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Extraordinary behavior of 5-(3,4-dihydroxyphenyl)-10,15,20-tris(*N*-methylpyridinium-3-yl)porphyrin triiodide in titration with bases and in albumin oxidation

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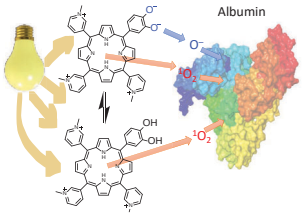
The observed rate constants of the photoinduced oxidation of albumin increased by a factor of 1.5–2 upon deprotonation of the peripheral hydroxyl groups of 5-(3,4-dihydroxyphenyl)-10,15,20-tris(*N*-methylpyridinium-3-yl)porphyrin triiodide compared to its phenolic form.



Keywords: hydroxy-substituted porphyrin, albumin, UV-VIS, fluorescence, ROS, photooxidation.

Hydroxy-substituted porphyrins are promising candidates for the use in photodynamic therapy (PDT)[1–5] as well as in the development of sensor devices.[6,7] The main scientific and practical interest in these porphyrins is associated with their pH-sensitivity. Hydroxy-substituted porphyrins are uncharged at pH ~ 7, while their electronic absorption spectra contain an intense Soret band at ~ 420 nm and four weak bands (Q-bands) in the visible spectral region. The presence of hydroxyl groups in the peripheral substituents in porphyrin creates new possibilities, since their deprotonation can lead to the charge transfer (CT) from the peripheral anionic hydroxide substituents to the porphyrin core. This entails a change in the energy state of the macrocyclic system and the manifestation of non-trivial spectroscopic and photocatalytic properties. For example, hydroxyphenyl-substituted porphyrins become hyperporphyrins in alkaline media demonstrating noticeable additional absorption bands (ε > 1000 dm^3 mol^{-1} cm^{-1}) at λ > 320 nm, which are not attributed to π–π* transitions.[8] These additional bands are supposed to originate from the π(phenoxide anion)–π*(porphyrin) transitions with CT.[8,9] The study of hydroxy-substituted porphyrins in the context of CT and the resulting photochemical properties is required for the creation and development of artificial photosynthetic systems and drugs with different mechanisms of action, both applications being based on photosensory properties and acid–base interactions. The spectral properties of hydroxy-substituted porphyrins, additionally containing alkyl and aryl substituents and hence soluble in organic media, have been studied. We assume that the introduction of cationic *N*-methylpyridinium groups will provide water solubility of porphyrins[10] together with the appearance of effects connected with pH-sensitivity or hydrogen bonding involving hydroxyl-containing substituents. The existence of peripheral substituents having opposite charges can promote CT with the formation of ion pairs and associates.

The hydroxyphenyl substituent in the porphyrin periphery should be converted into anionic phenoxide or keto form in order to promote CT.[11,12] Hydroxyphenyl substituents are also found in enol form in porphyrins. The activation energy of the tautomeric transition can be lowered by the proton removal from hydroxyl by external base. Therefore, the aim of this work was to explore the acid–base transformations of 5-(3,4-dihydroxyphenyl)-10,15,20-tris(*N*-methylpyridinium-3-yl)porphyrin triiodide from phenolic (OHP) to anionic phenoxide forms [OHP(O$^-$)]. The following bases were used to shift the equilibrium: NaOH, pyridine, aqueous ammonia and DABCO, and the spectrophotometric titrations of OHP solutions was carried out. The additional aim of this work was to evaluate the photodynamic activity of OHP and OHP(O$^-$) towards albumin, which is widely used as a model protein.[13]

The UV-VIS spectral changes of OHP during titrations with NaOH and aqueous ammonia are shown in Figure 1. The addition of a relatively small amount of base (up to pH 7.5) leads to a decrease in the absorption intensity of the Soret band and its blue shift from 420 to 418 nm in the case of NaOH and from 420 to 408 nm in the case of aqueous ammonia. The spectra in the long-wavelength region also undergo changes. The intensity of the

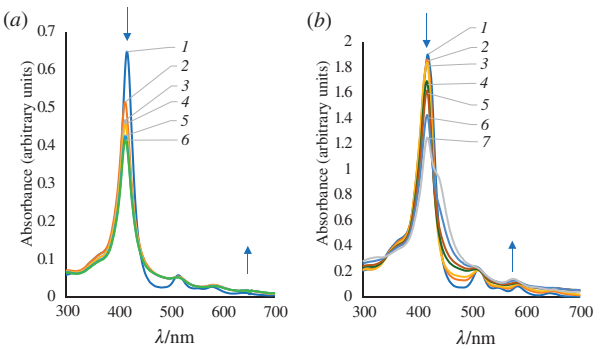


Figure 1 (*a*) Titration of (*1*) OHP (2 ml, 5.8 × 10^{-6} M) with (*2*) 10 μl, (*3*) 20 μl, (*4*) 30 μl, (*5*) 40 μl and (*6*) 50 μl of 25% aqueous ammonia in water–DMF (50:50 v/v). (*b*) Titration of (*1*) OHP (2 ml, 5.8 × 10^{-6} M) with (*2*) 0.1 mg, (*3*) 1 mg, (*4*) 3 mg, (*5*) 5 mg, (*6*) 10 mg and (*7*) 20 mg of NaOH in water–DMF (50:50 v/v).

