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THE COMBINED EFFECTS OF ASCORBIC ACID AND BOVINE SERUM ALBUMIN ON PHOTOTRANSFORMATIONS OF HEMATOPORPHYRIN DERIVATIVE IN AQUEOUS MEDIUM: ABSORPTION AND EPR SPECTROSCOPY STUDY

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There is a constant interest to increase the efficacy of photosensitized therapy by combining it with other modalities in order to boost the oxidative stress in tumour tissues, and L-ascorbic acid (AscA) could serve as a potential candidate. The photoinduced transformations of a hematoporphyrin-type photosensitizer (HpDiA) were chosen as a model system to monitor the effects of AscA on oxygen-dependent photoreactions in aqueous model solutions of different pH. Additional data on the role of the ascorbate radical in photoreactions initiated by HpDiA, as well as on the mutual activity in samples containing bovine serum albumin (BSA), including participation in Type I reactions, were obtained performing electron paramagnetic resonance (EPR) spectroscopy measurements, done on the mixed aqueous solutions poured into capillary tubes of a carefully selected diameter, both in the dark and under illumination with a laser beam in the absence of additional trapping molecules. A strong interaction between BSA and porphyrins was determined as a main factor in the observed photoreactions, not only boosting the photooxidation and photoreduction pathways, but also leading to the enhanced photoactivity in combination with AscA, especially, in the acidic medium.

Keywords: hematoporphyrin derivative, L-ascorbic acid, bovine serum albumin, EPR and absorption spectroscopies, photoproducts

1. Introduction

The illumination of a selected photosensitizer (PS) using an appropriate light source in order to induce photochemical reactions generating reactive oxygen species or free radicals, which subsequently attack surrounding biomolecules and bring damage to the malignant cells, constitutes the essence of photosensitized tumour therapy (PDT) [1]. The anticancer efficacy of PDT, however, is affected by tissue oxygenation and the capacity of malignant cells to endure the induced oxidative stress. A proper dynamic balance between oxidation and reduction processes in the living organism seems to be a key condition ensuring normal homeostasis of tissues and organs [2], while its imbalance could reflect an early stage of various inflammatory diseases. It is known that malignancy and resistivity of some tumours to various treatment modalities depend on the level of the tissue oxidative state [3]. To enhance the efficacy and applicability of PDT, various methods were suggested to maintain the supply of oxygen into the tumour tissue [4], or to avoid its photoinduced depletion [5]. It is not by chance that PDT is often combined with other treatment modalities seeking for the best outcome [1].

Many of the clinically approved PS are not photostable and can directly participate in photoreactions. Hematoporphyrin-type tetrapyrroles representing the first PS generation are known for the ability to initiate both Type I or II photoreactions, including generation of free radicals or energy transfer processes [6–9. The extent of phototransformations (PT), especially those occurring

in vivo, depends not only on the initial concentration of PS and the delivered light dose, but also on the level of oxygenation in the surrounding environment [10]. Changes in PS spectral properties, therefore, can be considered as a prognostic indication to monitor and forecast the photoinduced damage during the treatment [1].

The presence of other substances can also have a strong effect on the reactivity of photoexcited PS, which can then initiate both photooxidation and photoreduction processes, forming the reactive intermediate species [11]. As it has been reported in earlier studies, the formation of photoproducts (PhP) involving both electron and proton transferring has been observed in the case of solutions of porphyrins illuminated under hypoxic conditions [8, 12]. Declining availability of oxygen in photosensitized tumour tissues, which are often distinguishable from the normal surroundings by lower pH and hampered circulation of liquids, is usually stated as a serious obstacle during the irradiation procedure. It has been also accounted for as one of the important factors limiting the treatment success [4]. Thus, to achieve the efficient anti-tumour effect, the requirements for the applied photoactivable agents include the ability to induce the extensive photochemical reactions, which boost the existing oxidative stress and, at the same time, to overcome or deplete the internal antioxidative defence mechanisms of cancer [13]. As different photochemical reactions have varying dependence on the oxygenation level [14], such approach could involve the photoprocesses that would endure the depletion of oxygen species, but, nevertheless, would modify biomolecules via a free radical mechanism and enhance the oxidative stress to the critical level for tumour cells [15].

Recently, there is a resurgent interest in the anticancer activity of vitamin C or L-ascorbic acid (AscA) [16], especially with growing evidence that an intravenous application allows one to achieve its significantly higher concentration in the bloodstream [17, 18]. This antitumour modality is based on the modification of the oxidation state in tumour tissues [19, 20]. In the presence of various PS and oxygen the ascorbic acid is mostly accounted for its photoprotective and antioxidant activities [21]. The biological activity of AscA, as a reducing agent, manifests itself as donation of electrons and protons occurring during molecular interactions. Yet, under certain circumstances, which may arise in the microenvironment of cancerous tissues, AscA, like many compounds possessing antioxidant features, can be involved into prooxidative processes [22], especially in the vicinity of additional reducing agents [16, 23], and can generate hydrogen peroxide leading to cytotoxic effects in tumour cells [24, 25]. Such beneficial effects for cancer treatment can be boosted in the presence of PS and the light [26, 27], which leads to the formation of ascorbate radical (Asc•) [28, 29] and the enhancement of photodynamic action [30, 31]. However, which of multiple intersecting reaction pathways becomes predominant in each particular case strongly depends on the oxygenation and pH of the surrounding medium as well as on the availability of a sensitizing substance and oxidation states of the neighbour molecules.

In the present study, the phototransformations of hematoporphyrin-type PS (HpDiA) detected by means of absorption spectroscopy served as a model system to monitor the effects of AscA on the oxygen-dependent photoreactions induced in mixed aqueous solutions at different pH. Electron paramagnetic resonance (EPR) spectra were measured before and during the illumination of samples under carefully selected conditions to analyse the mechanisms of photoreactivity involving Asc•. The experimental evidence is provided on the distinct role of serum albumin in regulating the extent and the pathways of the photochemical reactions initiated by both HpDiA and AscA.

