Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 77 (2) 187–199 (2012)
JSCS–4260
UDC 546.472'562+547.979.733+
544.032.6:66.022.362
Original scientific paper

Zinc(II) and copper(II) complexes with pheophytin and mesoporphyrin and their stability to UV-B irradiation: Vis spectroscopy studies

JELENA B. ZVEZDANOVIĆ*, DEJAN Z. MARKOVIĆ and SANJA M. MILENKOVIĆ

University of Niš, Faculty of Technology, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia

(Received 10 March, revised 20 July 2011)

Abstract: The stability of Zn(II) and Cu(II) complexes of porphyrin derivatives (pheophytin and mesoporphyrin) to UV-B irradiation was studied by absorbance spectroscopy in 95 % ethanol. The chosen porphyrins and their heavy metal complexes underwent first-order photochemical decomposition. In general, pheophytin was more stable than mesoporphyrin to UV-B irradiation. Moreover, the stabilities of the Zn(II) complexes were lower than those of the Cu(II)-complexes for both pheophytin and mesoporphyrin. However, while the Cu(II)-complex with pheophytin was more stable than the one with mesoporphyrin, the situation was *vice versa* for the Zn(II)-complexes.

Keywords: pheophytin; mesoporphyrin; heavy metal; complexes; UV-B kinetics.

INTRODUCTION

Pheophytin and mesoporphyrin belong to the family of porphyrin compounds. The structure of mesoporphyrin IX (MP), 7,12-diethyl-3,8,13,17-tetramethyl-porphyrin-2,18-dipropionic acid, is shown in Fig. 1A.[1,2] Insertion of heavy metals (HM) such as zinc or copper (in the form of ions) into the center of the porphyrin structure leads to the formation of a heavy metal–mesoporphyrin (HM–MP) complexes, in which the new metal coordinates the four symmetric pyrrole rings.[3] On the other hand, pheophytin (Pheo – shown in Fig. 1B) is a derivative of the major photosynthesis pigment, chlorophyll (Chl), with an isocyclic cyclopentanone ring fused to a C-pyrrole ring of the porphyrin core between the C-13 and C-15 positions. In chlorophyll, the central magnesium (Mg) atom is bonded with the N-atoms from the four symmetric pyrrole rings by two covalent and two coordination bonds.[3] Pheophytin is formed when chlorophyll is depleted of magnesium (Fig. 1B). It is known that heavy metals can replace the labile bonded central Mg-atom

* Corresponding author. E-mail: jelite74@yahoo.com
doi: 10.2298/JSC110310175Z

of chlorophyll to form substitutional or "central" complex of chlorophyll, *i.e.* heavy metal complex of pheophytin, HM–Pheo.[4,5]

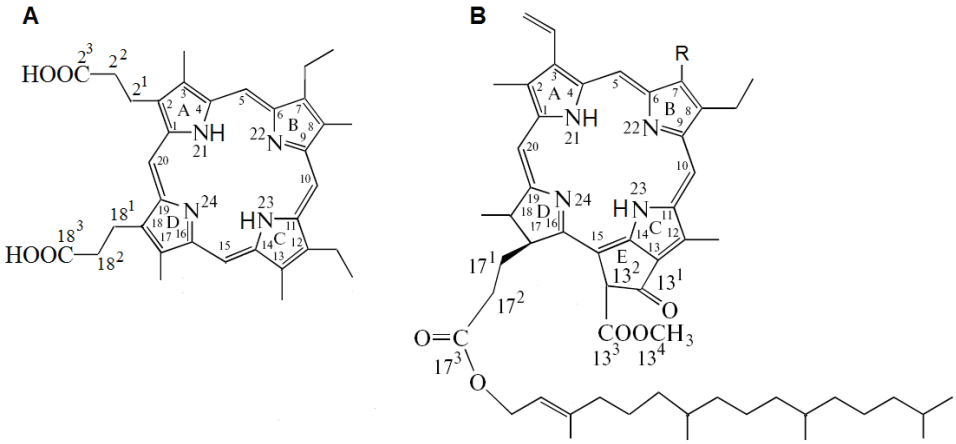

Fig. 1. Structure of mesoporphyrin (A), and pheophytin (B) with numerated C-positions; R = CH_3 in pheophytin *a*; R = CHO in pheophytin *b*.

It is well known that porphyrins and their metal derivatives have attracted significant attention in many areas because of their spectral properties; their characteristic absorption spectrum consists of an intense (Soret or *B*) band close to 400 nm and a less intense (Q) band in the region 500–700 nm.[6,7] They make a core in many other types of molecules, such are chlorophyll and hemoglobin, as well as in many enzymes where they play a heme role.[8] In numerous biological and solar energy applications,[3,9] it is often important to examine the basic properties of porphyrins and their metal-complexes when they are irradiated by UV light (*e.g.*, UV-B). Treatment of chlorophyll with strong UV and visible light in solutions and in isolated photosynthetic organelles results in irreversible breakdown of chlorophyll, *i.e.*, bleaching.[10–12]

This work deals with the stability of two porphyrin derivatives, *i.e.*, pheophytin and mesoporphyrin, and their heavy metal complexes to continuous UV-B irradiation. The irradiation was performed in 95 % ethanol for different irradiation periods, providing possibilities for kinetic analysis.

