

Copper Complexes of Chlorin Derivatives of Chlorophyll *a* as Potential Photosensitizers for Medical Purposes

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*The absorption and fluorescence properties, singlet oxygen photosensitization and photoinduced cytotoxic activity in the HeLa cells line were investigated for copper complexes of chlorins (methylpyropheophorbide *a* and exocycle-free 13-amide derivatives of chlorin e_6 dimethyl ether) obtained by chemical modification of chlorophyll *a*. The results are compared with the properties of metal-free analogs. It is shown that both copper complexes and metal free chlorins generate singlet oxygen under photoexcitation in solutions and show distinct phototoxicity in living cells. The photosensitizing effect of copper complexes is 4–13 times smaller, but remains rather high. According to the biological experiments, the activity of copper complexes is sufficient for photodynamic killing of HeLa cells. At the same time, the dark toxicity of copper complexes is lower than that of metal free chlorins. These data allow us to consider copper complexes of chlorins as a new class of photosensitizers, potentially suitable for the use in PDT.*

Keywords: Copper complexes, chlorin e_6 , pyropheophorbide *a*, chlorin e_6 derivatives, methylpheophorbide *a*, singlet oxygen generation, phototoxicity *in vitro*.

Медные комплексы хлоринов, производных хлорофилла *a*, как потенциальные фотосенсибилизаторы медицинского назначения

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*Исследованы абсорбционные и флуоресцентные свойства медных комплексов и безметалльных аналогов некоторых хлоринов, полученных при химической модификации хлорофилла *a* (комплексы метилпирофеофорбида *a* и не содержащих экзоцикла 13-амидных производных диметилового эфира хлорина e_6), а также их способность генерировать синглетный кислород и фотоиндуцировать токсическую активность по*

отношению к клеткам линии HeLa. Установлено, что медные комплексы, как и безметалльные хлорины, генерируют синглетный кислород при фотовозбуждении в растворах и обладают выраженной фототоксичностью в клетках. Эффективность фотосенсибилизирующего действия медных комплексов хлорина в 4–13 раз меньше, но остается достаточно высокой. Согласно биологическим экспериментам, активность медных комплексов достаточна для фотодинамического уничтожения клеток HeLa. При этом темновая токсичность медных комплексов меньше, чем у безметалльных аналогов. Эти свойства позволяют рассматривать медные комплексы хлоринов как новый класс фотосенсибилизаторов, потенциально пригодный для применения в ФДТ.

Ключевые слова: Комплексы меди, хлорин e_6 , пиррофеорбид a , производные хлорина e_6 , метилпиррофеорбид a , генерация синглетного кислорода, фототоксичность *in vitro*.

Introduction

The porphyrin-photosensitized oxygenation of biologically important compounds (photodynamic action) is widely used in medicine for treatment of cancer and infectious diseases (photodynamic therapy, PDT).^[1–16] Usually, oxygenation is due to the reactivity of singlet oxygen, which is generated by triplet molecules of photosensitizers. In some cases, the triplet molecules can oxidize biosubstrates directly, but the efficiency of the porphyrin involvement in these reactions is usually very low.^[1,2] It has been shown that metal free chlorophyll derivatives: pheophytin and pheophorbide a , chlorin e_6 , chlorin p_6 and their complexes with some metals are effective photodynamic sensitizers. Certain compounds from this group are recommended for clinical use in PDT (Phoscan, Photoditazin, Photolon, Radachlorin)^[1–16] and references cited therein). To our knowledge, the systematic studies of the photodynamic activity of copper chlorins have never been carried out, because the lifetime of the triplet state in copper complexes is known to be several orders shorter than that of the metal-free analogs,^[17–19] that should cause the strong reduction in the efficiency of singlet oxygen generation.^[4,18,19] Nevertheless, it was reported that Cu-mesoporphyrin retains the ability to moderately generate singlet oxygen upon photoexcitation in aerobic solutions.^[15,16] Preliminary data in favor of the ability of the copper complex of chlorin e_4 to photosensitize formation of singlet oxygen were recently reported in ref.^[20] However, this assumption does not seem convincing since as follows from the reported absorption and fluorescence spectra, the preparations of copper chlorins used by the authors contained at least 30 % admixture of metal-free chlorins and, possibly, their aggregated dimeric forms. We believe that it is extremely difficult to reliably identify in such a system the generation of singlet oxygen by copper complexes. Moreover, the authors did not provide reliable experimental evidence in favor of their hypothesis.^[20] Therefore, the question of the photosensitizing ability of copper complexes of chlorins essentially remained open. In addition, it should be noted that for the selection of photosensitizers for PDT both high photosensitizing activity and low dark toxicity of photosensitizers are very important.^[2] As shown in ref.^[21], the insertion of copper cations into the coordination sphere of a -series chlorins - chlorophyll a derivatives, might significantly reduce the dark toxicity of these compounds. In this regard, investigation of copper complexes of chlorins is of interest in view of their potential application as

