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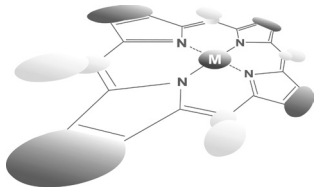

Hydroxyalkyloxy substituted tetraphenylporphyrins: Mechanism and superoxide scavenging activity

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ABSTRACT: The novel electrochemical approach based on coulometric response of electrogenerated superoxide ($O_2^{\bullet-}$) was used to determine scavenging properties of 2H-5,10,15,20-tetrakis (4-hydroxyphenyl)porphyrin (H_2T(4-OHPh)P); 2H-5,10,15,20-tetrakis[4-(2-hydroxyethyloxy)phenyl] porphyrin (H_2T(4-OH(CH_2)$_2$OPh)P); 2H-5,10,15,20-tetrakis[4-(4-hydroxybutyloxy)phenyl]porphyrin (H_2T(4-OH(CH_2)$_4$OPh)P); Zn-5,10,15,20-tetrakis[4-(4-hydroxyethyloxy)phenyl]porphyrin (ZnT(4-OH(CH_2)$_2$OPh)P) superoxide. It has been identified that the porphyrins under study possess good antioxidant properties. The analysis of possible interactions between porphyrins and superoxide anion-radical has shown that high values of superoxide scavenging activity of tetraphenylporphyrins with alcohol chains can be explained by the nucleophilic attack of $O_2^{\bullet-}$ on the C–C bonds of alcohol.

KEYWORDS: *para*-substituted tetraphenylporphyrins, superoxide scavenging activity, coulometric approach, mechanisms.

INTRODUCTION

Oxygen cellular metabolism is realized *via* formation of reactive oxygen species (ROS) including free radicals such as superoxide ($O_2^{\bullet-}$), hydroxyl radical (HO$^{\bullet}$), *etc*. [1]. As a rule, the radicals are aggressive oxidants, so ROS overproduction induces damage at various sites in the cell, especially cell membranes, and pathologies of biochemical processes [2–4]. Different enzymatic and nonenzymatic antioxidants prevent the damaging effects of free radicals on living cells [4–6]. At the same time, an urgent problem of therapeutic compounds development is finding natural and synthetic compounds with a high level of antioxidant activity.

Synthetic metalloporphyrins are an appropriate model of an enzyme catalytic center [7–10] where the metal ion is able to form superoxo complexes in the superoxide presence [11–14]. Transition metal complexes demonstrate superoxide dismutase (SOD) activity [15–22] which is explained by the redox activity of the metal ion and the electron transfer mechanism. On the other hand, it is well-known that there are nonenzymatic antioxidants with a heterocyclic structure [23–25], as well as antioxidants with phenol and polyphenol moieties [26–30]. Therefore, it is resonable that some porphyrin ligands [31–36] display a high antioxidant activity. Phenyl-substituted porphyrins have been investigated by Milgrom *et al.* [37–41] who have found that porphyrin phenyl groups contain a labile hydrogen atom that is lost at the first oxidation stage. This results in the formation of phenoxyl groups that promote further macrocycle oxidation.

Antioxidant activity of enzymatic and nonenzymatic types makes porphyrins a promising investigation object. Earlier, we showed the antioxidant properties of some amino- and hydroxyphenylporphyrins and their mechanism of superoxide scavenging activity [32–36]. This paper is focused on determining superoxide scavenging activity and its mechanisms in tetraphenylporphyrins with hydrocarbon chains between the phenyl rings and the OH groups. The high absorption coefficients of porphyrins

◊SPP full member in good standing

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[42] make spectrometric measurements of antioxidant activity [43] more complicated; for that reason, the electrochemical assay was found to be most suitable for such purpose. The determination of radical scavenging properties of the compounds is based on monitoring the elecrochemical response of superoxide in the presence of an antioxidant [32–36, 44–50].

Procedure of synthesis

H_2T(4-OHPh)P was synthesized by the two-step method *via* demethylation of 2H-5,10,15,20-tetrakis(4-methoxyphenyl)porphyrins [51] obtained in high yield by condensation of benzaldehydes with pyrrole [52, 53]. Then the H_2T(4-OHPh)P was used to synthesize porphyrins and complexes shown in Fig. 1.

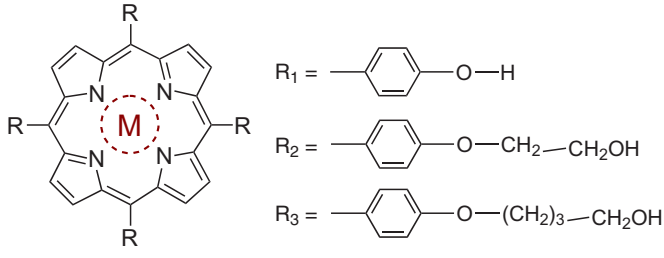
The purified products were studied by thin-layer chromatography (silufol plates), UV-vis spectrometry (Varian Cary 50 spectrometer) and ^{1}H NMR spectrometry (Bruker AVANCE-500 spectrometer) methods. The mass spectra were recorded on a Shimadzu Axima Confidence (MALDI-TOF) mass spectrometer.