band IV decreases, the band III is not detected, the bands I and II increase in their intensity. In addition, there is an increase in absorption in the region of 650–750 nm, indicating the so-called hyperporphyrin effect,[14] probably caused by a change in the LUMO and HOMO energies,[15–17] as well as CT from the substituent to the macrocycle.[18] The second stage of titration, which is observed only in the case of NaOH, is characterized by the splitting of the Soret band and the appearance of intense absorption near 578 nm, along with a decrease in intensity of the bands at 516 and 580 nm. The spectrum resembles the two-band spectra characteristic of porphyrins with D_{4h} symmetry [see Figure 1(*b*)]. To attribute the observed spectral changes to the processes of deprotonation of hydroxyl groups at the periphery of the macrocycle and deprotonation of NH-groups in the reaction center, the titration of 5,10,15,20-tetrakis(*N*-methylpyridinium-3-yl)porphyrin tetraiodide (TMPyP3) with NaOH solution was carried out, where deprotonation is possible only at the macrocycle (Figure 2). The spectral changes are detected upon addition of a large excess of NaOH, as the spectrum became two-band.

The similarity of the spectral changes observed during the titration of TMPy3P (see Figure 2) and during the second stage of OHP titration [see Figure 1(*b*)] suggests the deprotonation of pyrrolic NH-groups of the macrocycle. The relative acidities of the groups under consideration additionally confirm the order of deprotonation. According to the published data, pyrrolic NH-groups have a very low acidity ($pK_a > 15$),[19] while the acidity of phenol and its derivatives is much higher (pK_a = 8–10).[20] Therefore, the initial stage of titration of OHP with NaOH corresponds to the deprotonation of peripheral hydroxyls with the formation of anionic phenoxide substituents.

Titration of OHP with pyridine and DABCO (Figure 3) causes changes in the long-wavelength region of the spectrum resembling those depicted in Figure 1(*a*). This probably reflects the formation of H-complexes between the peripheral hydroxyl groups of porphyrin and amines.

The position of the Soret band in the UV-VIS spectrum of OHP during titration depends on the nature of the used base. The blue shift of the Soret band position is observed when DABCO is used, while the red shift occurs in the case of pyridine. It should be noted that the fluorescence spectra of OHP during titration with DABCO and pyridine are fundamentally different. The fluorescence of porphyrin decreases upon addition of pyridine, along with an increase in reflection intensity. Probably the change in the dielectric constant of the obtained water–DMF–pyridine (0–10%) solvent mixture affected in this case. This leads to a decrease in the degree of polarization of *N*-methylpyridinium–iodide ion pairs by solvent, a decrease in electrostatic repulsion between substituents of neighboring porphyrin molecules and π–π aggregation of porphyrin as reflected in the UV-VIS spectra [see Figure 3(*a*)].

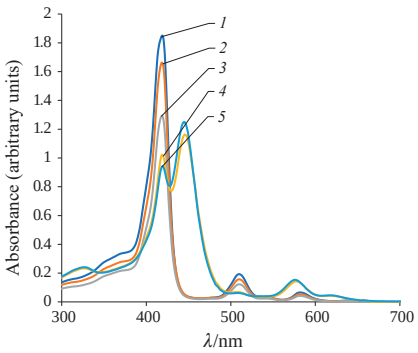


Figure 2 Titration of (*1*) TMPyP3 (2.2×10^{-5} M) with (*2*) 1 mg, (*3*) 2 mg, (*4*) 4 mg and (*5*) 10 mg of NaOH in water–DMF (50:50 v/v).

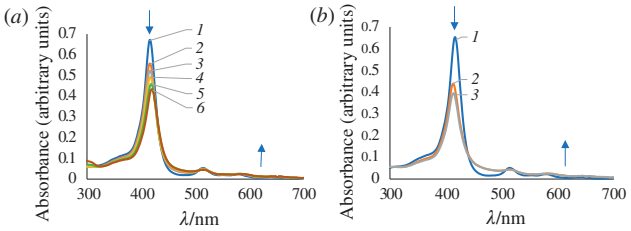


Figure 3 (*a*) Titration of (*1*) OHP (5.8×10^{-6} M) with (*2*) 5 μl, (*3*) 10 μl, (*4*) 20 μl, (*5*) 80 μl and (*6*) 200 μl of pyridine in water–DMF (50:50 v/v). (*b*) Titration of (*1*) OHP (5.8×10^{-6} M) with (*2*) 1 mg and (*3*) 5 mg of DABCO in water–DMF (50:50 v/v).