photosensitizers for medical purposes. Primary evaluation of the photosensitizing potential of these compounds can be obtained from testing their abilities to generate singlet oxygen and kill living cells (in culture) under the action of light. Apparently, the high yield photosensitized generation of singlet oxygen indicates that the photosensitizer has an active triplet state, which can oxidize biomolecules through both involvement of the reactive singlet oxygen or by direct reaction with an oxidized substrate leading to formation of free radicals (^[1] and the references therein). Phototoxicity manifested in experiments with cell cultures indicates the ability of a photosensitizer to interact with cellular structures and cause their damage. In the present work, we studied the spectral properties of copper complexes of some chlorins obtained by chemical modification of chlorophyll a (Figure 1), and the ability of these compounds to generate singlet oxygen and photosensitize toxic activity in HeLa cells. As noted above, the incorporation of the copper cation into the coordination sphere of chlorins affects the spectral, photochemical, and biological properties of these compounds; therefore, the comparative study of copper chlorins (**Cu-1-Cu-3**) and corresponding metal free analogs (**1-3**) was carried out. Since the spectral and photosensitizing properties of chlorins are known to strongly depend on the presence of exocycle in their molecules,^[15,16] we also compared the compounds with exocycle (methylpyropheophorbide a (**1**) and its copper complex (**Cu-1**) and chlorin e_6 derivatives (**2**, **Cu-2**, **3**, **Cu-3**) having no exocycle.

Experimental

Compounds (**1**, **2**) and the corresponding copper complexes (**Cu-1**, **Cu-2**) were synthesized according to refs.^[22,23] The chlorin e_6 derivative (**3**) was synthesized according to ref.^[24]

Cu-chlorin e_6 13-N-2-hydroxyethylamide 15,17-dimethyl ester (Cu-3) was prepared as follows. Copper acetate (365.6 mg) dissolved in 15 ml of methanol was added to a solution of 73.1 mg of the starting ligand (**3**) in 15 ml of chloroform. The resulting solution was stirred at room temperature for 1 hour. The formation of the reaction product was monitored by TLC on Sorbfil plates; the eluent was a mixture of CCl_4 with acetone 2 to 1 by volume. The reaction mixture was diluted with chloroform (50 ml), washed with water from an excess of copper acetate, dried with anhydrous Na_2SO_4 , and the resulting solution was evaporated to dryness under reduced pressure. The pellet obtained after evaporation was chromatographed on silica gel (eluent is a mixture of CCl_4 with acetone in volume ratios from 50:1 to 1:1). As a result, 47.4 mg (60 %) of **Cu-3** was obtained. In the IR spectrum (SpecordM-80,

