

Fig. 4. Fluorescence spectra of $TPPS_4$ in the presence of dopa melanin at different pH; excitation wavelengths: (A) 412 nm, (B) 420 nm, (C) 434 nm, (D) 490 nm, (E) 646 nm, (F) 694 nm; curves: (1) pH 2, (2) pH 4, (3) pH 5, (4) pH 9.

species [1]. Some changes in relative band intensities in the presence of dopa melanin are observed and this indicates some differences in the interaction between various spectral forms of $TPPS_4$, when they are in their excited states and are in the presence of dopa melanin. The intensities of the Ms and the Ds decrease slightly. One of the reasons for such a small decline could be the rise in the yield of the nonradiative processes in $TPPS_4$-M and $TPPS_4$-D in the presence of dopa melanin. The AD (the fluorescence maximum at 714 nm; Fig. 4D and F) is one exception: its 714-nm fluorescence increases about two to three times after dopa melanin is added. The explanation of this observation could lie in the energy transfer (ET) from dopa melanin to this porphyrin form at low pH (2 and 4). In the light of the results about the weak fluorescent properties of melanin presented in reference [18] and its strong thermal deactivation [16], as well as the relative localizations of energetical levels of dopa melanin and $TPPS_4$-AD, energy transfer cannot be neglected.

Very interesting results are obtained for the system of TAP and dopa melanin. It follows from our observation (Fig. 5) that the spectra are not changed at different excitation wavelengths for the sample with the lowest pH. The fluorescence spectra of this sample are characterized by one very intensive band at about 665 nm, which is assigned to the D of TAP without dopa melanin [1]. The result presented in this article indicates that fluorescence of this spectral species responsible for the 665 nm band is not quenched by dopa melanin. This suggests that the interaction between dopa melanin and Ds under acidic condition is rather weak. Otherwise, for the samples at pH $\geq$ 4, a very strong quenching of TAP fluorescence in the presence of dopa melanin is monitored. This observation is very much consistent with our previous results relating to the fluorescence quenching of TAP complexed with dopa melanin at neutral pH [16,17]. Thus, on the basis of our previous results for the spectral properties of TAP in the presence of dopa melanin at neutral pH [16,17] and the results presented in this article, we can conclude that the TAP–dopa melanin coulombic complex does not survive in acidic conditions. This complex is destroyed when pH is 5 (or lower) and Ds are formed instead of the coulombic complex. This dicationic form does not create a complex with dopa melanin, which does not quench its fluorescence as we observe for the TAP–dopa melanin complex at high pH. This result shows that at the lowest pH the dicationic species exist independently of the dopa melanin presence. The reason for such an observation could be because of the strong interaction between dopa melanin and proton from a buffer of low pH, rather than the interaction between dopa melanin and free-base TAP. This result confirms our absorption measurements presented above.

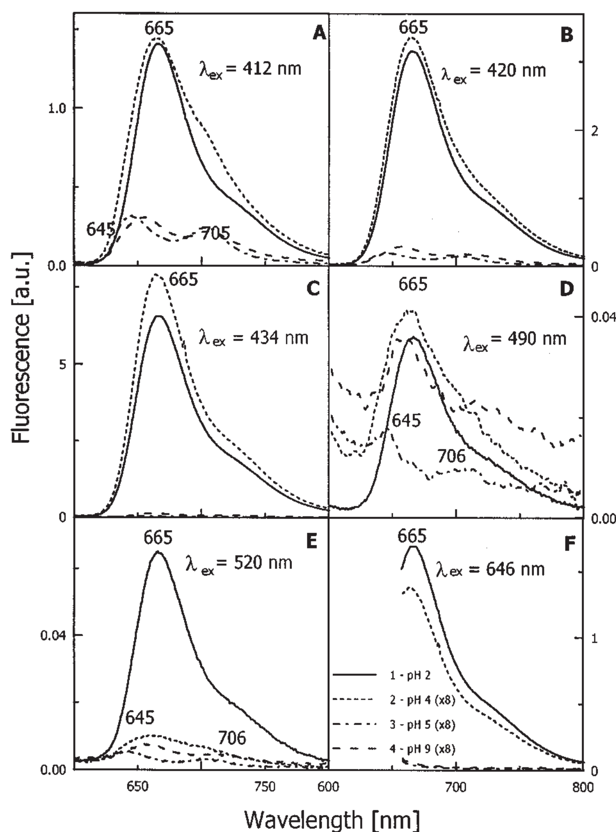

Fig. 5. Fluorescence spectra of TAP in the presence of dopa melanin at different pH; excitation wavelengths: (A) 412 nm, (B) 420 nm, (C) 434 nm, (D) 490 nm, (E) 520 nm, (F) 646 nm; curves: (1) pH 2, (2) pH 4, (3) pH 5, (4) pH 9.