2. Materials and methods

2.1. Preparation of solutions

The stock solution of a hematoporphyrin IX derivative (Hp diacetate, HpDiA [32]) was prepared by dissolving crude powder in 20 μl of 0.2 M NaOH and then diluting it to 10^{-3} M using a phosphate buffer solution (PBS, 0.05 M, pH 7). The stock solution of L-ascorbic acid (*Sigma Aldrich*) was prepared by dissolving crude powder in distilled water to a concentration of 0.1 M just before experimental measurements. The stock solution of bovine serum albumin (BSA, fraction V, *Carl Roth GmbH + Co*) was also prepared by dissolving it in a phosphate buffer to 10^{-3} M. H_2O_2 solution (3%,

Greenice, *Gemi*, Poland) was diluted in samples before usage.

2.2. Experimental setup for absorption spectroscopy

The experimental samples for spectroscopic measurements were prepared from the stock solution by a simple dilution with a 0.05 M phosphate buffered solution or subsequent mixing with stock solutions of other substances to get a final PS concentration of 7.5×10^{-5} M. The pH value of a particular buffered solution was adjusted with droplets of either 0.2 N NaOH or HCl measuring with a pH-meter (IQ150, *IQ Scientific Instruments, Inc.*). PT of HpDiA were induced by periodically exposing the samples in 10 mm quartz cuvettes to a beam of the green light (532 nm, 60 mW, expanded with a lens to a diameter of 1 cm^2) from a diode-pumped solid-state laser. A magnetic stirrer was applied continuously during exposure. The absorption spectra were recorded in-between exposures using a fibre-optics based spectrometer AvaSpec2048 (*Avantes*).

2.3. Experimental setup for EPR spectroscopy

The final concentrations of substances in the samples, which were mixed from the stock solutions for EPR spectroscopy, were the following: HpDiA and BSA, $c = 10^{-4}$ M, AscA and H_2O_2, $c = 10^{-2}$ M. The EPR spectra were recorded by placing the samples injected in 50 µl micropipettes (*Brand GmbH + Co KG*) inside a spectrometer Elexsys-E580 (*Bruker*) and continuously exposing to a beam of the green laser light through the tip of an optical fibre with a diameter of 1 mm, which was fixed to the special holder at the bottom of the resonator. The size of micropipettes was carefully chosen to ensure a balance between the sufficient signal amplitude and the suppressive absorption effect of the aqueous medium. The beam power was adjusted in-between spectral registrations from the lowest to the highest value unless otherwise indicated. The EPR X-band with the amplitude of modulation set to 0.8 G and the high frequency attenuation of 17 dB was used for measurements. The signals were recorded 3–5 times (about 1 min per scan) to get the averaged spectra, while the number of scans was chosen depending on the detected amplitude of a spectral signal.

3. Results and discussion

3.1. Absorption spectroscopy data

The photoinduced changes in the absorption spectra of the HpDiA that were detected in acidic (Fig. 1(a–d)) and neutral (Fig. 1(e–h)) aqueous buffered solutions after a total exposure to 108 J/cm^2 revealed strong effects of added BSA and AscA, also shown in a more detailed manner in Fig. 2. The well-reported PT of hematoporphyrin in aqueous neutral solutions comprise the autooxidation of PS, which is realized via energy transfer from its photoexcited triplet state towards molecular oxygen generating reactive oxygen species [33] (ROS, a Type II photochemical reaction), and the subsequent collision leading to the oxygen-dependent formation of red-absorbing chlorin-type photoproducts (Chl-PhP) with a distinct spectral band at about 640 nm [34]. Photooxidation of a porphyrin ring can also lead to its opening and formation of biliverdin-type products possessing a broad absorption band in the red spectral region and bilirubin-type products absorbing in the blue spectral region [35], as well as further structural degradation into smaller pyrroles. The PT of HpDiA were expectedly much more efficient in the neutral medium than in the acidic one (Figs. 1, 2). A shift in the ionic equilibrium as well as the increased aggregation of HpDiA in acidic solutions are among the most probable reasons for the decreased formation of a Chl-PhP at pH 5 (Fig. 1(a)).

AscA had a strong inhibitory effect on the photoinduced changes throughout the whole spectral region from 400 to 800 nm, including the formation of a spectral band at 640 nm, while a slight increase at about 700 nm underwent no changes in both cases (Fig. 2(a, b): 2). It has to be noted that the addition of AscA to the acidic buffered solutions of HpDiA caused further decrease in the medium pH, which is reflected in the lowered intensity of absorption bands and an increased background signal of light scattering, implying the extensive aggregation of porphyrins taking place in the solutions (Fig. 1(b), 1). The illumination of acidic HpDiA solutions apparently had a minimal effect on the intensity of absorption bands, but mostly diminished the scattering component in the spectra (Fig. 1(b), Fig. 2(a)).