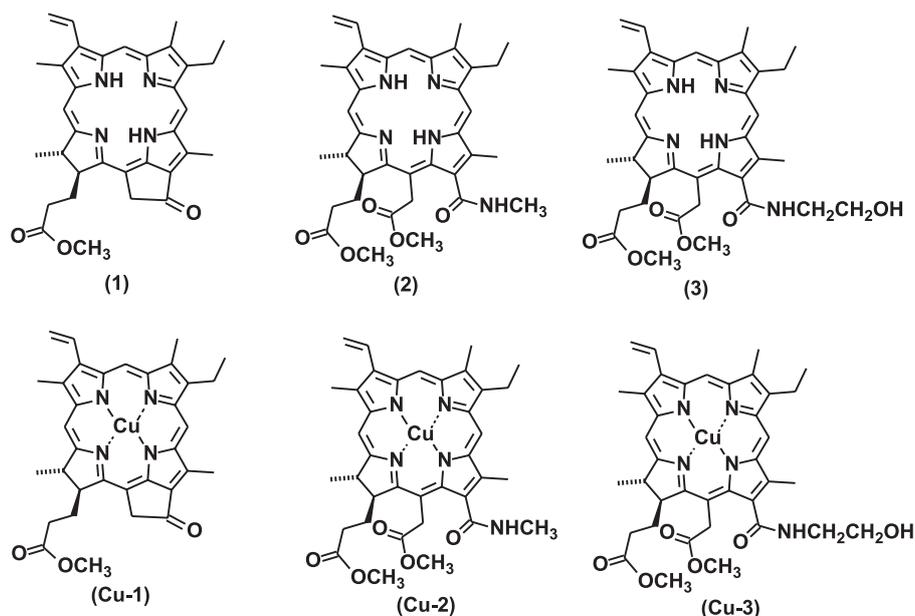


Figure 1. Structures of the compounds studied: (1) methylpyropheophorbide *a*; (2, 3) 13-amide derivatives of chlorin *e₆* dimethyl ether having no exocycle; (Cu-1), (Cu-2) and (Cu-3) are the copper complexes of these compounds.

KBr), cm^{-1} the following characteristic bands were observed: 1734 ($\nu\text{C}=\text{O}$, ester), 1634 (“amide-I”), 1595 (“chlorin band”), 1562 (“amide-II”). Parameters of the UV-vis spectra are listed in Table 1. MS (Thermo Finnigan LCQ Fleet, ESI) m/z : calculated for M^+ ($\text{C}_{38}\text{H}_{43}\text{CuN}_5\text{O}_6$): 728.3, found: 728.7.

Spectral properties of chlorins and their ability to generate singlet oxygen were studied in acetone (Khimmed, RF, puriss. spec.) Absorption spectra were measured using an SF-56 spectrophotometer (LOMO Spectrum, RF) in the 1 cm rectangular quartz cells. Fluorescence spectra were recorded by a Perkin Elmer MPF-44B computerized spectrofluorometer, which allows measurement of the fluorescence spectra and fluorescence excitation spectra of the samples.

Two methods were used for studies of singlet oxygen generation. One is based on detection of photosensitized IR phosphorescence of singlet oxygen at 1270 nm using the phosphorescence spectrometer assembled at the A.N. Bach Institute of Biochemistry (FRC Biotechnology RAS).^[25,26] The phosphorescence was excited by a LED with the emission band maximum at 405 nm and a half-width of 30 nm. Pulse repetition rate was 3–10 kHz, flash duration was 2 μs . Phosphorescence was recorded by a photomultiplier (FEU-112) cooled by liquid nitrogen vapor to -30°C . The phosphorescence spectrum was analyzed using a set of three interference light filters with the transmission maxima at 1230, 1270, and 1310 nm. The photomultiplier signal was passed through the preamplifier and then, processed by a computer photon counter synchronized with the LED pulse. The spectrometer allowed for estimation of the emission spectrum and recording the phosphorescence decay traces by averaging a signal after an unlimited number of exciting pulses using the method of multichannel photon counting. Typically, the signal accumulation time did not exceed 30 minutes. The average power of the exciting light was about 8 mW.

Another method for singlet oxygen detection was based on measuring the rate of chemical trapping of singlet oxygen by 1,3-diphenylisobenzofuran (DPIBF). The modification of this method applied at present work was described in detail elsewhere.^[25, 27–29] The maximum of the absorption spectrum of DPIBF in acetone is at 414 nm. In the region of 500–700 nm, DPIBF has no absorption bands. The reaction of DPIBF with singlet oxygen

caused accumulation of colorless products having no absorption bands in the visible spectral range. The rate of singlet oxygen formation was determined from the reduction of optical density in the maximum of the DPIBF absorption band.

The quantum yields of singlet oxygen generation were determined using reference compounds. As references, phenaleneone was employed in phosphorescence measurements and *meso*-tetraphenylporphine (TPP) – in chemical trapping experiments. According to the literature, the most probable values for the quantum yields of singlet oxygen generation are 0.70 ± 0.05 for TPP and 0.95 ± 0.05 for phenaleneone^[25–27] and references therein). Both methods yielded similar results.