EXPERIMENTAL

All experiments, beginning with the extraction, were performed under dim light as far as possible, and inside vessels and equipment covered with aluminum foil or black cloth, thus preventing exposure of the pigments to light.[13]

Mesoporphyrin IX (Sigma Aldrich) was a gift from the Hugo Scheer Laboratory, Botanisches Institute, LMU University, Munich, Germany.

Chlorophyll isolation and preparation of pheophytin

The chlorophylls were isolated from plant pigments (extracted from spinach leaves, *Spinacia oleracea*) using an already published method.[4,14] Chlorophyll fraction (containing Chl*a* and Chl*b* in molar ratio ≈ 6:1, respectively) was isolated by column chromatography with silica gel as the adsorbent (silica gel 60, Merck, 0.063–0.200 mm) and an *n*-hexane/acetone mixture as the eluent.[15] The *n*-hexane/acetone ratio was changed from initial 1:0 to final 1:1, to permit easier elution of the polar fractions. Chlorophyll was eluted at an eluent composition 1:0.1 (*n*-hexane/acetone, respectively). To prove the purity of the Chl-fraction, high pressure liquid chromatography (HPLC) was employed using a Chla-standard (Sigma–Aldrich) in acetone; the chlorophyll fractions were then run through HPLC as reported, proving large content of Chl*a* in each of them and a minor contribution of Chl*b* (the peaks ratio 8.5:1).[15] The total Chl content (Chl*a* + Chl*b*) in the isolated Chl-fraction has been calculated as reported.[16]

Pheophytins (*a* and *b*) were made from the collected chlorophyll in 95% ethanol by the dropwise addition of 1.0 M HCl.[17] Conversion was completed in approximately 2 h in the dark as observed by the color change from green to olive brown. The freshly made Pheo matter was then extracted by *n*-hexane and then dissolved in 95 % ethanol. The Pheo*a* and Pheo*b* content in the pheophytin fraction in 95% ethanol was calculated as reported previously.[16]

Preparation of pheophytin and mesoporphyrin heavy metal complexes

Zinc(II) and copper(II) complexes of pheophytin – denoted as Cu–Pheo and Zn–Pheo*, were prepared from Chl using a modified method proposed by Kupper.[18] Chlorophyll, dried at room temperature, was dissolved in 95 % ethanol and a solution of $CuSO_4$, or $ZnSO_4$, was then added. The substitution reaction of the central Mg in the Chl molecule by Zn, or Cu, (formation of the central Zn– or Cu–Pheo complexes) was performed by heating the reaction mixture in a reflux apparatus for 1 h at 40 °C, followed by 24 h at room temperature.

The copper and zinc complexes of mesoporphyrin (Cu–MP and Zn–MP) were prepared using the patent method of Inoue.[19] Mesoporphyrin was placed in 95 % ethanol and a 50 % ethanolic solution of $CuSO_4$, or $ZnSO_4$, was then added. Formation of the Cu–, or Zn–MP, complex was performed by heating the reaction mixture in a reflux apparatus for 1 h at 40 °C, followed by 24 h at room temperature.

Before metal ions addition to the pigment solutions, control experiments were performed in order to check the stability of the pigment solutions, and to assign all potential absorption changes to other factors other than metal–porphyrin interactions. The controls were realized in a period of 3 days (with porphyrin solutions only) by controlling their absorption spectra over the indicated period.

* Strictly speaking, those complexes should be denoted as Cu(II)–Pheo and Zn(II)–Pheo. However, essentially the pheophytin complex with Cu(II) or Zn(II) and chlorophyll (Chl) complex with Cu(II) or Zn(II) are basically the same, and the manner of their formation is practically the same: with chlorophyll, the formation these complexes is preceded by the removal of the central Mg atom (*i.e.*, pheophytin formation); then Cu(II) or Zn(II) ions (from surrounding solution) fill “the hole” and the complexes are formed. Therefore in most citations, Cu(II)–Pheo and Zn(II)–Pheo are denoted like Chl-complexes with these metals, *i.e.*, Cu(II)–Chl and Zn(II)–Chl, or just Cu–Chl and Zn–Chl.