In the case of titration of OHP with DABCO, the fluorescence spectra change differently. The first addition of DABCO causes a decrease in fluorescence and a change in the intensity ratio of the bands at 650 and 700 nm. Further titration leads to a gradual increase in fluorescence. This is an unexpected result, especially considering the previously published data.[21] The fluorescence quenching of hydroxy-substituted porphyrins by amines with different basicity has been investigated. It was concluded that amines quench the fluorescence of hydroxy-substituted porphyrins through both the static and dynamic pathways. The quenching effect intensified with an increase in the basicity of amines. The dynamic quenching process was identified as an electron transfer from the H-linked peripheral hydroxyphenyl substituent to the porphyrin macrocycle in the singlet state. This transfer is due to H-bonding and subsequent movement of the proton of the hydroxyphenyl substituent to pyridine. The basicity constant of DABCO ($K_b = 10^{-3}$–10^{-4}) is much higher than the basicity constant of pyridine ($K_b = 2.3 \times 10^{-9}$); however, only pyridine quenches the fluorescence of OHP.

As shown above, the changes in the UV-VIS spectrum of OHP upon titration with pyridine indicate the π–π aggregation of porphyrin [see Figure 3(*a*)]. This conclusion is confirmed by the OHP fluorescence decrease upon titration with pyridine.

The spectral changes obtained in the system OHP–DABCO (an increase in the reflection and fluorescence intensities) correspond to the phenomenon of J-aggregation.[22–24] Usually the J-aggregation in porphyrins is provoked by ionization of peripheral substituents, for example, by their protonation.[25] In the case of deprotonation of OHP, the ionization of OH groups due to hydrogen bonding is unlikely. Probably, there is another reason for the formation of J-aggregates. The strong blue shift of the Soret band in the UV-VIS spectra of OHP during the titration with DABCO [see Figure 3(*b*)] resembles that observed during the titration with ammonia [see Figure 1(*a*)]. Therefore, we analyzed the fluorescence spectra of the systems OHP–ammonia ($K_b = 1.8 \times 10^{-5}$) and OHP–NaOH ($K_b = 1.59$).[26] A monotonic decrease in fluorescence intensity associated with deprotonation of the porphyrin reaction center was observed during the titration of OHP with NaOH. Moreover, the addition of a strong base can lead to a partial neutralization of the charges of the peripheral cationic *N*-methylpyridinium groups, a decrease in the electrostatic repulsion between neighboring macrocycles, and thereby promote their association.

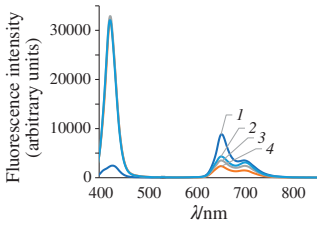


Figure 4 Fluorescence spectra of (*1*) OHP (5.8×10^{-6} M) upon addition of (*2*) 10 μl, (*3*) 30 μl and (*4*) 60 μl of 25% aqueous ammonia, λ_{ex} = 425 nm in water–DMF (50:50 v/v).

Titration of OHP with ammonia causes the changes in the fluorescence spectra (Figure 4) resembling those described above for titration with DABCO. Therefore, it can be concluded that J-aggregates are also formed in the OHP–aqueous ammonia system.

In general, the titrations of OHP with four bases lead to aggregation of the macrocycles. The addition of pyridine and NaOH to porphyrin solutions results in π–π aggregation, while DABCO and aqueous ammonia provide J-aggregation. There is no correlation between the strength of a base and the resulting destabilizing effect. Probably, the influence of the bases on the aggregation mechanism depends on their nature. DABCO and aqueous ammonia can axially bind the porphyrin macrocycle, sterically preventing π–π stacking. In the case of NaOH, the negative charge and high basicity of hydroxide anion makes it preferable to neutralize the peripheral cationic groups of OHP. The aromatic nature of pyridine, along with the macrocyclic effect of π-electron shielding, probably prevents the solvation of the reaction center of porphyrin by pyridine molecules.