However, for measurement of phosphorescence, chlorins were excited by a relatively broad-band LED radiation with the maximum at 405 nm. This radiation corresponds to the spectral regions, in which the chlorins and reference compounds absorb light. However, LED radiation does not match the maxima of the chlorin spectral bands. This complicated the calculation of the quantum yields and reduced the accuracy of the results.

When the chemical trapping method was applied, the solutions were irradiated by monochromatic light of the xenon lamp eliciting the monochromator of the Perkin Elmer MPF-44B fluorimeter. The wavelength of the exciting light corresponded exactly to the maxima of the long-wavelength band of each tested compound (for compounds (1–3) – 662 nm, for the (Cu-1) complex – 650 nm, for the (Cu-2, Cu-3) complexes – 632 nm, for TPP – 515 nm). The spectral width of the monochromator slits corresponded to 5 nm in the experiments with compounds (1–3) and TPP and 15 nm in the experiments with compounds (Cu-1–Cu-3). The concentration of DPIBF was set so that its optical density in the absorption maximum was 0.9–1.1 before illumination. Under illumination, optical density at the DPIBF absorption maximum decreased, whereas the optical density of chlorins remained unaltered.

The power of the exciting light was measured using ThorLabs PM-100D with a S120VC sensor head (ThorLabs, USA). The quantum yields were calculated using the program MS Excel and equations given in refs.^[27–29]

Cancer cells from the HeLa line of the uterine cervix that were cultured in a DMEM/F12 nutrient medium (PAA Labora-

tories GmbH, Austria) containing 10 % calf fetal serum (FBS) (Thermo Scientific HyClone, UK) were used for biological tests without adding antibiotics at 37 °C and 5 % CO₂. The dark and photoinduced toxic activity of chlorins was investigated as described in refs.^[21,30,31]

Results and Discussion

Absorption and fluorescence spectra

The absorption spectra of methylpheophorbide and other chlorins are shown in Figures 2 and 3. The spectral maxima are indicated in Figures 2 and 3, and listed in Table 1. It is seen that the maxima of the main spectral bands of exocycle-free chlorins (2, 3) are shifted to the shorter wavelengths compared to methylpyropheophorbide *a* (1). In copper complexes, the Soret band shifted to longer wavelengths, whereas the red band moved to shorter wavelengths as compared to metal-free macrocycles.

Metal-free macrocycles emitted strong fluorescence with similar quantum yields and the main maximum at 666–673 nm (Figures 2, 3), the excitation spectra coincided with the absorption spectra of these compounds (not shown). In solutions of copper complexes, a very weak fluorescence was also detected. However, the fluorescence intensities in the solutions with equal optical density in the excitation region was about 1000 times weaker than in solutions of the metal-free analogues. The spectrum of this fluorescence in all cases coincided with that of the metal-free chlorins. In the fluorescence excitation spectra of the solutions of the copper complexes, the Soret band was estimated to be at 405–410 nm that corresponds to the absorption band of the metal-free analogues. Fluorescence corresponding to the absorption spectrum of copper complexes was not found. These data indicate that the copper complexes practically do not fluoresce themselves, but the solutions of these complexes contain an admixture of about 0.1 % of the metal-free analogues.

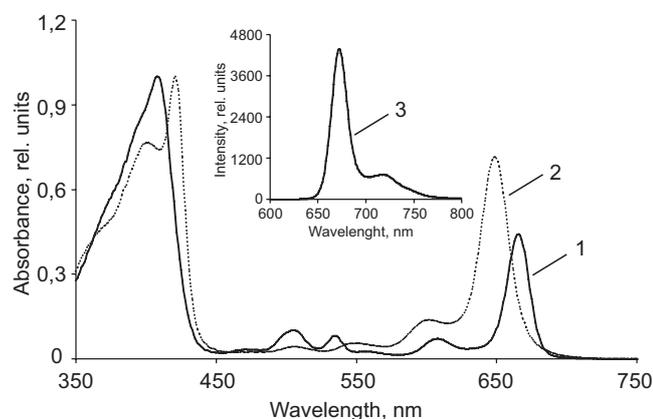


Figure 2. The absorption spectra of compounds (1) (curve 1) and (Cu-1) (curve 2) in acetone normalized at the maxima of the Soret bands. The main maxima of the curve 1 are at 408 and 666 nm; the main maxima of the curve 2 are 421 and 649 nm. Curve 3 is the fluorescence spectrum of the compound (1) (the main maximum is at 673 nm), under excitation at 408 nm.