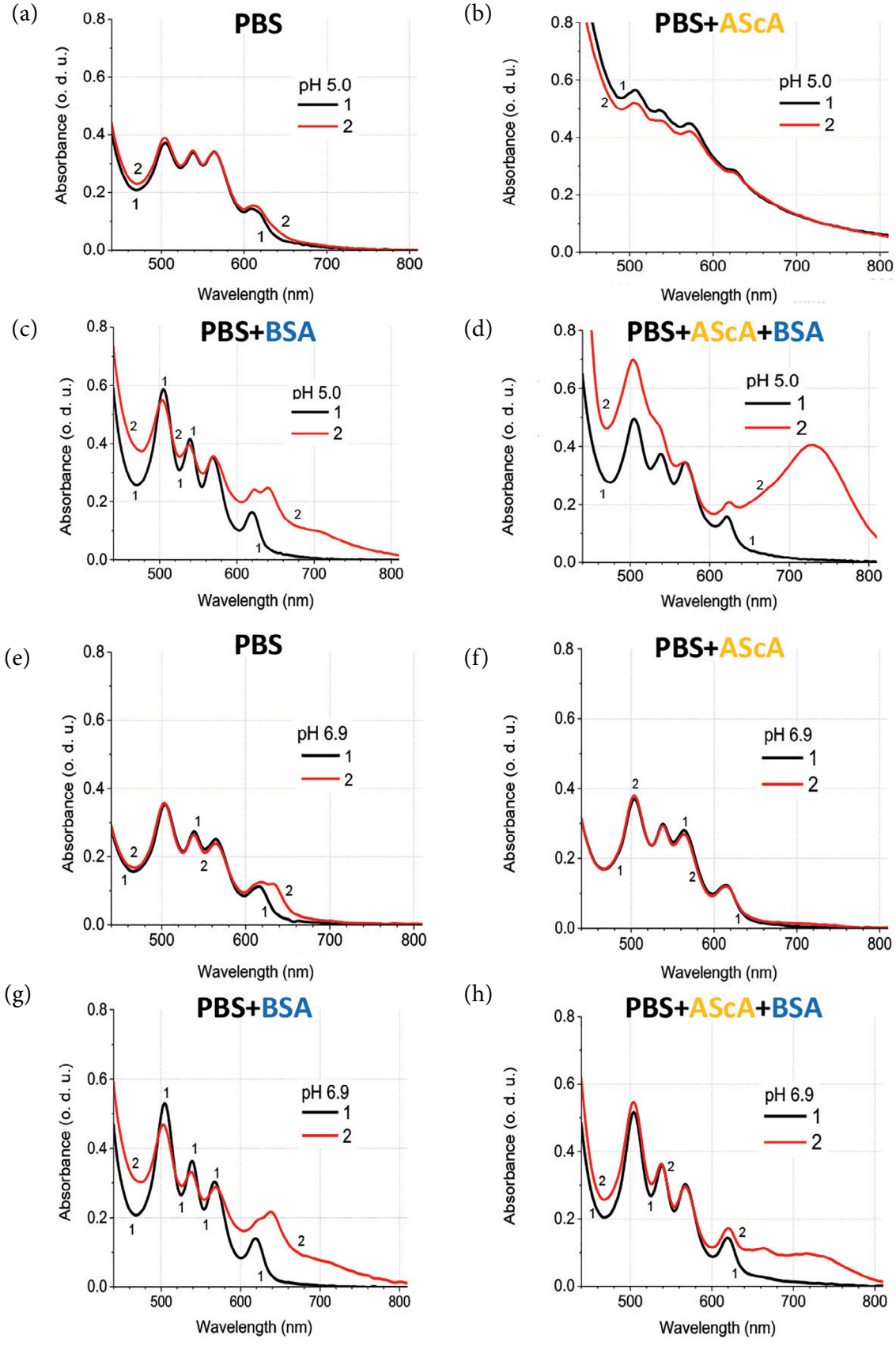

Fig. 1. The absorption spectra of HpDiA ($c = 7.5 \times 10^{-5}$ M) in (a–d) acidic (pH 5.0, 0.01 M) and (e–h) neutral (pH 6.9, 0.05 M) buffered solutions recorded (1) before and (2) after illumination for 30 min, reaching a dose of 108 J/cm^2: (a, e) control samples, (b, f) with AscA ($c = 5 \times 10^{-3}$ M), (c, g) with BSA ($c = 7.5 \times 10^{-5}$ M), (d, h) a mixture.

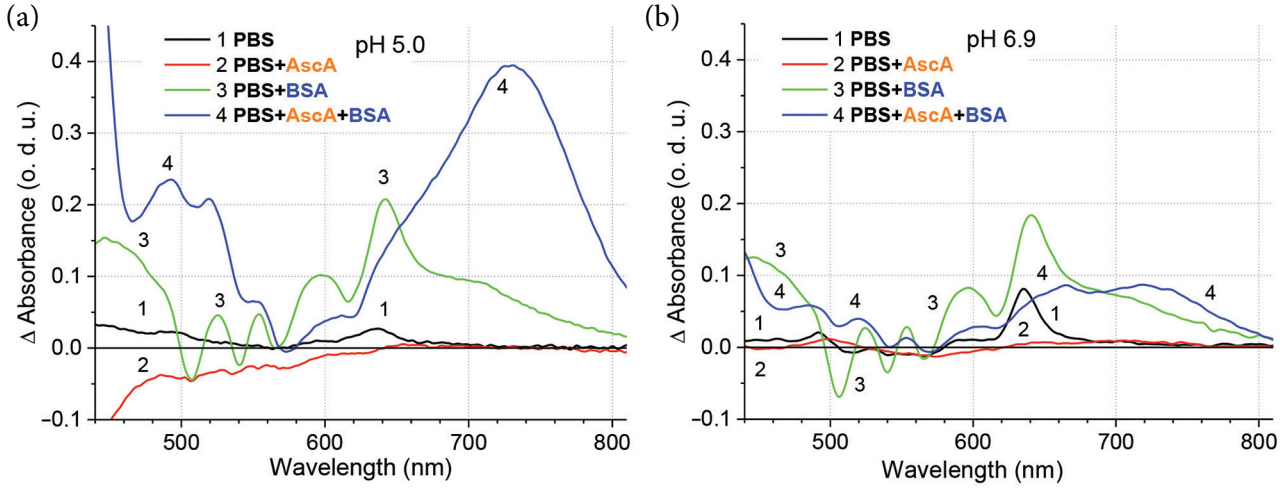

Fig. 2. The absorption difference spectra of (1) HpDiA in (a) neutral (pH 6.9, 0.05 M) and (b) acidic (pH 5, 0.01 M) phosphate buffered solutions, in the presence of (2) AscA ($c = 5 \times 10^{-3}$ M), (3) BSA ($c = 7.5 \times 10^{-5}$ M) and (4) both compounds, illumination as in Fig. 1.

BSA, in contrast to AscA, boosted the spectral changes of HpDiA (Fig. 2(a, b): 3), both in the blue and the red spectral regions. Appearance of the Chl-PhP band at 640 nm was followed by a rise of a broad spectral plateau from 580 up to 800 nm and its amplitude became similar in neutral (Fig. 1(g)) and acidic (Fig. 1(c)) solutions. The formation of a broad absorption band at about 710 nm as well as one in the blue region of the spectrum became even more pronounced in acidic mixtures (Fig. 2(a)).