2H-5,10,15,20-Tetrakis(4-methoxyphenyl) porphyrin {intermediate compound}. A solution of 5.0 mL (72.2 mmol) of pyrrole and 8.8 mL (72.2 mmol) of anisaldehyde was drop added to a boiling solution of 16 g of chloroacetic acid in 300 mL of an isomeric xylenes mixture for 20 min. The resulting mixture was refluxed by air bubbling for 1 more hour. After xylene steaming, the deposit was filtered off, washed with water and dried in air at 80 °C. The deposit was dissolved in chloroform and purified on aluminum oxide (Brockmann Activity III) eluting with chloroform. The first red zone was collected, the eluate was evaporated, and the porphyrin was precipitated with methanol, filtered and dried at room temperature in air. The yield was 5.6 g (42%). R_f = 0.33 ($CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ_H, ppm -2.79 (2H, s, pyrrole-NH), 4.03 (12H, s, -O-CH_3), 7.22 (8H, d, *m*-H-Ph), 8.06 (8H, d, *o*-H-Ph), 8.79 (8H, s, pyrrole-H 8H). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 652 (3.87), 595 (3.78), 557 (4.07), 520 (4.25), 423 (5.69).

2H-5,10,15,20-Tetrakis(4-hydroxyphenyl) porphyrin. A solution of 0.5 mL (5.29 mmol) of boron tribromide in 10 mL of methylene chloride was added to the stirring and cooling solution of 1.0 g (1.36 mmol) of 5,10,15,20-tetrakis(4′-methoxyphenyl) porphyrin in 200 mL of dried methylene chloride. The mixture was stirred at room temperature for 2 h, and then 5 mL of methanol was added. The mixture was neutralized by ammonia until the color changed from green to dark cherry, washed with water, dried over sodium sulfate and evaporated to dryness. The deposit was dissolved in ethyl acetate and purified on silica gel by eluting with ethyl acetate. The eluate was evaporated and precipitated with petroleum ether. The yield was 0.9 g (98%). R_f = 0.33 ($CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ_H, ppm -2.92 (2H, s, pyrrole-NH), 7.70 (8H, d, m-H-Ph), 8.08 (8H, d, o-H-Ph), 8.79 (8H, s pyrrole-H). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 650 (3.72), 595 (3.71), 556 (3.90), 519 (4.06), 423 (5.43).

The 2H-5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin characteristics agree quite well with the reported data [54, 55].

2H-5,10,15,20-Tetrakis[4-(2-hydroxyethyloxy) phenyl])porphyrin. A mixture of 0.3 g (0.44 mmol) of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin, 1.0 mL (15.2 mmol) of 2-chloroethanol and 0.5 g (3.62 mmol) potassium carbonate in 10 mL of dry DMF was refluxed for 10 h. Then 1.0 mL (15.2 mmol) of 2-chloroethanol was added and the mixture was refluxed again for 10 h. The mixture was poured into water, the precipitate was filtered off, washed with water and dried in air at room temperature to constant weight. The precipitate was Soxhlet extracted with methanol, the solution was chromatographed on silica gel, eluting with methanol, the eluate was evaporated, diluted with water and the precipitate was filtered off and dried. The yield was 250 mg (66.5%). R_f = 0.61 (MeOH). UV-vis (MeOH): λ_{max}, nm (log ε) 650 (3.90), 593 (3.93), 554 (4.14), 517 (4.24), 418 (5.57). IR (KBr tablet): λ_{max}, cm^{-1} 3423, 2923, 2850, 1607, 1509, 1471, 1245, 1172, 967, 802.



(1) M = 2H R = R_1 H_2T(4-OHPh)P
(2) M = 2H R = R_2 H_2T(4-OH$(CH_2)_2$OPh)P
(3) M = 2H R = R_3 H_2T(4-OH$(CH_2)_4$OPh)P
(4) M = Zn R = R_2 ZnT(4-OH$(CH_2)_2$OPh)P

Fig. 1. Structural formulas of the studied porphyrins

^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ_H, ppm -2.91 (2H, s, pyrrole-NH), 3.43 (8H, m, $CH_2O(H)$), 4.38 (8H, m, $CH_2O(Ph)$), 5.27 (4H, s, OH), 7.55 (16H, m, C_6H_4), 8.89 (8H, s, β-pyrrole). MALDI-TOFF-MS: *m/z* $[M + H - H_2O]^+$ experimental 838.01, calcd. 837.95.

The 2H-5,10,15,20-tetrakis[4-(2-hydroxyethyloxy) phenyl])porphyrin characteristics agreed well with the reported data [56].