UV-B Treatment

In all the experiments, the final concentration of pheophytin and mesoporphyrin was adjusted to 2.4 μM. Continuous irradiation of Pheo and MP solutions, as well as their Zn(II)- and Cu(II)-complexes in 95 % ethanol was performed in a cylindrical photochemical reactor "Rayonnet" with 8 symmetrically placed UV-B lamps having an emission maximum at 300 nm. The samples were irradiated in quartz cells (1 cm×1 cm×4.5 cm) placed on a circular rotating holder. The total measured energy flux (hitting the samples) was about 12.0 W m^{-2} at a distance of 10 cm from the lamps.

Vis spectroscopy

The spectrophotometric measurements were made on a Varian Cary-100 spectrophotometer equipped with 1.0 cm quartz cells. All spectra before and after irradiation were recorded from 300 to 750 nm with a 1.0 nm bandwidth.

HPLC Analysis

HPLC analysis of the isolated chlorophyll was performed under isocratic conditions; apparatus: Agilent 1100 series, Waldborn, Germany; column: Zorbax Eclipse XDB-C18; mobile phase: acetonitrile/methanol/ethyl acetate, 60:20:20; flow rate: 0.5 cm^3 min^{-1}; temperature: 25 °C. The monitoring wavelengths were 430 and 660 nm.

RESULTS AND DISCUSSION

Vis (absorption) spectra of UV-B-irradiated porphyrin derivatives

Absorption spectra of pheophytin (A), Zn–Pheo complex (B) and Cu–Pheo complex (C) following increasing periods of continuous UV-B irradiation in 95 % ethanol are shown in Fig. 2. Similarly, absorption spectra of mesoporphyrin (A), Zn–MP complex (B) and Cu–MP complex (C) following increasing periods of UV-B irradiation in 95 % ethanol are shown in Fig. 3*.

It is well known that porphyrins have two major absorption bands in the visible range, due to extended π-delocalization at the edge of the cyclic tetrapyrrole (porphyrin) skeleton: a ''red'' (Q) band and a ''blue'' (Soret or B) band.[6,7,20] HM–Pheo complexes showed spectroscopic behavior different from those of Chl and Pheo themselves, both qualitatively (shifts of the characteristic bands maximums, A_{Qmax}), as well as quantitatively (different intensities of the corresponding bands).[6,7,18,21] For example, the formation of the Cu–Pheo complex was followed by a characteristic hypsochromic ("blue") shift of the Q absorption band, compared to Chl itself;[4,21] this effect was not clearly seen with the Zn–Pheo complex.[4,21] The absorption maximum of the Q-band (A_{Qmax}) for Pheo a in 95 % ethanol was located at 665.0 nm, while same absorption maximum for Zn–Pheo and Cu–Pheo in 95 % ethanol were located at 664.0 and 654.0 nm, respectively (Fig. 1S of the Supplementary material and Fig. 2).[16] On the other hand, the Cu–MP and Zn–MP complexes expressed very different spectral behavior in comparison to

* The absorption spectra of: pheophytin (a), Zn–Pheo (b), Cu–Pheo (c) and mesoporphyrin (d), Zn–MP (e), Cu–MP (f) in 95 % ethanol are shown in Fig. 1S of the Supplementary material to this paper.