The influence of medium acidity on the complexity of photoinduced transformations was most clearly manifested in the mixed samples of HpDiA containing both BSA and AscA (Fig. 1(d, h); Fig. 2(a, b): 4). The dual nature of the photoinduced activity of AscA in neutral solutions was reflected in the redistribution of amplitudes of the spectral bands: the peak of Chl-PhP at about 640 nm (Fig. 2(b): 1, 3) was replaced by a broad shoulder with two minor crests at about 640 and 660 nm (Fig. 2(b): 4) reflecting the suppressive action. In contrast, the broad absorption bands at the blue-green (around 500 nm) and the plateau in the far-red (around 720 nm) spectral regions had further increased in comparison with those detected in the samples with BSA only (Fig. 1(h), Fig. 2(b)). The promoting action was especially clearly seen in acidic solutions, where illumination resulted in dominance of a far-red spectral band at about 720 nm with an accompanying increase of absorbance in the blue-green spectral region (Fig. 1(d), Fig. 2(a)). A small new peak at about 660 nm (Fig. 2(a)) might reflect a next stage of the photoinduced oxidation reactions leading to the formation of bacteriochlorin-type products [34], however, the rise of a broad peak of PhP at 720 nm was a clear indication of the reducing AscA contribution [36]. Thus, this band can be attributed to the produced metastable phlorin-type photoproduct (Phl-PhP) [12, 37, 38].

It is generally accepted that the alkalisation of aqueous solutions or the strong interaction with albumins, which has been reflected in characteristic spectral changes of HpDiA absorption bands before illumination (Fig. 1), leads to the disruption of porphyrin equilibrium aggregates. Hence, it was no surprise that the presence of BSA in acidic solutions affected the absorption properties of porphyrins even stronger than in a neutral solution, and the spectra of the mixed samples became very similar to each other (Fig. 1(c, g)). Consequently, the photoinduced changes in spectral HpDiA properties in acidic solutions with BSA reminded those in neutral solutions. The spectrum of the acidic mixture of HpDiA, BSA and AscA (Fig. 1(d)) also looked more like that recorded in the absence of AscA (Fig. 1(c)). It seems that the strong interaction with BSA not only monomerizes porphyrins in the mixed solutions, thus increasing photoreactivity of PS and making it more susceptible towards AscA, but also can directly involve proteins into the photoreactions. Forming Phl-PhP and Chl-PhP seems

to compete for the same source initiating a chain of interconnected oxidation-reduction photoreactions. Apparently, the oxidation prevails in pure solutions of HpDiA. In the absence of ascorbate BSA enhances both pathways, while the reducing activity of AscA sways a whole set of photoreactions towards the formation of Phl-PhP, leading to a drastic increase in the intensity of a spectral band forming at 720 nm in the acidic mixture. Presumably, this indicates the direct participation of AscA in the photoinduced donor–acceptor reactions occurring between BSA and porphyrins. Thus, under acidic conditions (and, probably, under anoxic conditions), when prevailing photosensitization reactions become inefficient, the photoactivated HpDiA in the presence of both BSA and AscA can enhance certain Type I reactions involving the surrounding biomolecules.

3.2. EPR spectroscopy data

To obtain more detailed information on the significant role of AscA in the photoreactions initiated by HpDiA, as well as on possible pathways of mutual activity in the presence of BSA, including Type I electron transfer reactions, the samples prepared in aqueous solutions of various acidity were studied using EPR spectroscopy, both in the dark and under illumination with a laser beam. It is well known that the EPR signal consisting of a duplet of peaks located in a spectral range of 3510–3516 G belongs to Asc• [39], which appears in the samples during the oxidation [40, 41] of AscA. The initial measurements of the AscA samples being prepared by dilution in distilled water or PBS showed no spectral signal of Asc• (Fig.3: 1, 2), probably, due to a lack of oxidizing molecules [42]. However, the presence of H_2O_2 in a non-illuminated sample induced a stable spectral signal of 4G in a width at about 3510–3514 G. H_2O_2 is known to be a good oxygen donor [43], therefore, its presence raises the oxidative potential of the medium, which accelerates the oxidation of AscA through the formation of a radical intermediate [44]. A similar signal has also been recorded in an alkaline sample of AscA with added NaOH (0.2 M). While similar amplitudes of the initial EPR signals (Fig. 3) can be considered as coincidence, all samples demonstrated an obvious insensitivity to the applied illumination.

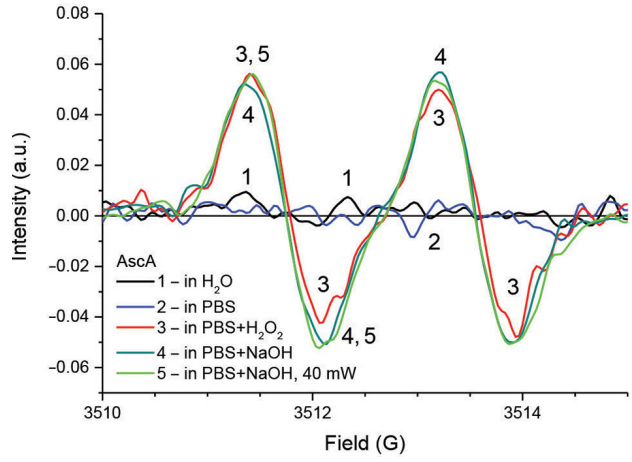

Fig. 3. The EPR spectra of the ascorbic acid samples ($c = 10^{-2}$ M) prepared (1) in distilled water or (2) in a phosphate buffered solution (0.05 M, pH = 7.0 to pH 6.2), in the presence of (3) H_2O_2 ($c = 10^{-2}$ M) and (4) NaOH ($c = 4 \times 10^{-2}$ M, pH = 11.2). Spectra were scanned 5 times, one scan per minute. Spectra (4) and (5) represent the same sample, but the latter was recorded under exposure to a laser beam (40 mW).

3.2.1. Formation of ascorbate radical in unilluminated mixed solutions

The control measurements of the EPR spectra of neutral HpDiA solutions or in the presence of BSA revealed no signal in a region of 3500–3530 G, neither in the case of the non-exposed samples (Fig. 4(a, b)) nor during illumination. Weak spectral EPR signals of Asc• were detected in each of HpDiA solutions only in the presence of AscA (solution pH changed from 7.0 to 6.2), and their amplitudes were about the same. Since HpDiA might act as an acceptor of protons and electrons forming intermediate ionic states [45–47], such reactivity can promote the formation of Asc• even in a weakly acidic medium, apparently, without interference from the interaction with BSA. The observed stability of Asc• can be related to the dynamic equilibrium of AscA ionic forms, which involves a dismutation reaction [16] resulting in a slower rate of the AscA autooxidation at lower pH and makes the solution more stable in the presence of oxygen.