The aim of the next stage of the work was to estimate the effect of the deprotonation of hydroxyphenyl substituents on the photocatalytic activity of OHP in the oxidation of 1,3-diphenylisobenzofuran (DPBF) and bovine serum albumin (BSA). The rate of singlet oxygen generation by OHP was determined using DPBF in air-saturated DMF with tetraphenylporphyrin as a standard. Deprotonation of OHP was achieved by addition of NaOH. The formation of anionic phenoxide substituent in OHP has the similar effect on its UV-VIS spectra in water and in organic solvents, but in DMF the CT band is shifted to the long-wavelength region with its maximum at about 725 nm. It is obvious that OHP is not effective in generating singlet oxygen. The deprotonation of dihydroxyphenyl substituents forming the anionic OHP(O^-) negatively affects the ability of porphyrin to generate singlet oxygen (Table 1).

The BSA oxidation was carried out in aqueous media with addition of NaOH to convert OHP into OHP(O^-), while the pH of the resulting solution was less than 7.5. This parameter is essential for proteins, because the conformation of polyelectrolytes is pH-dependent. It is generally known that the N–B conformational transition occurs at pH 9.[27] Typical protein fluorescence spectra obtained upon irradiation at 425 nm in aqueous BSA–OHP solutions at pH 7 and 7.5 are shown in Figures 5(*a*) and 5(*b*), respectively.

The obtained data indicate that the BSA photooxidation in the presence of OHP(O^-) proceeds much faster than in the case of OHP. The addition of sodium azide into the system slows down

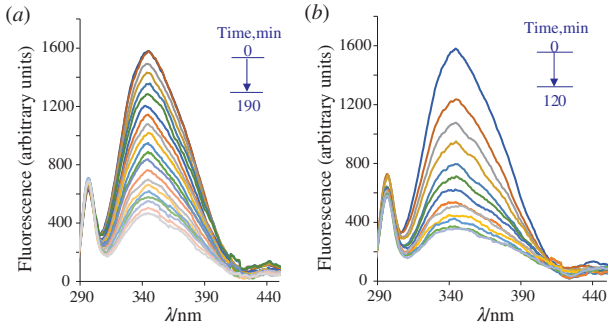


Figure 5 BSA fluorescence spectra under irradiation of BSA–OHP solutions with 425 nm light at (*a*) pH 7 and (*b*) pH 7.5 in 0.05 M aqueous NaCl. Time step is 10 min.

Table 1 Singlet oxygen quantum yields of porphyrins in DMF at λ_{ex} = 525 nm.

	OHP	OHP(O^-)
$\Phi(^1O_2)$	0.07	0.02

Table 2 Observed rate constants of BSA oxidation by porphyrins.

Porphyrin	K_{obs}(BSA) × 10^5	
		NaN_3
OHP(O^-)	20	3
OHP	10	2
TMPyP3 (pH 7)	70	
TMPyP3 + NaOH (pH 7.5)	80	

the protein oxidation but does not completely block it. This means that some other reactive oxygen species are involved in protein oxidation. The observed reaction rate constants calculated according to the known procedure[28] are given in Table 2.

The observed rate constants of BSA oxidation with the participation of OHP are twice as low compared to that observed in the presence of OHP(O^-). These differences may be caused by the fact that CT from the anionic phenoxide substituent to the macrocycle does not affect its ability to generate reactive oxygen species. In addition, despite the preservation of the native conformation, the protein can undergo conformational changes depending on pH or background electrolyte. To test this assumption, the rate constants of BSA photooxidation in the presence of TMPyP3 at pH 7 and 7.5 were measured (see Table 2). With an increase in pH, a slight increase in the rate constant is observed. Therefore, the twofold difference in the rate constants in the case of OHP and OHP(O^-) when pH changes from 7 to 7.5 is associated mainly with the effect of porphyrin, and, to a lesser extent, with a change in the protein conformation. The negative charge on the oxygen atoms in OHP(O^-) can abstract protons from the amino acid residues of BSA.

In conclusion, the spectral properties of 5-(3,4-dihydroxyphenyl)-10,15,20-tris(*N*-methylpyridinium-3-yl)porphyrin triiodide (OHP) were explored when NaOH, pyridine, ammonia and DABCO were added to its water–DMF solution. It has been found that all the above bases can induce a hyperporphyrin effect and promote the deprotonation of hydroxyphenyl substituents in OHP with the formation of the corresponding anionic phenoxide groups. In addition to the hyperporphyrin effect, electron donor compounds cause a shift in the aggregation equilibria of porphyrin towards association. J-aggregates are formed upon addition of ammonia and DABCO, while π–π-aggregates are formed in the case of pyridine. The photochemical measurements showed that OHP is not effective in generating singlet oxygen. The conversion of dihydroxyphenyl substituents into the anionic form by the action of NaOH decreases the quantum yield of singlet oxygen, but at the same time accelerates the photooxidation of BSA by a factor of 1.5–2. Apparently, the negative charge on the oxygen atoms in OHP(O^-) can lead to the removal of protons from amino acid residues of the protein, making an additional contribution to photodegradation.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.07.040.

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