5,10,15,20-Tetrakis[4-(4 acetoxybutyloxy)phenyl] porphyrin (intermediate compound). A mixture of 0.3 g (0.44 mmol) of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin, 1.0 mL (7.7 mmol) of 4-chlorobutyl acetate and 0.5 g (3.62 mmol) potassium carbonate in 10 mL of dry DMF was boiled for 4 h, then 1.0 mL (7.7 mmol) of 4-chlorobutyl acetate was added and the mixture was boiled again for 10 h. Then the mixture was poured into water, the precipitate was filtered, washed with water and dried in air at room temperature. The precipitate was dissolved in dichloromethane and chromatographed on silica gel eluting with the dichloromethane-methanol, then the eluate was evaporated, precipitated with methanol and dried. The yield was 230 mg (45.9%). R_f = 0.85 (ethyl acetate), 0.77 (benzene-methanol, 20:1). ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ_H, ppm -2.73 (2H, bs, pyrrole-NH), 2.00–2.13 (16H, m, CH_2), 2.16 (12H, s, CH_3CO), 4.28–4.34 (16H, m, -O-CH_2), 7.29 (8H, d, *J* = 8.2 Hz, *m*-H-Ph), 8.14 (8H, d, *J* = 8.2 Hz, *o*-H-Ph), 8.89 (8H, s, pyrrole-H). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 651 (3.89), 594 (3.89), 557 (4.14), 519 (4.29), 423 (5.69). MALDI-TOFF-MS: *m/z* $[M - H]^+$ experimental 1134.61, calcd. 1134.31.

2H-5,10,15,20-Tetrakis[4-(4-hydroxybutyloxy) phenyl]porphyrin. A solution of 220 mg (0.19 mmol) of 5,10,15,20-tetrakis[4-(4 acetoxybutyloxy)phenyl]porphyrin in 20 mL THF was mixed with a solution of 0.5 g (8.91 mmol) of potassium hydroxide in 1.0 mL of water, then the mixture was refluxed for 20 h. The mixture was poured into water and the precipitate was filtered off, washed with water and dried. The yield was 183 mg (98.0%). R_f = 0.13 (ethyl acetate). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 651 (3.89), 594 (3.85), 557 (4.10), 520 (4.24), 423 (5.63). ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ_H, ppm -2.76 (2H, bs, pyrrole-NH), 1.95 (8H, qv, *J* = 6.8 Hz, -CH_2), 2.10 (8H, qv, *J* = 6.8 Hz, -CH_2), 3.88 (8H, t, *J* = 6.1 Hz, -CH_2O), 4.31 (8H, t, *J* = 6.1 Hz, -CH_2O), 7.29 (8H, d, *J* = 8.4 Hz, *m*-H-Ph), 8.13 (8H, d, *J* = 8.4 Hz, *o*-H-Ph), 8.87 (8H, s, pyrrole-H). MALDI-TOFF-MS: *m/z* $[M - H]^+$ experimental 966.33, calcd. 966.16.

Zn-5,10,15,20-Tetrakis[4-(2-hydroxyethyloxy) phenyl]porphyrin. A mixture of 0.3 g (0.44 mmol) of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin, 1.0 mL (15.2 mmol) of 2-chloroethanol and 0.5 g (3.62 mmol) potassium carbonate in 10 mL of dry DMF was refluxed for 10 h. Then 1.0 mL (15.2 mmol) of 2-chloroethanol was added and the mixture was refluxed again for 10 h. The mixture was poured into water, the precipitate was filtered off, washed with water and dried in air at room temperature to constant weight. The precipitate was Soxhlet extracted with methanol, then 0.5 g (2.28 mmol) of zinc acetate dihydrate was added and the mixture was boiled for 1 h. The solution was chromatographed on silica gel, eluting with methanol, the eluate was evaporated, diluted with water and the precipitate filtered off. The yield was 250 mg (61.9%). R_f = 0.76 (MeOH). UV-vis (DMSO): λ_{max}, nm (log ε) 600 (4.12), 559 (4.30), 424 (5.74). UV-vis (MeOH): λ_{max}, nm (log ε) 605 (4.14), 563 (4.14), 431 (5.49). IR (KBr tablet): λ_{max}, cm^{-1} 3449, 1608, 1509, 1245, 1173, 997, 803, 722. MALDI-TOFF-MS: *m/z* $[M]^+$ experimental 918.28, calcd. 918.33. The low solubility of Zn-complex in deuterochloroform did not allow us to obtain ^{1}H NMR data.

Electrochemical procedure

Dimethylsulfoxide (DMSO ≥ 99.5, ALDRICH) was purified by zone melting and then stored over molecular sieves in a dry box before use. Tetrabutylammonium perchlorate (TBAP ≥ 98.0, ALDRICH) was purified by recrystallization from ethanol. Concentrated solutions of porphyrins containing 0.02 M TBAP as the supporting electrolyte were prepared by the gravimetric method using the electronic analytical balance «Sartorius» ME215S (the mass determination error did not exceed 3%). The solutions of smaller concentrations were prepared by the method of serial dilution.