Emission Excitation

Figures 6 and 7 show the influence of dopa melanin on $TPPS_4$ and TAP samples when monitored by the excitation spectra. At the highest pH, dopa melanin has only little influence on the fluorescence behavior of $TPPS_4$: it only changes the relative intensities of the observed bands, which could be connected with partial energy absorption by dopa melanin (a filter effect) and energy transfer. The relationship of the bands in the sample at pH 5 (413/434 nm) is changed at different observation wavelengths and can be explained by the coexistence of the M and D forms in the sample. In our previous article, we demonstrated the Förster energy transfer between M and D forms [1], and on the basis of the above results and

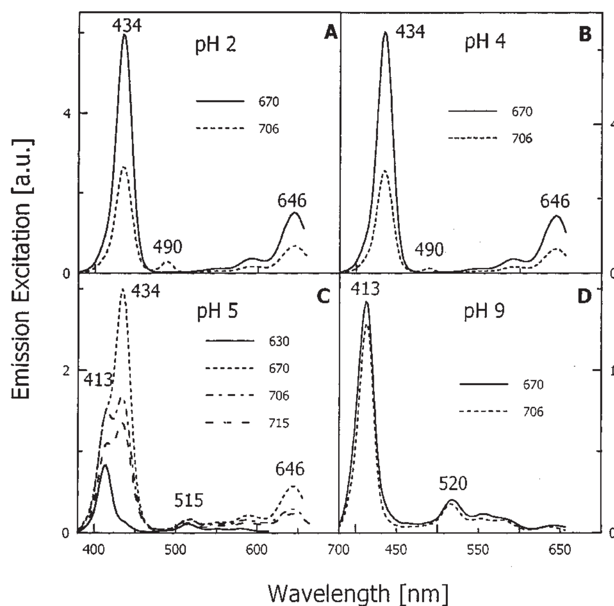

Fig. 6. Excitation spectra of $TPPS_4$ in the presence of dopa melanin at different pH; (A) pH 2, (B) pH 4, (C) pH 5, (D) pH 9.

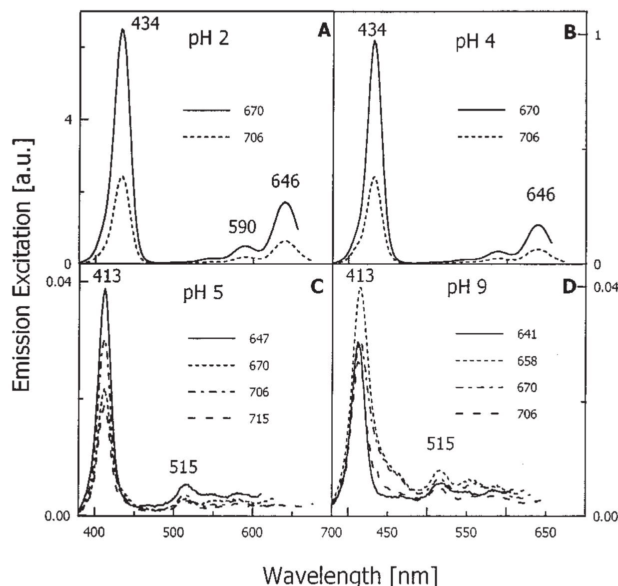

Fig. 7. Excitation spectra of TAP in the presence of dopa melanin at different pH; (A) pH 2, (B) pH 4, (C) pH 5, (D) pH 9.

those presented in reference [1], we can conclude that dopa melanin can influence this process because of the interaction with both spectral species. Otherwise the band at 490 nm (Fig. 6A and B), which is found to be less intensive than that in the absorption spectra (pH 4 and 2), can confirm the participation of dopa melanin in energy transfer, as mentioned. The diminishing of the 490 nm band in the excitation spectra could also be associated with the effective thermal deactivation occurring in AD's as discussed previously and in reference [1]. We can thus conclude that M and D are affected by dopa melanin, and energy transfer between dopa melanin and ADs could be involved, as mentioned.