Interestingly, H_2O_2 increased the amplitude of the EPR signal in the mixed acidic solutions of AscA and HpDiA in the dark (Fig. 4(c): 1) to about the same value as that detected in the absence of porphyrins (Fig. 3: 3), revealing no extra reactivity

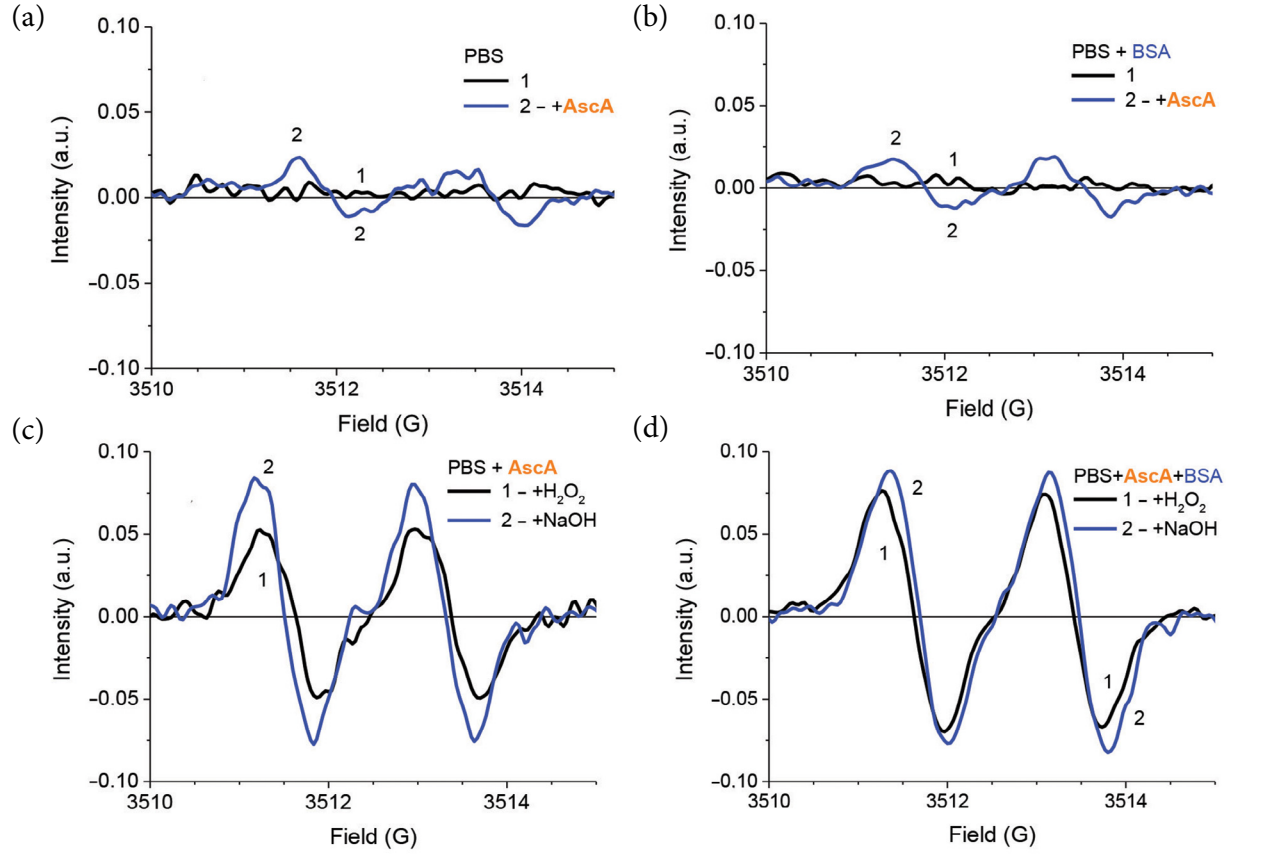

Fig. 4. The EPR spectra of the unexposed Hp diacetate samples ($c = 10^{-4}$ M) in phosphate buffered solutions (0.05 M, pH 7.0) mixed with different additives: (a) ascorbic acid, $c = 10^{-2}$ M, pH 6.2; (b) BSA ($c = 10^{-4}$ M) and AscA; (c) AscA and hydrogen peroxide, $c = 10^{-2}$ M, and in the presence of NaOH, $c = 4 \times 10^{-2}$ M, pH 11.2; (d) the full mixture. The triple scan of spectral signals has been performed to get the spectral curves.

of the porphyrins in the mixture without illumination. On the other hand, the alkalinisation (up to pH 11.2) caused even higher increase in the spectral intensity of the Asc• signal (Fig. 4(c): 2), which is consistent with the dependence of Asc• formation on pH given in [40]. The EPR signal was higher in the mixed alkaline solutions (Fig. 4(c): 2; Fig. 3: 4), implying a more efficient interaction between HpDiA and AscA being in a more reactive ionic state.

The presence of BSA in an alkaline HpDiA solution did not increase the intensity of an initial signal, similarly as in a slightly acidic solution (Fig. 4(b, d): 2). Still, the signal was higher than that in alkaline solutions of AscA without BSA and porphyrins (Fig. 3: 4). The presence of both BSA and H_2O_2 in the slightly acidic solutions containing HpDiA and AscA (Fig. 4(d): 1) induced a stronger EPR signal in comparison with those samples, in which only one of those substances was present. Whether H_2O_2 makes porphyrins interacting with proteins more active towards AscA [48], or the BSA itself increases the capacity of AscA to interact with H_2O_2, remains to be clarified. Despite that, the amplitude of the Asc• signal measured in any of acidic solutions did not exceed the signal observed in alkaline solutions of AscA and HpDiA (Fig. 4(c)). In general, the initial signal intensity of Asc• before illumination increased more in aqueous solutions at higher pH values, indicating an enhanced degradation of AscA [49]. The signal was relatively more stable in alkaline solutions of AscA alone, but tended to decline faster in the presence of HpDiA.

3.2.2. Effect of illumination on the formation of ascorbate radical

To determine the influence of the applied illumination on the spectral amplitude of the Asc• signal, the samples of HpDiA and AscA and their mixtures with different additives were exposed to the increasingly higher intensities of a laser light. A small but steady EPR signal at slightly acidic pH strongly

increased under illumination until a beam power was set at about 7 mW (Fig. 5(a): 2). Further increase in power did not induce the corresponding increase of the Asc• signal. On the contrary, the signal had decreased (Fig. 5(a): 3, 4), and it started to regain the amplitude only when the highest beam power was used. The subsequent measurements of the Asc• signal in the dark showed that its amplitude did not decrease to the ground level.