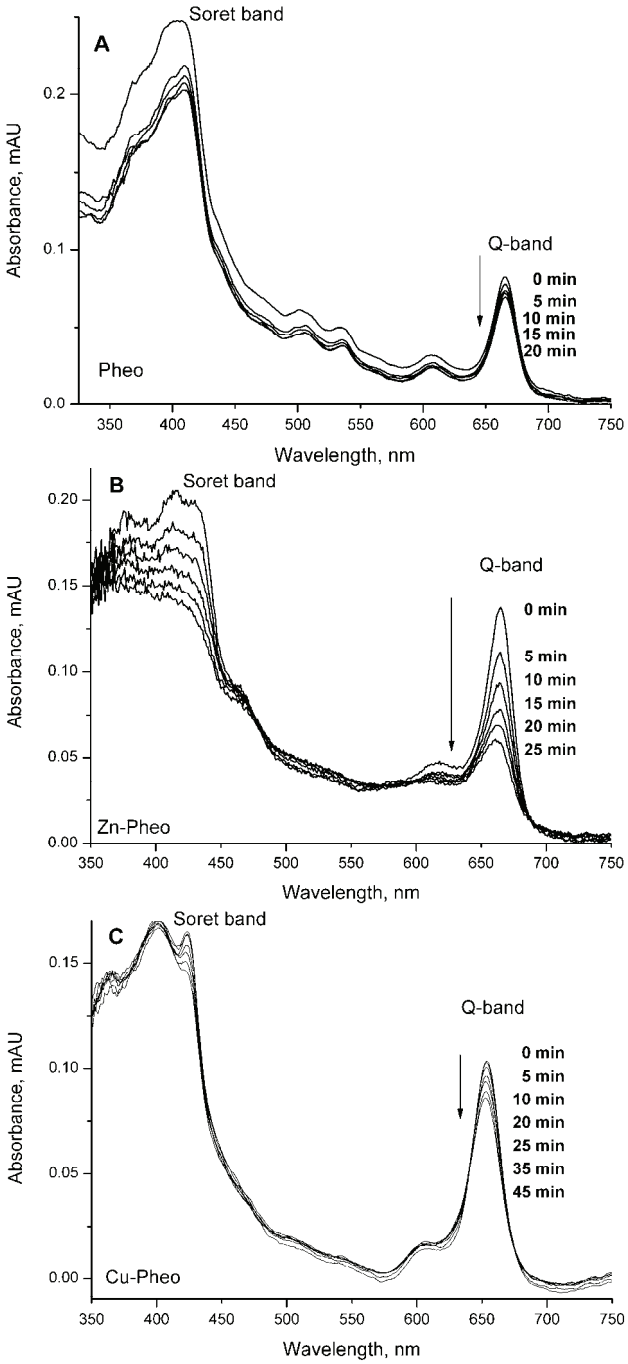

Fig. 2. UV-B-induced decomposition of Pheo (A), Zn––Pheo (B) and Cu–Pheo (C) in 95 % ethanol – changes in the absorption spectra followed their exposure to UV-B radiation. The exposure times are displayed for all three irradiated samples. The initial samples concentration was 2.4 μM.

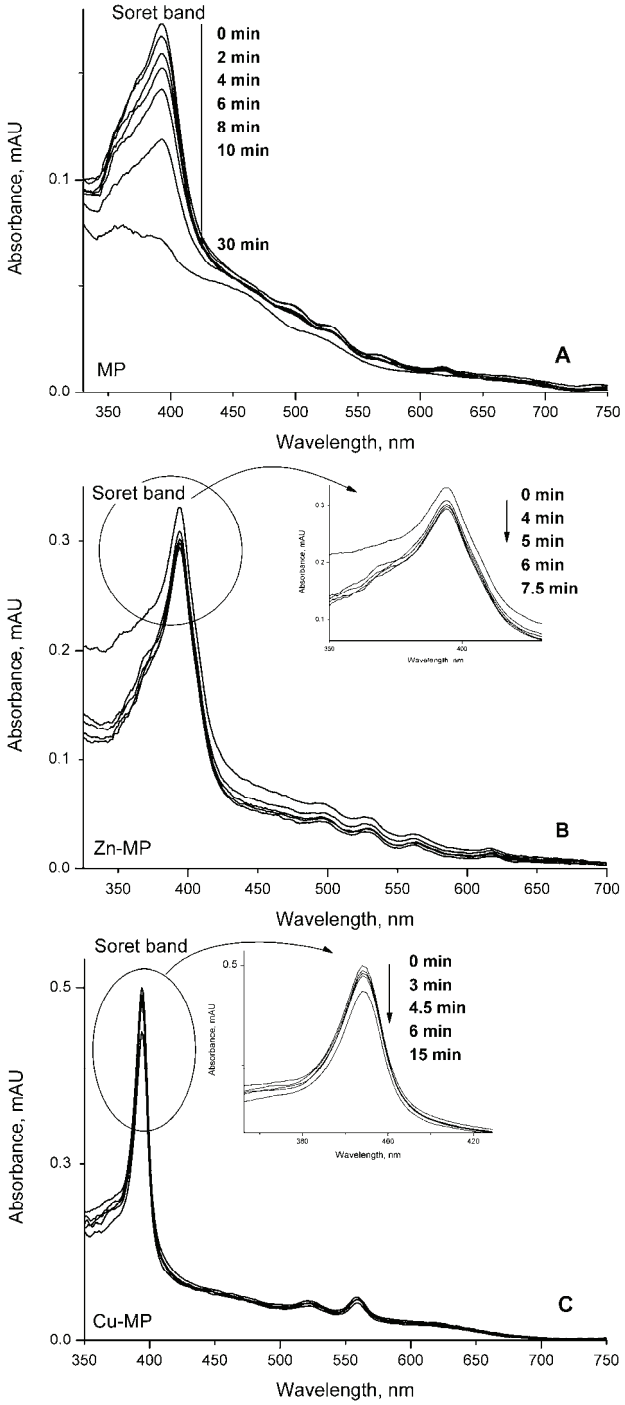

Fig. 3. UV-B-induced decomposition of MP (A), Zn–MP (B) and Cu–MP (C) in 95 % ethanol – changes in the absorption spectra followed their exposure to UV-B radiation. The exposure times are displayed for all three irradiated samples. The initial samples concentration was 2.4 μM.