A potentiostat PI-50PRO3 (Elins, Russia) was used for electrochemical measurements. The experiments were carried out in a three-electrode temperature-controlled (25 ± 0.5 °C) electrochemical cell in freshly prepared solutions. The saturated calomel electrode (SCE) inserted into the electrochemical cell through the Luggin capillary was used as the reference electrode. The Pt wire was used as an auxiliary electrode.

As the working electrode, we used a polishing Pt strip (the working surface equaled 1.2 cm^2) rigidly fixed in the fluoroplastic lid. Before every measurement, the active surface of the working electrode was mechanically mirror-polished, degreased with ethanol, etched with a chromic mixture for 20 min, carefully cleaned in distilled water and then in the solution under study. The working electrode was immersed in the cell with the test solution where the potential of the working electrode reached a steady value in 10 min. In order to degas or oxygenate solutions before the electrochemical measurements, argon or oxygen was bubbled through the capillary tube for 30 min.

The oxygen saturation condition was verified by the cyclic voltammetry (CV) method. In saturated conditions the concentration of the dissolved oxygen in DMSO at 25 °C was 2.1 mM [57, 58]. The free convection mode was reached 3 min. after the capillary removal from the solution. After that, the CV response was recorded at scan rates from 0.01 to 1.00 V/s. The CV data were corrected for Ohmic (iR) losses using the current interruption technique [59]. Only the first cycle was used

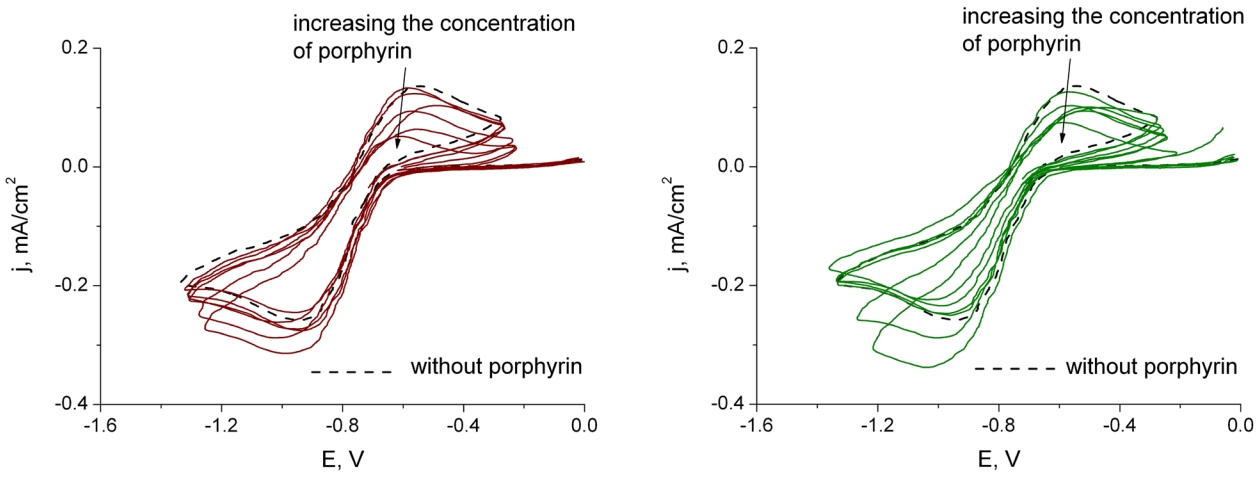


Fig. 2. Electrochemical response of the $O_2/O_2^{\bullet-}$ redox process at $H_2T(4\text{-}OH(CH_2)_2OPh)P$ (a) and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ (b) concentration of 0.00, 0.10, 0.25, 0.50, 1.00, 1.50 mM. The scan rate is 0.02 V/s

to study the interaction of the porphyrins and superoxide anion-radical.

RESULTS AND DISCUSSION

Figure 2 shows a typical influence of porphyrins admixture on the electrochemical response of the $O_2/O_2^{\bullet-}$ redox couple. With small porphyrin additions (up to 0.1 mM), the superoxide anion-radical electrochemical oxidation peak increases and shifts slightly, which indicates the increasing of heterogeneous electron transfer rate [60, 61]. A further increase in the concentration of $H_2T(4\text{-}OHPh)P$, $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_4OPh)P$ leads to a decay of the $O_2^{\bullet-}$ oxidation currents. The losses of $O_2^{\bullet-}$ current values allow us to determine the superoxide scavenging activity using the jC_{50} and K_b parameters [32–34, 48, 50]. But it has been shown [35, 36] that the coulometric response of electrogenerated superoxide allows us to obtain more suitable parameters for superoxide scavenging activity determination.