The initial illumination with the laser beam of low power had almost no influence on the increased amplitude of an EPR signal in the presence of H_2O_2 (Fig. 5(b): 2), while increasing the power further increased the signal, which approached saturation at the highest power value, in contrast to the earlier mentioned case. Interestingly, the amplitude of the signal, which was measured in the dark after the entire series of exposures (like one represented by curve 6), showed a tendency to increase, reaching even higher values after additional illumination and retaining them during the signal measurement time.

The alkaline medium of the mixed solutions of HpDiA and AscA resulted in an immediate rise of the EPR signal of Asc• at higher pH values, which was not stable and almost reached the ground level within several minutes spent on the repeated registrations (Fig. 6(a): 1, 2). The increasing pH has a direct effect on the dynamic equilibrium of ascorbate ionic species [42], resulting in the preferable conversion of ascorbate into a dianionic form [16, 50], which can directly generate Asc•, increasing the autooxidation rate of AscA and strongly diminishing its stability. Thus, the detection of a high Asc• signal in an alkaline solution and its subsequent decrease was not unexpected. The subsequent illumination of samples led to the reappearance of the free radical spectral signal. The initial increase in the spectral amplitude of the EPR signal under low intensities (up to 7 mW) was very similar to that observed in slighly acidic solutions (Fig. 6(a), Fig. 5(a)). The amplitude did not stop increasing until the highest beam power (70 mW) was used, similar to the trend observed in the presence of H_2O_2 (Fig. 5(b)); however, the cessation of exposure led to the disappearance of the signal. When the illumination power was increased again, it resulted in the EPR signal of almost the same amplitude. No other EPR bands were detected during the exposure of samples.

The initial EPR signal of Asc• being measured before the illumination in the mixed alkaline solutions in the presence of BSA (Fig. 6(b)) was even higher. It was also unstable and decreased by more than ten times within a few minutes of registration, subsequently reaching the background level. The illumination of samples resulted in the reappearance of two peaks of the Asc• signal; however,

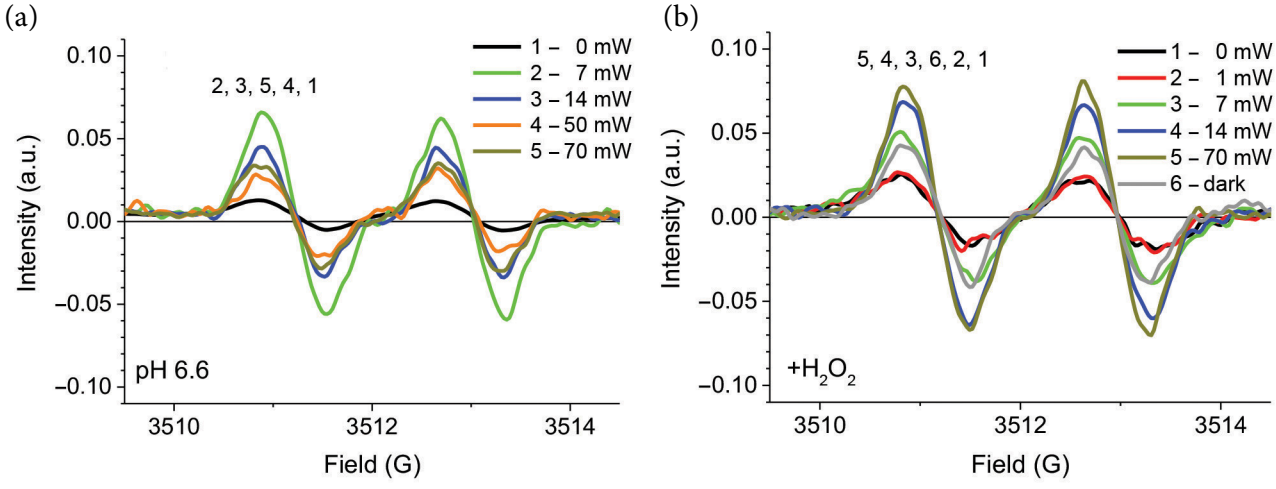

Fig. 5. The EPR spectra of HpDiA buffered solutions (c = 10^{-4} M) with AscA in (a) slightly acidic medium (pH 6.6) and (b) with added H_2O_2 recorded under exposure of the samples to a laser beam of varied intensity. Spectrum 6 was measured after spectrum 5. Five scans were recorded for each spectrum with exception of spectrum 1 (a) of a non-exposed sample, representing 50 scans. A row of numbers indicates the amplitudes of integral spectra in a descending order.