As expected, TAP fluorescence is very effectively quenched by dopa melanin, which is confirmed by the excitation spectra presented in Fig. 7C and D. At high pH the excitation spectra do not correspond to the absorption spectra; in the excitation spectra, the Soret band is localized at 413 nm, whereas absorption is observed mainly at 420 nm. The intensities of all TAP excitation spectra bands diminished strongly after dopa melanin was added, and the low signal originates from Ms that are not involved in the complex with dopa melanin. The shape of the spectra shown in Fig. 7A and B, positions of the bands, and their intensities confirm high fluorescence of Ds and very weak influence of dopa melanin. The comparison of the absorption and excitation spectra confirms the results presented in references [16–18] about nonfluorescent character of dopa melanin and its weak influence on TAP-D.

Table I. Fluorescence Lifetime of TAP and $TPPS_4$ in the Absence/Presence of Dopa Melanin

Sample	pH	Dye form	τ [ns]
TAP	5	M	8.58 ± 0.20
	9	M	8.63 ± 0.20
TAP + dopa melanin	5	M + C	8.73 ± 0.20
	9	M + C	7.84 ± 0.20
$TPPS_4$	5	M + D	7.69 ± 0.20
	9	M	8.16 ± 0.20
$TPPS_4$ + dopa melanin	5	M + D	8.06 ± 0.20
	9	M	9.10 ± 0.20

Observation wavelength: 645 nm, M, monomer; D, monomeric dication; C, coulomb complex.

Fluorescence Lifetimes

The fluorescence decay times for TAP and $TPPS_4$ and their mixtures with dopa melanin (observation wavelength of 645 nm and at pH 5 and 9) are presented in Table I. In this fluorescence region the emission originates predominantly from M dye forms.

For TAP and $TPPS_4$ we obtain the fluorescence decay with the lifetimes of 8–9 ns, as expected for monomeric porphyrin [19,20]. The fluorescence of the TAP–dopa melanin coulombic complex is strongly quenched, but the fluorescence decay times are found to be similar to the lifetime of the M form. This supports our excitation fluorescence results about the existence of the TAP–dopa melanin complex and M form of TAP in the sample. Some of the TAP molecules that do not create the complex show fluorescence. Small differences in the TAP monomeric lifetime are due to the pH conditions and rather weak interaction with dopa melanin.

Table II shows the fluorescence lifetimes for porphyrins and their mixtures with dopa melanin for the observation wavelength of 670 nm in the wide pH range. The 670 nm region is assigned to the fluorescence originating mainly from the Ds and partially from the Ms. The lifetimes for the D forms are about 2 and 3 ns for TAP and $TPPS_4$, respectively, and they are similar in the samples with dopa melanin. When the M and D forms coexist together in the sample (pH 4, TAP; pH 5, $TPPS_4$), excitation energy transfer between the M (energy donor) and D (energy acceptor) was shown to occur very effectively [1]. For that reason, the shortening of the M lifetime can be expected because of the interaction with the D form, which leads to energy transfer. Thus, we observed the decrease of the fluorescence lifetimes of TAP (at pH 4) and of $TPPS_4$ (at pH 5) from about 9 to 3.55 ns (TAP) and

Table II. Fluorescence Lifetime of TAP and $TPPS_4$ in the Absence/Presence of Dopa Melanin

Sample	pH	Dye form	τ (ns)	τ_1 (ns)	τ_2 (ns)
TAP	2	D	2.68 ± 0.20	—	—
	4	M + D	—	2.15 ± 0.20	3.55 ± 0.20
	5	M	7.81 ± 0.20	—	—
	9	M	9.52 ± 0.20	—	—
TAP + dopa melanin	2	D	2.58 ± 0.20	—	—
	4	D + C	—	2.96 ± 0.20	3.24 ± 0.20
	5	M + C	7.79 ± 0.20	—	—
	9	M + C	8.07 ± 0.20	—	—
$TPPS_4$	2	D	3.23 ± 0.20	—	—
	4	D	3.53 ± 0.20	—	—
	5	M + D	—	3.87 ± 0.20	6.08 ± 0.20
	9	M	8.51 ± 0.20	—	—
$TPPS_4$ + dopa melanin	2	D	3.38 ± 0.20	—	—
	4	D	3.54 ± 0.20	—	—
	5	M + D	—	4.03 ± 0.20	6.51 ± 0.20
	9	M	9.04 ± 0.20	—	—

Observation wavelength: 670 nm. M, monomer; D, monomeric dication; C, coulomb complex; τ, for monoexponential decay; τ_1, τ_2 for biexponential decay.

to 6.08 ns ($TPPS_4$), respectively. This decrease is caused by the energy transfer from M to the D form [1].