The quantity of electricity (Q) of reduction processes was determined by integrating dependences I(t) for the cathode branch of the CV curve. The quantity of the electricity spent on oxidation was calculated by integrating dependences I(t) for the anode branch of the CV curve. The region between curves 1 and 2 corresponds to the quantity of electricity Q_{1red} required for porphyrin electroreduction in a degassed porphyrin solution. The region between curves 1 and 3 determines the quantity of electricity Q_{2red}, of dissolved O_2 electroreduction in oxygenated DMSO region between curves 1 and 3 Q_{3red} — the quantity of electricity corresponding to the simultaneous porphyrin and oxygen reduction in an oxygenated solution. The region between curves 2 and 4 (gray colored) evaluates the quantity of electricity Q_{3red}–Q_{1red} of O_2 reduction in porphyrin presence. To calculate the quantity of electricity of $O_2^{\bullet-}$ oxidation in porphyrin presence, we found value Q_{3ox}–Q_{1ox} (the gray colored area between lines 2 and 4 for positive currents in Fig. 3).

The radical scavenging activity of the *para*-substituted tetraphenylporphyrins was determined using dimensionless parameters ω and k:

$$\omega = (Q_{3ox}–Q_{1ox})/(Q_{3red}–Q_{1red}) \quad (2)$$

where $(Q_{3ox}–Q_{1ox})$ is the quantity of electricity of $O_2^{\bullet-}$ oxidation in porphyrin presence; $(Q_{3red}\text{-}Q_{1red})$ is the quantity of electricity of O_2 reduction in the same experiment.

$$k = (\omega_0–\omega)/\omega_0 \quad (3)$$

where ω_0 is the value obtained by extrapolating the ω dependence to the zero porphyrin concentration.

The *k* value *vs*. antioxidant concentration plots are shown in Fig. 4. The antioxidant activity of the compound under study is characterized by the slope (A_k) (the bigger is the slope, the higher is the activity).

According to the A_k value, the antioxidant activity series can be arranged as follows:

$$\begin{aligned} &H_2T(4\text{-}OHPh)P \geq H_2T(4\text{-}OH(CH_2)_2OPh)P \\ &\quad \approx H_2T(4\text{-}OH(CH_2)_4OPh)P \\ &\quad > ZnT(4\text{-}OH(CH_2)_2OPh)P \end{aligned} \quad (4)$$

Comparing the binding constants of superoxide anion radical of the studied porphyrins and those of flavanoids [48, 50], we can describe $H_2T(4\text{-}OHPh)P$ as molecular systems with a high antioxidant activity. Our results also indicate the considerable activity of $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_2OPh)P$.

Porphyrins antioxidant activity has not been studied well enough yet, only a few works deal with this problem [15–22, 31–36]. It is assumed that the antioxidant action of the most common antioxidants (aromatic amines,

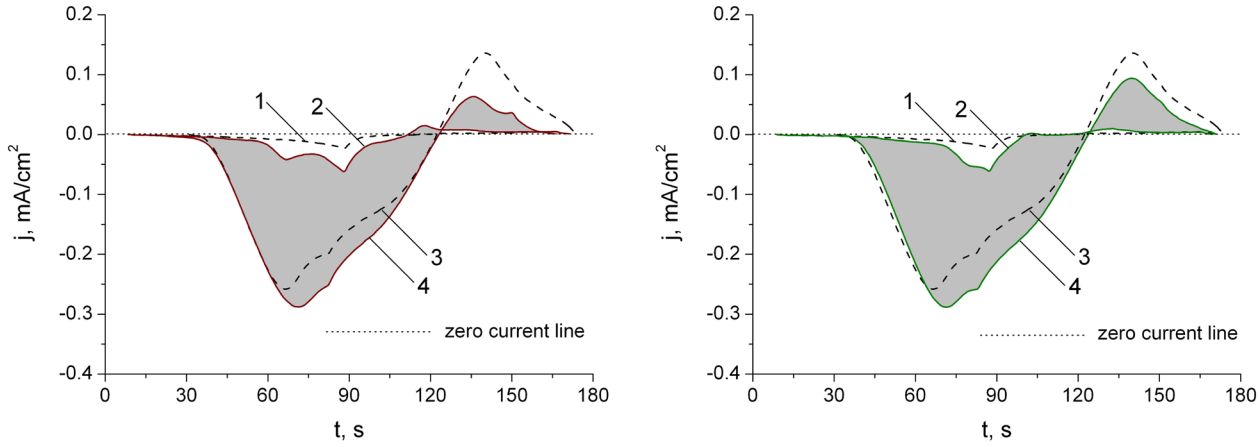


Fig. 3. Redox currents *vs*. time plots: (1) background; (2) porphyrins $H_2T(4\text{-}OH(CH_2)_2OPh)P$ (a) and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ (b) in degassing solution; (3) oxygen in oxygenated solution; (4) respective porphyrins and oxygen in oxygenated solution. The scan rate is 0.02 V/s. The porphyrins concentrations are 1 mM