MP itself; seen through the higher absorption in "blue" region, *i.e.*, the Soret (B) band (Fig. 1S of the Supplementary material and Fig. 3).[3,22,23] The absorption maximum of the Soret band, ($A_{\text{Soret-max}}$) of MP and the Zn– and Cu–MP complexes in 95 % ethanol were found at ≈ 394 nm and the maxima were, respectively, ≈ 2 and ≈ 3 times higher for Zn– and Cu–MP, compared to MP. The metal coordination of the four N-atoms leads to an increase of the porphyrin symmetry (in comparison to the free base porphyrin) and consequently to a rise in the intensity of the Soret band.[24,25]

The UV-induced changes of chlorophylls were already detected in various solvents, such as acetone and *n*-hexane, using the Q-band as a sensitive indicator.[10] Similar to this, in the present study, it was found that UV-B irradiation induced a gradual decrease of the absorption over the whole measured spectral range (300–750 nm), *e.g.*, a hypochromic effect was clearly observed for irradiated pheophytin, and the Zn– and Cu–Pheo complexes in 95 % ethanol, as shown in Figs. 2A–2C. In addition, it was also found that UV-B irradiation of mesoporphyrin and its Zn– or Cu–MP complexes induced a clear decrease of the Soret band intensity ($A_{\text{Soret-max}}$), as shown in Fig. 3. Clearly, UV-B irradiation of pheophytin and its Zn(II)- and Cu(II)-complexes, as well as of MP and its Zn(II)- and Cu(II)-complexes in 95 % ethanol results in their irreversible photochemical decomposition – bleaching.

Kinetics of the UV-B-induced photochemical decomposition the porphyrin derivatives

Photochemical decomposition kinetics of pheophytin and HM–Pheo complexes (Cu–Pheo and Zn–Pheo), as well as of MP and the HM–MP complexes (Cu–MP and Zn–MP), *i.e.*, logarithmic (ln) plots of the absorption *vs*. time of irradiation with UV-B light, are shown in Figs. 4 and 5, respectively. The decomposition (bleaching) rate constants, *k*, for Pheo and the HM–Pheo complexes, and for MP and the HM–MP complexes were calculated from the slopes given in Figs. 4 and 5:

$$y = n - kx$$

where *y* is the ln of the absorbance maximum, ln A_{Qmax} and ln $A_{\text{Soret-max}}$, respectively, and *x* is the UV-B irradiation time (in min). The decomposition kinetics of Pheo and the Zn– and Cu–Pheo complexes were followed by recording the value of the absorption maximum of the Q band, A_{Qmax} (665 nm for Pheo, 664 nm for Zn–Pheo and 654 nm for Cu–Pheo, Fig. 2). On the other hand, the decomposition kinetics of MP, and the Zn– and Cu–MP complexes were followed by recording absorption the maximum of the Soret band, $A_{\text{Soret-max}}$ at ≈ 394 nm (Fig. 3). The UV-B-induced decomposition (bleaching) of the porphyrin derivatives fits the first order kinetic model, as shown in Figs. 4 and 5. The calculated rate constants,