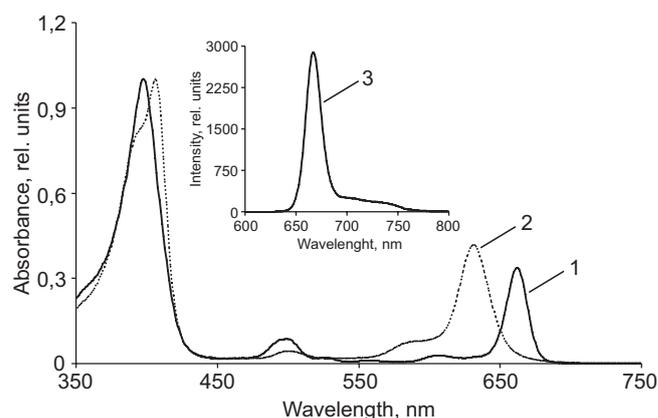


Figure 3. The absorption spectra of the compounds (3) (curve 1) and (Cu-3) (curve 2) in acetone normalized at the maxima of the Soret bands. The main maxima of the curve 1 are at 398 and 662 nm; the main maxima of the curve 2 are at 406 and 631 nm. Curve 3 is the fluorescence spectrum of compound (3) (the main maximum is at 667 nm) under excitation at 400 nm. Similar absorption and fluorescence spectra were obtained for compounds (2) and (Cu-2) (not shown).

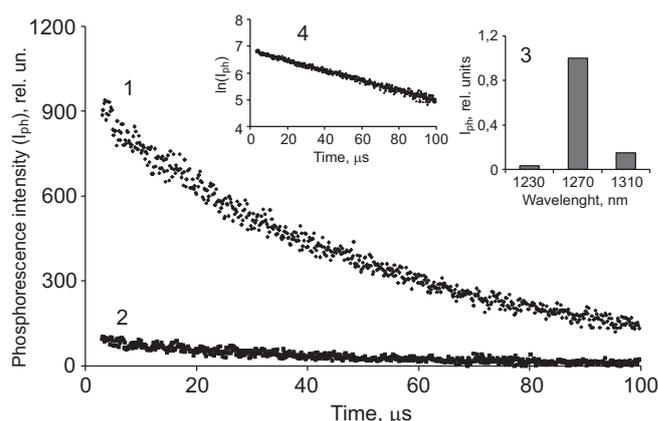


Figure 4. Decay kinetics (1, 2) and spectrum (3) of photosensitized phosphorescence of singlet oxygen under excitation by pulses of the LED radiation in solutions of metal-free chlorin (3) (curve 1) and its copper complex (Cu-3) (curve 2) in acetone; (4) curve 1 in the semi-log scale. The excitation wavelength is 405 nm, the flash duration is 2 μs, the flash repetition rate is 10 kHz, the average power of the exciting light is 7.6 mW, the signal accumulation time is 30 min. Measured in a 5 mm cuvette. The optical density of the pigment solutions was about 1.0 at the maximum LED emission.

Photosensitizing effect of chlorins

As seen from Figure 4, illumination of the acetone solutions of both metal-free macrocycles and their copper complexes, is accompanied by IR phosphorescence of singlet oxygen. In solutions of all chlorins the phosphorescence spectrum showed a pronounced maximum at 1270 nm (Figure 4, diagram 3). The phosphorescence decays after LED pulses are also similar in acetone solutions of all metal-free chlorins and their copper complexes (at the chlorin concentration of less than 5 μM) (Figure 4, curves 1 and 2). They are well approximated by exponentials with the decay time