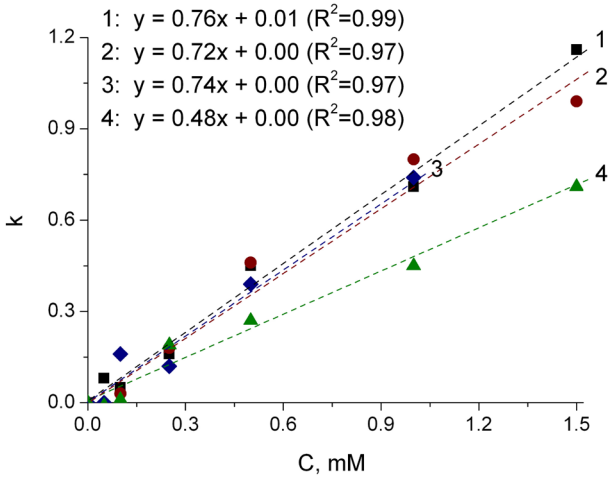


Fig. 4. Coulometric parameter *k* of $H_2T(4\text{-}OHPh)P$ (1); $H_2T(4\text{-}OH(CH_2)_2OPh)P$ (2); $H_2T(4\text{-}OH(CH_2)_4OPh)P$ (3); $ZnT(4\text{-}OH(CH_2)_2OPh)P$ (4)

$$O_2^{\bullet-} + AO \rightarrow AO^+ + O_2^{2-}$$

Scheme 1. Electron donating mechanism of superoxide scavenging

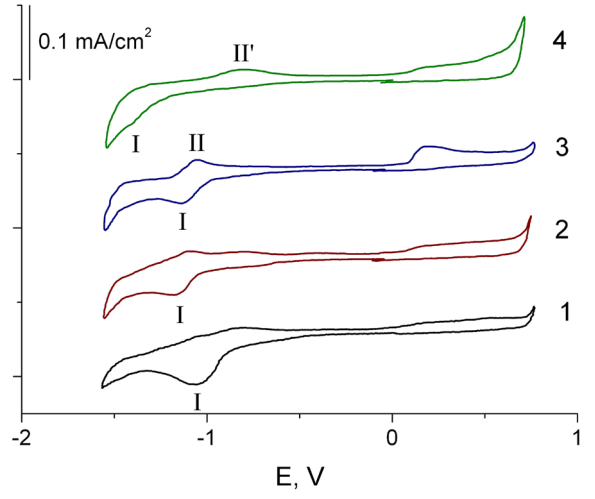


Fig. 5. CV of redox processes in DMSO: (1) $H_2T(4\text{-}OHPh)P$, (2) $H_2T(4\text{-}OH(CH_2)_2OPh)P$, (3) $H_2T(4\text{-}OH(CH_2)_4OPh)P$, (4) $ZnT(4\text{-}OH(CH_2)_4OPh)P$. The scan rate is 0.02 V/s. The porphyrins concentration is 1 mM

phenols, naphthols, *etc*.) consists in breaking the reacting chains. Antioxidants neutralize free radicals donating (accepting) an electron or a hydrogen atom. Antioxidant interaction with active radicals results in formation of low activity radicals, which reduces the oxidation rate. A number of works have reported a correlation between oxidation potential and antioxidant activity of compounds. This correlation is thought to be connected with electron transfer, namely: easier antioxidant oxidation leads to a more efficient process of superoxide scavenging according to Scheme 1, where AO is the antioxidant molecule.

Figure 5 presents redox behavior of the studied porphyrins in degassed solutions DMSO. As the represented CV curves show, the electrochemical response of oxidation of *para*-substituted tetraphenylporphins is within the electrochemical window (up to +0.8 V *vs*. SCE). The oxidation of $H_2T(4\text{-}OHPh)P$, $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ is irreversible due to the fast intermolecular electron transfer and π-cation radical formation [62, 63]. $H_2T(4\text{-}OHPh)P$ oxidation includes two peaks with the maximum potentials of +0.20 and +0.48 V described elsewhere [35]. In case of oxidation, the first oxidation wave of $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ occurs, with the maximums around +0.30, +0.24, and +0.20 V, respectively. The oxidation

current passes through a plateau and then transforms into the second oxidation wave at the borders of the electrochemical window. Thus, the oxidation potential decreases as follows:

$$H_2T(4\text{-}OHPh)P \approx ZnT(4\text{-}OH(CH_2)_2OPh)P < H_2T(4\text{-}OH(CH_2)_4OPh)P < H_2T(4\text{-}OH(CH_2)_2OPh)P \quad (5)$$

The series of porphyrins oxidation potential differs from that of antioxidant activity, which means that the process described in Scheme 1 cannot explain the differences in antioxidant properties of the studied porphyrins.