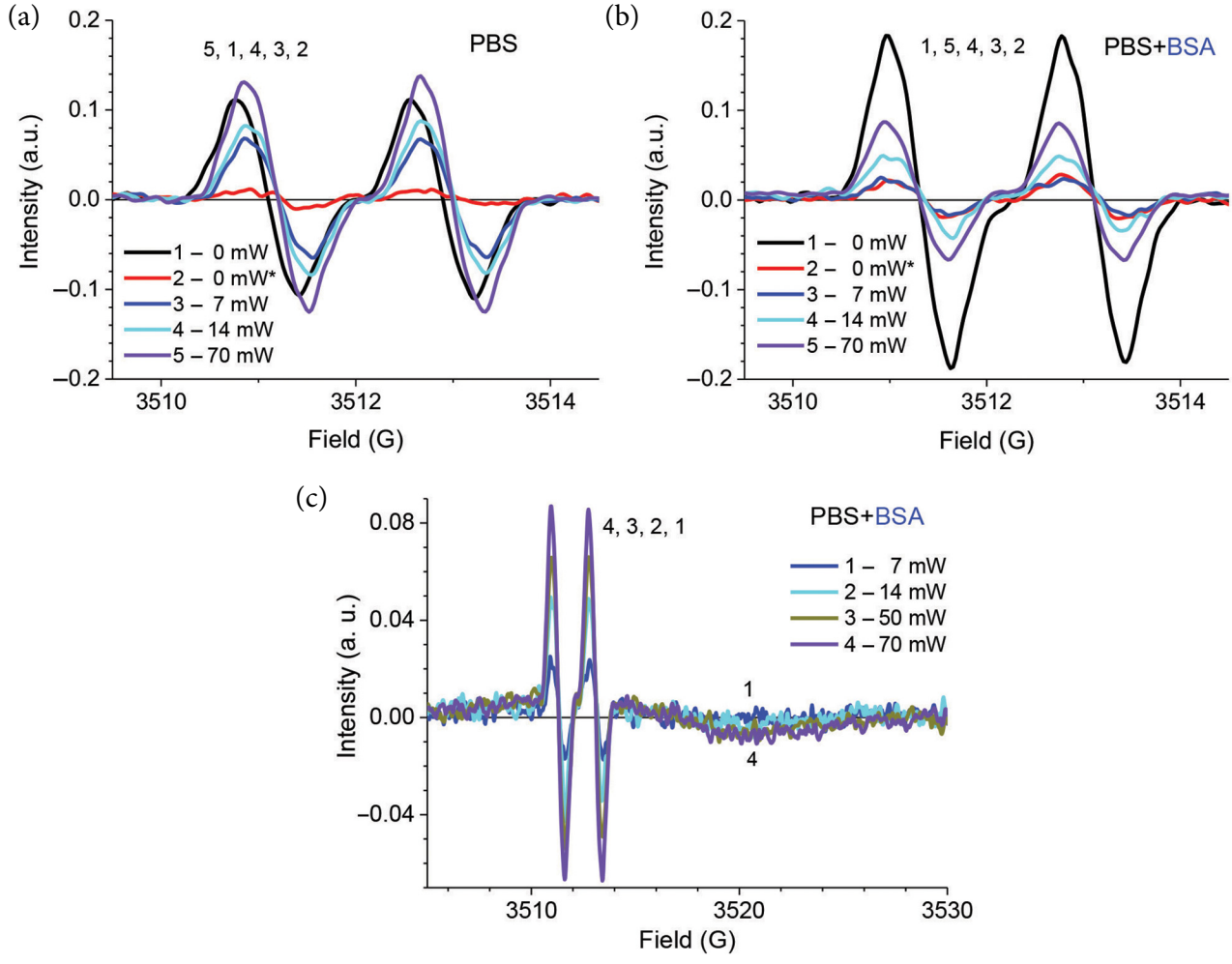

Fig. 6. The EPR spectra of HpDiA buffered solutions ($c = 10^{-4}$ M) with AscA in the alkaline medium, pH 12.5 (a) without and (b) with added BSA ($c = 10^{-4}$ M) and in addition (c) rescaled to show a new band. Five scans were recorded for each spectrum under exposure of the samples to a laser beam at different powers. Spectra (2) were registered 5 min after the corresponding spectrum (1).

with an increase in the beam power, a broad additional signal was detected in a spectral region of 3505–3530 G (Fig. 6(c)). The enhanced formation of another radical species in the presence of BSA could be one of the reasons for the lower amplitudes of the EPR signal of Asc• in comparison with those being measured without BSA when the corresponding powers of a laser beam were applied.

3.3. The role of ascorbate radical in photoinduced transformations

The registered EPR data confirmed a stimulating effect of increased pH on the reactions involving the production of Asc• in the mixed HpDiA solutions in the dark. Although its significance was also demonstrated in the illuminated samples, AscA and BSA appear to play a major role in modifying the photochemical activity of HpDiA. Their influence on the yield of two different red-absorbing PhPs in aqueous solutions of different acidity is summarized in Fig. 7.

The enhanced photostability of HpDiA in acidic solutions indicates a lowered generation of ROS as well as the supressed oxidation-reduction reactivity leading to a low production of Chl-PhP and Phl-PhP. The higher pH of the solution affects the relative intensity of these reactions by facilitating oxidation and impeding reduction pathways when solutions become alkaline.

Not only PT of the samples containing PS and AscA were supressed by the latter, but the illumination also significantly enhanced the registered EPR signal of Asc• (Fig. 5(a)). On the one hand, AscA can

be oxidized by the photogenerated $^1O_2$ producing Asc•. On the other hand, the photoexcitation of PS in the vicinity of AscA can increase the likelihood of their direct interaction. The suppressed formation of a Chl-PhP as well as an increased signal of Asc• during the exposure of HpDiA thus reflect the antioxidative capacity of an AscA anion.

The enhancement of PT in the presence of BSA seems to be related with the BSA structure, which provides a special reactive microenvironment [51, 52] for attached porphyrins boosting the photoactivity and diminishing the influence of the increased medium acidity. Thus, while AscA alone in acidic and neutral solutions of porphyrins reacts mostly as an antioxidant, suppressing the formation of Chl-PhP, it reveals a strong reducing capacity in combination with BSA resulting in the formation of Phl-PhP (Fig. 7). The higher concentration of AscA with respect to BSA under the conditions of adjusted neutral pH can still change the PT balance controlled by BSA towards Phl-PhP, simultaneously limiting the oxidation pathway.

The yield of Asc• produced in the samples under illumination might reflect the dynamic competition occurring between two reactions: the direct interaction of AscA and HpDiA in the excited state, leading to the photoreduction of PS, and the interaction of AscA with ROS produced by HpDiA. The reduced amplitude of the Asc• signal that was observed in slightly acidic mixed solutions at higher intensities of a laser beam (Fig. 5(a)) could reflect a faster quenching of the radical by increased amounts of ROS and/or the formation of Phl-PhP, which is more favoured in the acidic environment.

An increased alkalinity of the medium adversely affects the stability of AscA, increasing the pool of dianionic ascorbate forms, and simultaneously increases the photochemical activity of HpDiA, which, in contrast to observations in weakly acidic solutions, facilitates the enhanced generation of Asc• via a photooxidation pathway under the conditions of a reduced probability to form Phl-PhP (Fig. 6(a)). In contrast, the enhancing effect of BSA alone on the PT of HpDiA was reflected in the increased absorption bands of Chl-PhP and Phl-PhP. The formation of the latter, which requires donation of two protons and two electrons, might be the main reason why the presence of BSA in the alkaline medium did not increase the amplitude of the Asc• signal under illumination, and even led to its diminution at the same illumination conditions (Fig. 6(b)). At the same time, the suppressive effect of alkaline medium on the second stage of phlorin-type PhP formation created favourable conditions to detect the EPR signal of an intermediate reactive species, most probably, the porphyrin anionic radical [17, 39, 46, 47], the appearance of which seems to be related with the depletion of Asc• in the medium.