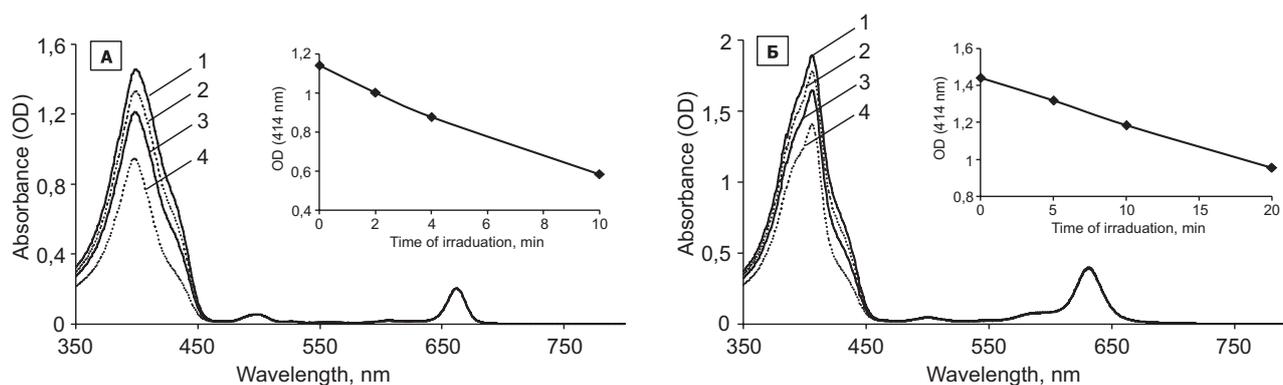


Figure 5. Changes in the absorption spectrum of DPBIF (curves 1-4) in the mixture of DPIBF with compound (3) (A) and Cu-3 (B) in acetone during irradiation of chlorins. For A, the excitation wavelength is 662 nm, power is 79 μ W. For B, the excitation wavelength is 632 nm, power is 272 μ W. Inset shows the time course of the fall in optical density of DFIBP at the absorption maximum 414 nm.

of about 50 μ s corresponding to the lifetime of singlet oxygen in acetone. In solutions of the copper complexes (Cu-1-Cu-3), the phosphorescence of singlet oxygen is weaker than in solutions of the metal-free chlorins by about one order of magnitude (1–3). On the one hand, this fact, indicates a decrease in the quantum yield of singlet oxygen generation in solutions of copper complexes. On the other hand, it shows that the observed singlet oxygen phosphorescence is determined by the photosensitizing effect of copper complexes, and is not associated with an admixture of metal-free chlorins. Indeed, as follows from measurements of fluorescence, the residual amount of metal-free chlorin in solutions of copper complexes is only 0.1 %.

The experiments on photosensitized oxygenation of the singlet oxygen trap (DPIBF) (Figure 5) support this conclusion. As shown by Figure 5, irradiation of the mixtures of chlorins and DPIBF by red light corresponding to the main absorption maximum of chlorins, causes rapid fading of the trap absorption band at 414 nm. At the same time fading of chlorins was not observed. The fading rate depended linearly on the intensity of the actinic light and was slowed down with the addition of singlet oxygen quencher α -tocopherol. Both metal-free macrocycles and their complexes with copper exhibited the photosensitizing activity. In accord with the results of phosphorescence measurements, the trapping method indicates that the photosensitizing effect of copper complexes of chlorins was weaker by about an order of mag-

nitude. Besides, the generation of singlet oxygen by copper complexes, is not related to the admixture of metal-free chlorins, the content of which is less than the yield of singlet oxygen by two orders of magnitude.

Thus, the experiments with trapping and phosphorescence of singlet oxygen convincingly demonstrate that both metal-free chlorins and the corresponding copper complexes generate singlet oxygen upon photoexcitation. The photosensitizing effect of the copper complexes is markedly weaker.

Measurement of the quantum yields of singlet oxygen generation by chlorins

As mentioned in the “Experimental” section, the quantum yields of singlet oxygen generation by chlorins were analyzed using both the phosphorescence and chemical trapping methods. Qualitatively, the results of both methods reasonably correlate.