The lifetime results confirm our supposition about the weak influence of dopa melanin on $TPPS_4$ and much stronger interaction between dopa melanin and TAP. In the TAP–dopa melanin sample at the pH 4 condition, two observed lifetimes can be assigned to the D form (τ_1 = 2.69 ns) and the small trace remaining M form, which transfers energy to the D form (τ_2 = 3.24 ns). The weakening of the TAP–dopa melanin interaction is evidently observed under the very low pH conditions in which the coulombic complex is destroyed.

Photoacoustic

Figures 8 and 9 show the photoacoustic spectra (PAS) data for $TPPS_4$ and TAP, respectively. In $TPPS_4$ at low pH (spectra shown in Figs. 8A and B) the appearance of the particularly strong bands at 490 and 706 nm indicates the domination of the ADs in losing

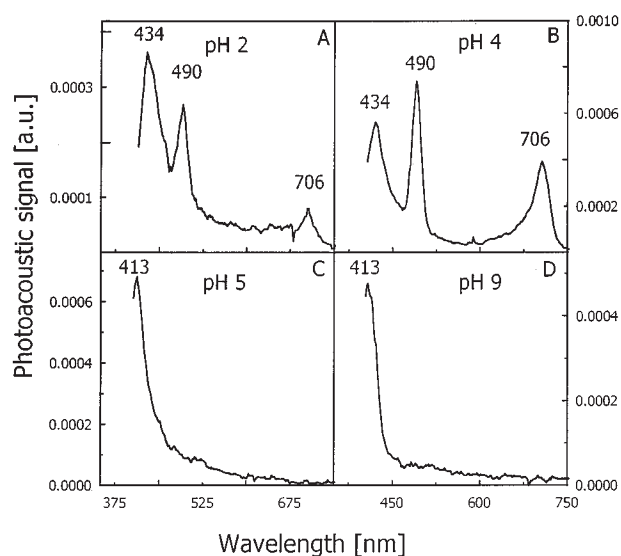

Fig. 8. Photoacoustic spectra of $TPPS_4$ in the presence of dopa melanin at different pH; (A) pH 2, (B) pH 4, (C) pH 5, (D) pH 9.

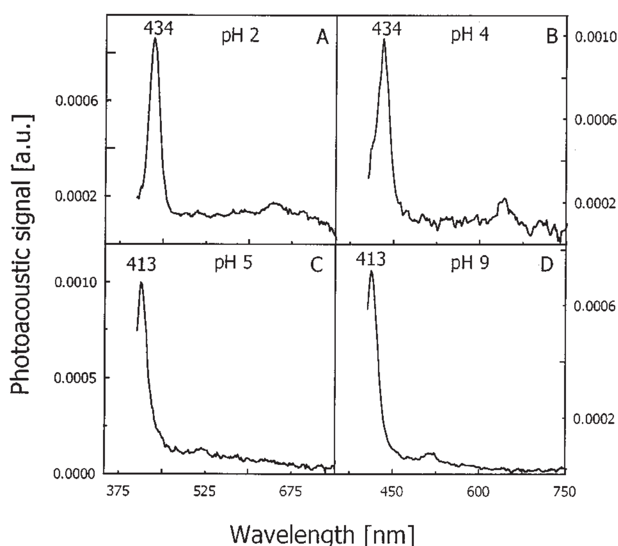

Fig. 9. Photoacoustic spectra of TAP in the presence of dopa melanin at different pH; (A) pH 2, (B) pH 4, (C) pH 5, (D) pH 9.

energy in the process of thermal deactivation. In the excitation spectra, the 490 nm band was observed as a small peak and its strong PAS confirms domination of the AD heat liberation. This process can occur as a result of the direct light excitation of the electronic state of the spectral form and also as a consequence of the indirect energy transfer from dopa melanin. The PAS intensity of the spectral form responsible for the signal at 490 nm in the presence of dopa melanin is changed when compared with PAS of this form without dopa melanin [1]. This result confirms our supposition made on the basis of the fluorescence study. In the remaining species of $TPPS_4$, thermal deactivation is only modestly increased in the presence of dopa melanin.