The deactivation mechanism of superoxide scavenging in case of electron transfer from the superoxide anion-radical to the antioxidant is represented in Scheme 2.

The antioxidant efficiency in such a mechanism must correlate with the electron affinity or reduction potential: the lower the antioxidant reduction potential is, the more preferable Scheme 2 is. As the CV curve shows (Fig. 5), the first reduction peaks of $H_2T(4\text{-}OHPh)P$, $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ reaches the maximum at -1.08, -1.17, -1.14 and -1.40 V potential values, respectively. Therefore, the reduction stability increases as follows:

$$H_2T(4\text{-}OHPh)P < H_2T(4\text{-}OH(CH_2)_4OPh)P < H_2T(4\text{-}OH(CH_2)_2OPh)P < ZnT(4\text{-}OH(CH_2)_2OPh)P \quad (6)$$

The reduction stability series differs from that of antioxidant activity of the porphyrins under study.

$$O_2^{\bullet-} + AO \rightarrow AO^- + O_2$$

Scheme 2. Electron accepting mechanism of superoxide scavenging

Additionally, the electrochemical reduction of such porphyrins takes place at more negative potential values (-1.0 ÷ -1.5 V, Fig. 5) than that of oxygen; therefore, the mechanism shown in Scheme 2 cannot be used to explain the series of antioxidant activity as the electron transfer is an energetically unfavorable process.

Figure 6 represents the influence of potential scan rate on the electrochemical response of the porphyrins oxidation-reduction processes.

In order to determine the nature of the anode peak observed at potential values of about -0.5 V, the potential was cycled within the range from 0 to -1.6 V at the scan rate of 0.01 V/s (20 cycles). As a result, the same electrochemical responses (similar to those in Figs 5 and 6 at low scan rates) were observed, without increasing of oxidation peaks with the potential values of around -0.5 V. If the scan rate is high, the potential cycling in the range -1.70 ÷ -0.80 V leads to a CV response (Fig. 6b, curve 2), which coincides with those obtained within a wider range (Fig. 6b, curve 1). If the potential is cycled in the range -0.90 ÷ +0.10 V (Fig. 6b, curve 3), no anode peak of about -0.4 V is observed either. Based on the facts given above, the occurrence of the anode peak II' should be interpreted as an intermediate oxidation.

Porphyrin reduction accompanied by intermediates formation was described elsewhere [35, 36]. The intermediates redox potentials allow us to assume intermediates involvement in the superoxide scavenging according to both Schemes 1 and 2. But in the case of $H_2T(4\text{-}OH(CH_2)_4OPh)P$ with high superoxide scavenging activity there are no observed intermediates. Therefore, the assumption of a primary role of intermediates in the superoxide scavenging activity is unfounded.

Thus, the mechanisms of direct electron transfer cannot explain the porphyrin antioxidant activity series. At the same time, the hydrogen transfer mechanism cannot explain the porphyrin antioxidant activity series in the case

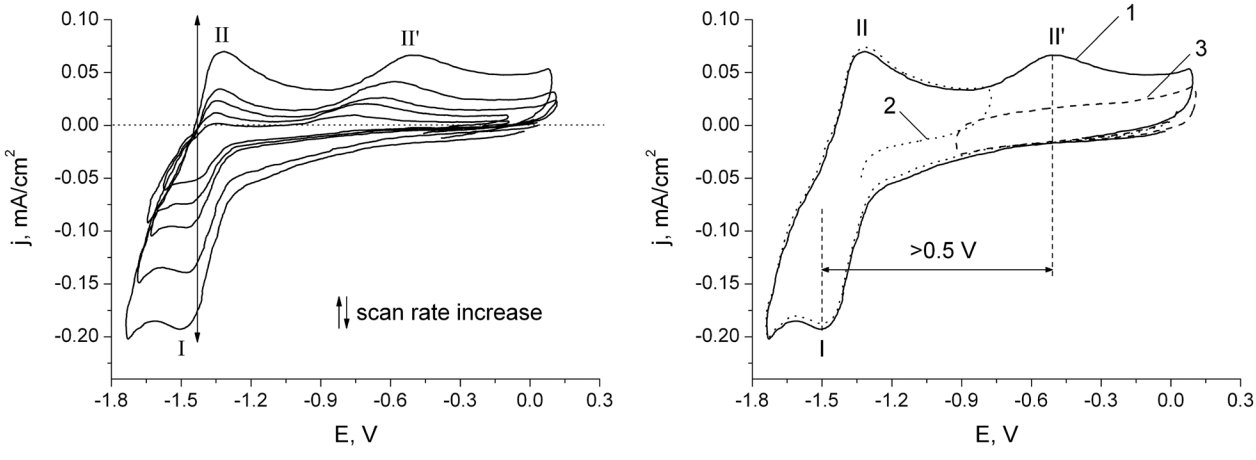
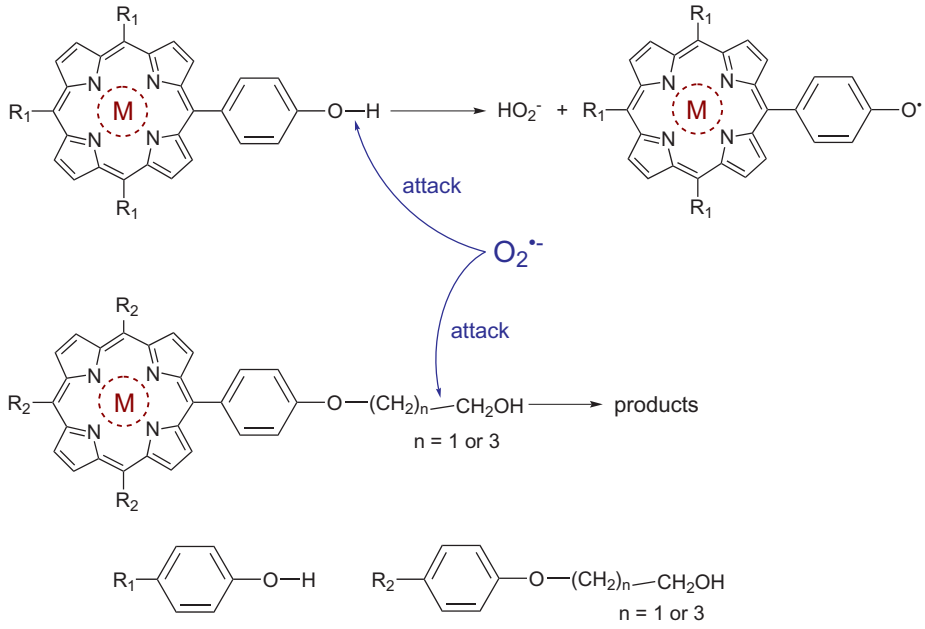