The quantitative measurements were performed using the chemical trap – DPIBF. The quantum yields (ϕ_{Δ}) were calculated using MS Excel and the following equation:

$$\phi_{\Delta} = \phi_{\Delta St} \frac{\Delta D_{Chl} \cdot t_{irrSt} \cdot I_{St} \cdot (1 - 10^{-D_{St}}) \cdot \lambda_{St}}{\Delta D_{St} \cdot t_{irrChl} \cdot I_{Chl} \cdot (1 - 10^{-D_{Chl}}) \cdot \lambda_{Chl}}$$

where $\phi_{\Delta St}$ is the quantum yields of singlet oxygen generation by the reference (standard) compound; ΔD_{Chl} is the reduc-

Table 1. Spectral characteristics, photochemical properties and biological activity of metal-free chlorins and copper complexes.

Compound	Absorption maxima in acetone, nm	Quantum yields of singlet oxygen generation	IC ₅₀ , μ mol / l Under LED irradiation ($\lambda = 660 \pm 20$ nm)	IC ₅₀ , μ mol / l, dark
1	408, 506, 535, 608, 666	0.65	1.42 \pm 0.16	> 100
Cu-1	400, 421, 507, 551, 602, 649	0.17	13.32 \pm 2.07	> 100
2	398, 498, 607, 662	0.72	0.025 \pm 0.003	4.01 \pm 0.80
Cu-2	406, 502, 632	0.055	0.22 \pm 0.02	7.90 \pm 1.32
3	398, 499, 607, 662	0.74	0.047 \pm 0.002	5.63 \pm 0.49
Cu-3	406, 500, 631	0.055	0.28 \pm 0.03	13.16 \pm 1.04

Note. The error of measuring of the quantum yields is estimated to be $\pm 5\%$.^[24,26-28] IC₅₀ is the concentration of half maximal inhibition of cell growth after exposure in the dark or under irradiation.

tion of DPIBF optical density during irradiation of chlorins; ΔD_{St} is the reduction of DPIBF optical density during irradiation of the reference compound (standard); t_{irrChl} and t_{irrSt} are the time of irradiation of the mixtures of DPIBF with chlorins or standards in seconds, I_{Chl} and I_{St} are the excitation rates (in μW) of chlorins and the reference (standard) compound. D_{Chl} and D_{St} are optical densities of the chlorins and TPP at the excitation wavelengths (λ_{Chl} and λ_{St} , in nm). The results are summarized in Table 1.

The IC_{50} values used for estimation of the dark and photoinduced toxicity in experiments on HeLa cells (Table 1) were determined similarly to refs.^[21,30,31] The metal-free chlorins (**1–3**) as well as their copper complexes (**Cu-1–Cu-3**) showed distinct photosensitizing effect. The photosensitizing efficiencies of metal-free chlorins, virtually coincides with the data reported earlier.^[21,30,31] IC_{50} in the cells incubated in the dark exceeds IC_{50} in illuminated samples by two or more orders of magnitude. The photodamaging effect of complexes (**Cu-1–Cu-3**) is somewhat weaker, however, the ability of these compounds to exert a photosensitizing cell killing is manifested quite clearly. With (**Cu-2**) and (**Cu-3**), the coefficients IC_{50} for photoinduced cytotoxicity were by 36 and 47 times smaller than for action of the same compounds in the dark (Table 1). With (**Cu-1**) no dark toxicity was revealed. Since, according to fluorescence measurements admixture of the metal-free chlorins in the preparations of copper complexes (**Cu-1–Cu-3**) is about 0.1 %, one can conclude that the biological effect is due precisely to the copper chlorins. It is noteworthy that the relative photodynamic activity of metal-free and copper-containing chlorins on HeLa cells and the relative activity of these compounds in photosensitization of the singlet oxygen formation reasonably correlate (Table 1). In both cases, the difference is within one order of magnitude. Therefore, it seems likely that it is the ability to generate singlet oxygen that determines the phototoxicity of copper-containing chlorins in biological experiments.

Conclusion

Thus, the studies of photosensitized oxygenation of DPIBF and IR phosphorescence of singlet oxygen show that metal-free chlorins (**1–3**) and their copper complexes (**Cu-1–Cu-3**) generate singlet oxygen during photoexcitation. The photosensitizing effect of copper chlorins is noticeably weaker, but remains reasonably high. Biological experiments demonstrate that the compounds (**Cu-1–Cu-3**) have pronounced phototoxicity, which is sufficient for photodynamic destruction of HeLa cells (IC_{50} for dark cytotoxicity is by about two orders of magnitude greater than for phototoxicity). At the same time, the dark toxicity of (**Cu-1–Cu-3**) is low compared to metal-free chlorins (IC_{50} rises by about one order during the transition from metal-free chlorins to the corresponding copper complexes). These properties of copper complexes of chlorins allow us to consider them as a new class of photosensitizers, potentially suitable for the use in PDT.