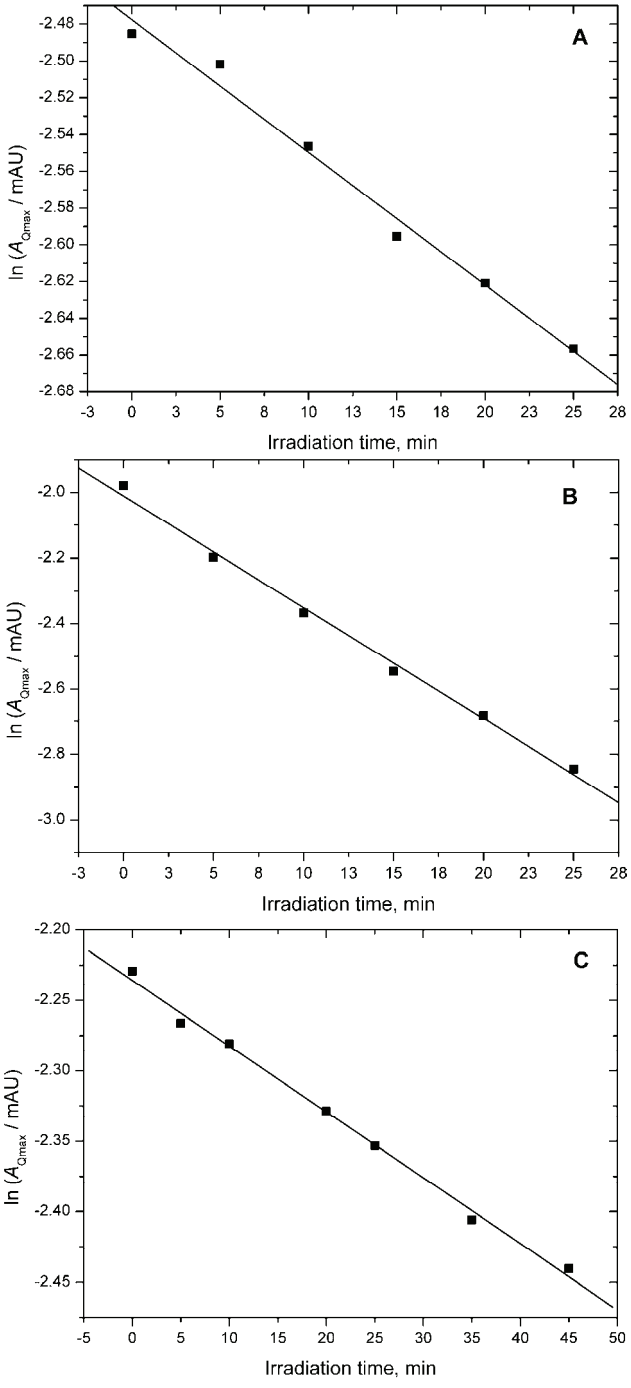

Fig. 4. First order kinetic plots for the photochemical decomposition of pheophytin (A), Zn–Pheo (B) and Cu––Pheo (C) in 95 % ethanol under UV-B irradiation. The absorbances were taken from the Q-band maximum.

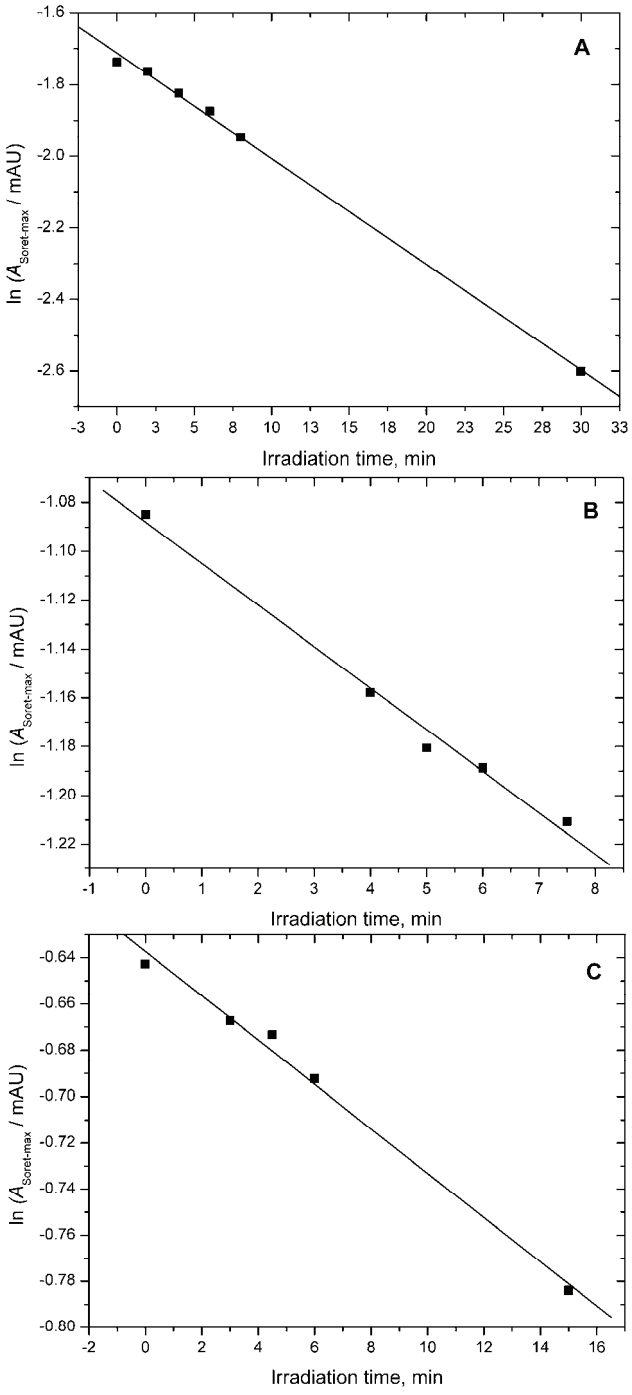

Fig. 5. First order kinetic plots for photochemical decomposition of mesoporphyrin (A), Zn–MP (B) and Cu––MP (C) in 95 % ethanol under UV-B irradiation. The absorbances were taken from the Soret-band maximum.

k (in min^{-1}), for Pheo and MP as well as for their Zn- and Cu-complexes in 95 % ethanol are given in Table I (correlation coefficient $r^2 \approx 0.99$).