Fig. 6. CV response of $ZnT(4\text{-}OH(CH_2)_2OPh)P$ in oxygen-free DMSO solutions: (a) at the scan rates: 0.02, 0.05, 0.10, 0.20, 0.50 V/s; (b) at the scan rate 0.50 V/s within potential ranges: -1.70 ÷ +0.10 (1), -1.70 ÷ -0.80 (2); -0.90 ÷ +0.10 (3) V. The porphyrin concentration is equal to 1 mM



Scheme 3. Hydrogen transfer mechanism of superoxide scavenging in case of hydroxy- and hydroxyalkyloxy substituted tetraphenylporphyrins.

of $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_2OPh)P$ porphyrins either. For antioxidants containing phenyl moieties, the hydrogen transfer mechanism is provided by OH bond dissociation, which leads to a phenoxyl radical formation. The dissociation energy of OH bonds is in the range of 75–90 kcal/mol (depending on the substituents and functional environment) [27–30]. The dissociation energy of alcohol OH groups is around 105 kcal/mol [64]. This dissociation energy value does not allow the hydrogen atom to get involved in effective superoxide scavenging. As a result, a strong decrease in the superoxide scavenging activity should be observed.

The high experimental values of superoxide scavenging activity of $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_4OPh)P$ can be explained by the effective nucleophilic attack of $O_2^{\bullet-}$ on the C–C bonds of alcohol. It is a more favorable process than OH dissociation (Scheme 3) because C–C bond dissociation leading to CH_2OH radical formation requires about 85 kcal/mol only [64]. OH bond dissociation energy in CH_2OH radical is equal to about 30 kcal/mol [64], so hydrogen atom transfer mechanism is easily realized.

The obtained higher level of antioxidant activity of the porphyrin-ligand than that of the Zn-porphyrin is similar to the effects observed for hydroxyphenyl porphyrins [36] previously. The phenomena, on the one hand, can be caused by metal influence on intramolecular transfer of unpaired electrons [37], which leads to a decrease in the porphyrin oxidation rate. On the other hand, the metal influences on the structure of the molecular orbitals and, as a result, on the bond dissociation energy of moieties involved in the superoxide scavenging processes.

CONCLUSION

A new approach to superoxide scavenging activity determination of hydroxyalkyloxy substituted tetraphenylporphyrins has been applied. It has been found that the tested porphyrins have a high activity despite the absence of the labile hydrogen atom in the porphyrin structure. The high experimental values of superoxide scavenging activity of $H_2T(4\text{-}OH(CH_2)_2OPh)P$, $H_2T(4\text{-}OH(CH_2)_4OPh)P$ and $ZnT(4\text{-}OH(CH_2)_4OPh)P$ can be explained by the effective nucleophilic attack of $O_2^{\bullet-}$ on the C–C bonds of hydroxyalkyloxy substitutes. It is supposed that the attack leads to the formation of CH_2OH radical with a labile hydrogen atom and the hydrogen transfer mechanism in what follows.

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