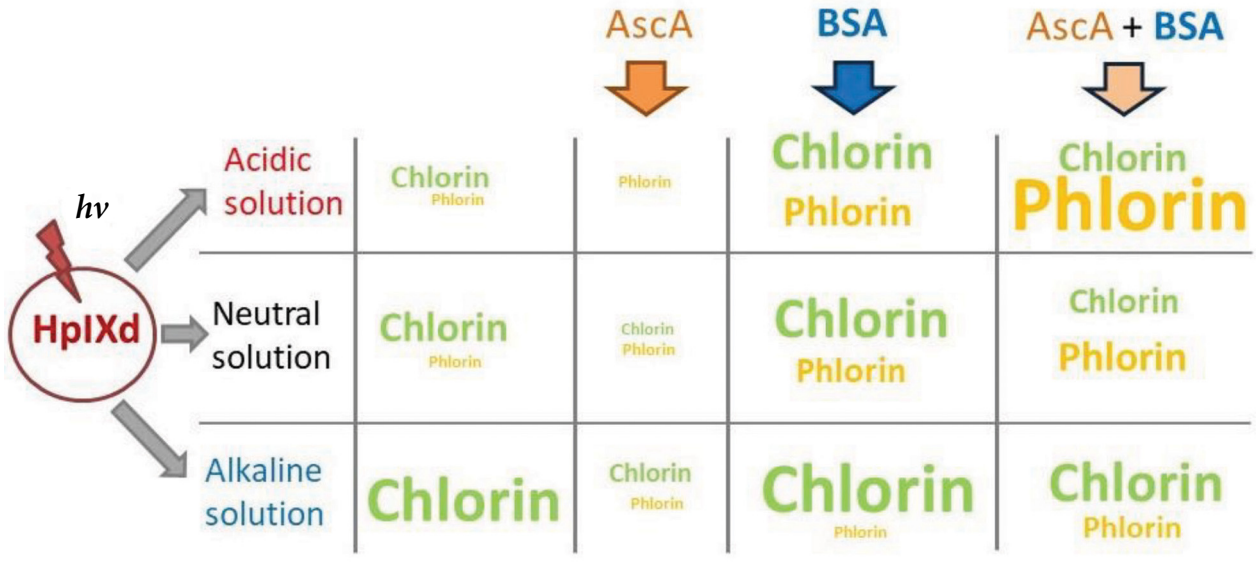

Fig. 7. The schema of formation of the main red-absorbing photoproducts from HpDiA in the presence of ascorbic acid or/and BSA (the text size serves for guidance only and reflects the tendency of the particular transformation).

4. Conclusions

The dynamic equilibrium of photoreactions determining phototransformation pathways in the case of hematoporphyrin-type PS shows a strong dependence on the medium pH, which directly affects the reactivity of PS itself, as well as on the presence of surrounding biomolecules like serum proteins and AscA, which can alter and expand the spectrum of photoinduced activities.

Thus, there is a direct competition between chlorin- and phlorin-type photoproducts, which are transformed from HpDiA via oxidation-reduction pathways sensitive to changes in the medium pH and local microenvironment. AscA itself suppresses (auto)photooxidative reactions, but facilitates the photoinduced formation of Phl-PhP, thereby exhibiting a dual antioxidative-reducing activity in the mixed solutions of HpDiA. The strong interaction between BSA and hematoporphyrin-type PS might be a key factor in the observed photoreactions, not only boosting PT, but also providing the microenvironment for additional pathways to emerge. The studied mixture of BSA and AscA shifted the dynamic equilibrium of HpDiA PT towards the photoreduction pathway, leading to the enhanced formation of Phl-PhP, most likely through a double-electron and double-proton mechanism, which became especially prominent at acidic pH. The prooxidant effect of alkaline medium, on the other hand, made it possible to detect the additional radical species being an intermediate of the photoreduction transformation, most plausibly, the anionic porphyrin radical.

These findings clearly show the special ability of serum proteins to alter the photochemical activity of the adjacent tetrapyrrolic PS in the presence of antioxidants such as ascorbic acid, and especially to enhance phototransformations in an acidic environment through a combined action, which is relevant to the respective biological conditions.

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BENDRAS ASKORBO RŪGŠTIES IR JAUČIO SERUMO ALBUMINO POVEIKIS HEMATOPORFIRINO DARINIO FOTOTRANSFORMACIJAI VANDENINĖJE TERPĖJE: SUGERTIES IR EPR SPEKTROSKOPIJOS TYRIMAS

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Santrauka

Siekis padidinti fotosensibilizuotos terapijos veiksmingumą įgyvendinamas derinant ją su kitais gydymo būdais ir bandant sukelti stipresnį oksidacinį stresą navikiniuose audiniuose. Tam potencialiai tiktų L-askorbo rūgštis (AscA). Šviesos sukeltos hematoporfirino tipo fotosensibilizatoriaus (HpDiA) transformacijos buvo pasirinktos kaip modelinė sistema tiriant AscA poveikį nuo deguonies priklausomoms fotoreakcijoms vandeniniuose modeliniuose skirtingo pH tirpaluose. Papildomi duomenys apie askorbato radikalo vaidmenį HpDiA sukeltose fotoreakcijose ir abipusį aktyvumą bandiniuose su serumo albuminu, įskaitant dalyvavimą I tipo reakcijose, buvo gauti atliekant elektronų paramagnetinio rezonanso spektroskopijos matavimus vandeniniuose mišinių tirpaluose be papildomų molekulinių radikalų gaudyklių kruopščiai parinkto skersmens kapiliariniuose vamzdeliuose tiek tamsoje, tiek apšviečiant lazerio spinduliuote. Nustatyta, kad stipri sąveika tarp jaučio serumo albumino ir porfirinų buvo pagrindinis veiksnys ne tik sustiprinantis fotooksidacijos ir fotoredukcijos vyksmus stebėtose fotoreakcijose, bet kartu su AscA didino fotoaktyvumą, ypač rūgštinėje aplinkoje.