TABLE I. First order rate constants (k / 10^{-3} min^{-1}) for photochemical decomposition of pheophytin, mesoporphyrin and their Zn(II)- and Cu(II)-complexes in 95 % ethanol under UV-B irradiation

Compound	Derivatives of pheophytin	Derivatives of mesoporphyrin
No metal	7.2	30
Zn(II)-complex	34	17
Cu(II)-complex	4.7	9.6

The bleaching rate constants of the Zn–Pheo and Zn–MP complexes generally exhibited higher values than those for the Cu(II)-complexes (Table I). The ratios of the UV-B induced decomposition rate constants of the Zn(II)- and Cu(II)--complexes of Pheo and MP, taken separately (*i.e.*, $k_{Zn–Pheo}/k_{Cu–Pheo}$ and $k_{Zn–MP}/k_{Cu–MP}$) were 7.3 and 1.8, respectively (Table I). In porphyrins, the energy levels of the corresponding electronic transitions are determined by the electrostatic interactions between the N (nitrogen)-tetrapyrolles-mediated π-electron densities and the metal center, and hence the effect of the central metal is related to its electronegativity.[26] In general, the stability of the metal complexes of pheophytin and mesoporphyrin in solution can be explained by theoretical analysis of the Falk "stability factor" (including charge number of the metal ion, effective radius of the metal ion in Å and the Pauling electronegativity).[13] According to the Falk equation, the stability of the complexes is ordered as: Mg(II)-complex ≈ ≈ Zn(II)-complex << Cu(II)-complex.[13] In addition, a similar order for the tendency of metal ions to fill "the hole" in the center of the chlorophyll molecule was already found in the cited reports.[18,21] On the other hand, metal complexes of porphyrins can be divided into two groups based on the properties of their electronic structure.[2] Complexes which contain closed-shell metal ions (d^0 or d^{10}), such as Zn(II), have relatively low energy in d–π metal-based orbitals. These interactions have very little effect on the porphyrin π–π* energy gap in electronic spectra. On the other hand, metal complexes of porphyrins which contain metals such as Cu(II) with a d^m configuration of the corresponding energy levels (m = 6–9, number of electrons) have significant metal to porphyrin orbital interaction (*i.e.*, metal to ligand d–π-backbonding), resulting in an increased π–π* energy separation, which is seen as a blue-shift in the appropriate spectra.[2–4] A heavy metal in the central position of the porphyrin structure (Fig. 1) seems to play a significant role in the stability of HM–Pheo and HM–MP complexes to UV-B irradiation; the affinity of heavy metals to the four nitrogen atoms (N-(21--24)) in the center of the porphyrin molecule could play the role of stability factor for HM-complexes against UV-B irradiation.

The photochemical decomposition rate constants for Pheo and its Cu-complexes (Cu–Pheo) generally showed smaller values than the same rate constants for MP and its Cu-complexes (Cu–MP), as given in Table I. The ratios of UV-B induced decomposition constants for MP and Pheo (k_{MP}/k_{Pheo}) and their Cu–MP and Cu–Pheo complexes ($k_{Cu–MP}/k_{Cu–Pheo}$) are 4.1 and 2.1, respectively. Mesoporphyrin differs from pheophytin by an additional double bond in the D pyrrole ring (Fig. 1A). In the mesoporphyrin structure, the extended delocalization at the edge of the cyclic tetrapyrrole (porphyrin) skeleton contains 11 π–bonds (Fig. 1A). In the pheophytin structure, the extended delocalization contains 10 π-bonds and two more π-bonds belonging to: 1) the C=O carbonyl group in position C--13[1] and 2) the C=C bond in position C-3[1]; the phytil chain is bonded to the propionic acid residue in position C-17[3] and isocyclic cyclopentanone ring fused to a C-pyrrole ring between the C-13 and C-15 positions (Fig. 1B) makes substantial differences between the Pheo and MP molecules. These differences between the two chosen porphyrin molecules and the related energy distributions seems to be related to the stability of the Pheo molecule against UV-B irradiation, compared to MP.

Pheophytin is more stable compared to its Zn(II) complex, but, on the other hand, the Zn–MP complex is more stable than MP itself to UV-B irradiation, as shown in Table I. The ratios of UV-B induced decomposition constants for Zn––Pheo and Pheo ($k_{Zn–Pheo}/k_{Pheo}$), as well as of MP and the Zn–MP complex ($k_{MP}/k_{Zn–MP}$) are 4.7 and 1.7, respectively (Table 1). It is known that the Zn––Pheo complex is more labile than Chl itself in solution: Zn(II) was shown to be involved in the formation of a peripheral complex at the right edge of the Chl molecule that includes at least two C=O groups in different positions.[4,21]

CONCLUSIONS

The chosen porphyrins, pheophytin and mesoporphyrin, and their heavy metal complexes undergo UV-induced photochemical decomposition obeying first--order kinetics. Pheophytin and its Cu(II)-complex are more stable to UV-B irradiation than mesoporphyrin and its Cu(II)-complex; contrarily, the Zn(II)-complex of mesoporphyrin is more stable than the same complex of pheophytin.