In our previous articles [16,17], the existence of the TAP–dopa melanin complex was confirmed by the fluorescence quenching and photoacoustic examination at high pH (7–9) and the particular thermal properties of this complex were observed [1]. We do not discuss this result in this article. We focus our attention on the PAS results for TAP at low pH. Figure 9 (A, B), which presents the results for TAP in the presence of dopa melanin in the acidic medium (pH 2 and 4), shows that the monomeric dication of this dye gives a large contribution to thermal deactivation since the intensive band with a maximum at 434 nm is clearly observed. We notice that PAS of the D form of TAP is not actually altered in the presence of dopa melanin and in the low-pH medium. We did not observe any changes in PAS shape or in the position of 434 nm band at acidic pH. When the PAS result is compared with the absorption spectrum, we can indicate the large contribution of monomeric dication in thermal processes, and dopa melanin hardly influences these properties.

DISCUSSION

We have shown the influence of pH and of the presence of dopa melanin on the photophysical behavior of charged porphyrins. A variation in pH originates the creation of different TAP and $TPPS_4$ forms: M (for both dyes), D (TAP and $TPPS_4$), and the AD form in the case of $TPPS_4$ [1]. The influence of dopa melanin on these forms is shown to be dependent on a dye form. A formation of the D form at low pH is due to the protonation of the nitrogen atoms in the porphyrin ring of dyes. The molecules change their symmetry from D_{2h} to D_{4h} [19,21–24]. Then the absorption spectrum of protonated porphyrins becomes similar to the spectrum of metalloporphyrins [25], as we also observed in reference [1]. The molar absorption coefficients of these forms in the red absorption region are higher that those of the monomeric forms. This ascertainment is essential in view of the biological tissue transparency in the visual absorption region. If the tissue transparency and the lower acidity of a cancerous tissue in comparison to healthy tissue is taken into account, the large molecular coefficient is a feature needed for an effective agent in PTD [22,26–29].

This work shows the fluorescence and thermal behaviour of two differently charged porphyrins in the presence of dopa melanin. These results were compared previous studies of the photophysical properties of these dyes presented in previous articles [16,17]. The presence of dopa melanin does not drastically change the spectral behavior of $TPPS_4$. All three forms still exist when mixed with melanin. The D forms of both $TPPS_4$ and TAP show strong thermal deactivation, which is slightly changed in the presence of dopa melanin.

Another interesting result is the existence of the possible process of energy transfer between dopa melanin and the AD form of $TPPS_4$, because the fluorescence intensity of the latter increases in the presence of dopa melanin and the thermal deactivation is also changed. A different situation is found for TAP with dopa melanin. This study shows that the TAP–dopa melanin coulombic complex is destroyed under acidic conditions (the lowest pH). A possible explanation for such a supposition is that the interaction between dopa melanin and a solvent is stronger than that between dopa melanin and TAP dye (at the lowest pH). This interaction is most probably because of the competition between the positive groups of TAP dye and protons in the acidic solvent. Moreover, the formation of the aggregated species of dopa melanin in the low-pH medium cannot be excluded, as indicated in reference [24]. However, our result indicates that the interaction with the acidic solvent leads to weakening in the interaction between dopa melanin and TAP. This is confirmed by the position of the absorption band of TAP-D (434 nm) in contrast to that of the TAP–dopa melanin complex (420 nm), as well as by the high fluorescence of the D form at 665 nm (whereas fluorescence of the complex is strongly quenched). The D form exists in the presence of dopa melanin and its fluorescence and thermal properties are only slightly altered by the latter.

CONCLUSIONS

We generally conclude as follows:

1. Acidic conditions lead to the destruction of the TAP dopa melanin complex because of the sat-

uration of the negative centers of dopa melanin by protons. There is a competition between protons and positively charged TAP in the interaction with dopa melanin.

2. Thermal deactivation of M and D (TAP and $TPPS_4$) forms under acidic conditions is hardly altered in the presence of dopa melanin. This conclusion can suggest that in the dyes a part of the absorbed light energy is exchanged into heat independently of the dopa melanin presence. This conclusion is important because of the protection of a biological tissue from excessive solar irradiation.

The presented results on photophysical properties of the porphyrin dyes under acidic conditions seem to be important because of the lower acidity of cancerous tissue when compared to that of healthy tissue.

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