SUPPLEMENTARY MATERIAL

Absorbance spectra of porphyrin derivatives are available electronically from http:////www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. This work was supported by the Ministry of Education and Science of the Republic of Serbia under project number TR 34012. We thank Dr Hugo Scheer who donated the mesoporphyrin standard.

И З В О Д

КОМПЛЕКСИ ФЕОФИТИНА И МЕЗОПОРФИРИНА СА ЦИНКОМ(II) И БАКРОМ(II) И ЊИХОВА СТАБИЛНОСТ ПРЕМА ДЕЈСТВУ UV-B ЗРАЧЕЊА: VIS СПЕКТРОСКОПСКА АНАЛИЗА

ЈЕЛЕНА Б. ЗВЕЗДАНОВИЋ, ДЕЈАН З. МАРКОВИЋ и САЊА М. МИЛЕНКОВИЋ

Универзитет у Нишу, Технолошки факултет, Булевар ослобођења 124, 16000 Лесковац

Стабилност Zn(II) и Cu(II) комплекса са дериватима порфирина (феофитином и мезопорфирином) према дејству UV-B зрачења у 95 % етанолу, анализирана је апсорпционом спектроскопијом. Изабрани порфирини као и њихови комплекси са тешким металима подлежу деструкцији (деградацији), пратећи кинетику првог реда. Генерално посматрајући, у односу на мезопорфирин, феофитин је стабилнији према дејству UV-B зрачења. Са друге стране, Zn(II) комплекси су мање стабилни од Cu(II) комплекса са оба испитивана порфирина – феофитином и мезопорфирином; ипак, док је Cu(II) комплекс феофитина стабилнији од оног са мезопорфирином, у случају комплекса са Zn(II) ситуација је обрнута.

(Примљено 10. марта, ревидирано 20. јула 2011)

REFERENCES

1. H. Fisher, *On haemin and the relationships between haemin and chlorophyll*, Nobel Lecture, 1930, p. 165
2. L. R. Milgrom, *The Colours of Life: An Introduction to the Chemistry of Porphyrins and Related Compounds*, OUP, Oxford, UK, 1997
3. A. Kay, M. Grätzel, *J. Phys. Chem.* **97** (1993) 6272
4. J. Petrović, G. Nikolić, D. Marković, *J. Serb. Chem. Soc*. **71**, 501 (2006)
5. L. J. Boucher, J. J. Katz, *J. Am. Chem. Soc*. **89** (1967) 4703
6. M. Gouterman, in *The Porphyrins*, D. Dolphyn, Ed., Academic Press, New York, 1978, p. 3
7. L. K. Hanson, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, 1991, p. 994
8. M. R. Pereira, A. J. Ferreira, G. Hungerford, *J. Photochem. Photobiol., A* **172** (2005) 7
9. A. Kay, R. Humphry-Baker, M. Graetzel, *J. Phys. Chem*. **98** (1994) 952
10. J. Zvezdanović, D. Marković, *J. Serb. Chem. Soc.* **73** (2008) 271
11. M. N. Merzlyak, S. I. Pogosyan, L. Lekhimena, T. V. Zhigalova, I. F. Khozina, Z. Cohen, S. S. Khrushchev, *Russ. J. Plant Physiol*. **43** (1996) 160
12. S. Santabarbara, *Arch. Biochem. Biophys.* **455** (2006) 77
13. P. H. Hynninen, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, 1991, p. 145
14. W. A. Svec, in *The Porphyrins*, D. Dolphyn, Ed., Academic Press, New York, 1978, p. 341
15. J. Zvezdanović, T. Cvetić, S. Veljović-Jovanović, D. Marković, *Radiat. Phys. Chem.* **78** (2009) 25
16. H. K. Lichtenthaler, *Meth. Enzymol*. **148** (1987) 350
17. H. Küpper, F. Küpper, M. Spiller, *J. Exp. Bot.* **47** (1996) 259
18. H. Küpper, M. Spiller, F. Küpper, *Anal. Biochem*. **286** (2000) 247
19. Y. Inoue, V. Borovkov, J. Lintuluoto, US patent, US 6,420,553 B1 (2002)
20. A. J. Hoff, J. Amesz, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, 1991, p. 723
21. J. Zvezdanović, D. Marković, Russ. *J. Phys. Chem., A* **83** (2009) 1542
22. F. C. Belanger, A. R. Constantin, *J. Biol. Chem*. **257** (1982) 1360

23. R. Franco, J.-G. Ma, Y. Lu, G. C. Ferreira, J. A. Shelnutt, *Biochemistry* **39** (2000) 2517
24. M. Gouterman, *J. Chem. Phys*. **30** (1959) 1139
25. W. Zheng, N. Shan, L. Yu, X. Wang, *Dyes Pigm*. **77** (2008) 153
26. A. Drzewiecka-Matuszek, A. Skalna, A. Karocki, G. Stochel, L. Fiedor, *J. Biol. Inorg. Chem*. **10** (2005) 453.