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References

1. Krasnovskii A.A. Singlet Oxygen and Primary Mechanisms of Photodynamic and Laser Medicine. In: *Basic Science for Medicine: Biophysical and Medical Technology* (Grigoriev A.I., Vladimirov Yu.A., Eds.) Vol. 1, Moscow: MAKS Press, **2015**. p. 173–217 (in Russ.) [Красновский А.А. Синглетный кислород и первичные механизмы фотодинамической и лазерной медицины. В кн.: *Фундаментальные науки – медицине. Биофизические медицинские технологии* (Григорьев А.И., Владимиров Ю.А., ред.) Т. 1, М.: Макс Пресс, 2015. с. 173–217].
2. Stranadko E.F. *ibid.* p. 218–263 (in Russ.) [Странадко Е.Ф., там же, с. 218–263].
3. Krasnovsky A.A. *Biophysics* **1977**, 22, 927–928 (in Russ., see English version on Research Gate).
4. Venediktov E.A., Krasnovskii A.A. *Zh. Prikl. Spektrosk. (J. Appl. Spectrosc. Minsk)* **1982**, 36(1), 152–154 (in Russ., see English version on Research Gate).
5. Krasnovsky A.A., Jr., Neverov K.V., Egorov S.Yu., Roeder B., Levald T. *J. Photochem. Photobiol. B: Biology* **1990**, 5, 245–254.
6. Kochubeyev G.A., Frolov A.L., Gurinovich G.P. *Russ. Chem. Phys.* **1989**, 8, 1184–1190 (in Russ.).
7. Frolov A.A., Zenkevich E.I., Gurinovich G.P. *J. Photochem. Photobiol. B: Biology* **1990**, 7, 43–56.
8. Zenkevich E., Sagun E., Knyukshto V., Mironov A., Efremova O., Bonnet R., Songca S.P., Kassem M. *J. Photochem. Photobiol. B: Biology* **1996**, 33, 171–180.
9. Bonnett R., Charlesworth P., Djelal B. D., Foley S., McGarvey D. J., Truscott T. G. *J. Chem. Soc., Perkin Trans.* **1999**, 2, 325–328.
10. Parkhats M.V., Lapina V.A., Butorina D.N., Sobchuk A.N., Lepeshkevich S.V., Petrov P.T., Krasnovskii A.A., Dzhagarov B.M. *Opt. Spekt.* **2005**, 98, 517–425.
11. Feofanov A., Sharonov G., Grichin A., Karmakova T., Pljutinskaya A., Lebedeva V., Ruziyev R., Yakubovskaya R., Mironov A., Refregier M., Maurizot J.-P., Vigny P. *Photochem. Photobiol.* **2004**, 79, 172–188.
12. Mojzisoava H., Bonneau S., Maillard P., Berg K., Brault D. *Photochem. Photobiol. Sci.* **2009**, 8, 778–787.
13. Parkhats M.V., Galievskii V.A., Zharnikova E.S., Knyukshto V.N., Lepeshkevich S.V., Stashevskii A.S., Trukhacheva T.V., Dzhagarov B.M. *J. Appl. Spectrosc. (Minsk)* **2011**, 78, 299–306.
14. Belik V.P., Gadzhiev I.M., Semenova I.V., Vasyutinskii O.S. *Spectrochim. Acta, Part A* **2017**, 178, 181–184.
15. Ormond A. B., Freeman H. S. *Materials* **2013**, 6, 817–840.
16. Dąbrowski J.M., Arnaut L.G. *Photochem. Photobiol. Sci.* **2015**, 14, 1765–1780.
17. Dongbo K., Holten D., Gouterman M. *J. Am. Chem. Soc.* **1984**, 106, 2793–2798.

18. Dzhagarov B.M., Salokhiddinov K.I. *Opt. Spektrosk.* **1981**, 51, 841–847 (in Russ.).
19. Dzhagarov B.M., Gurinovich G.P. Mechanisms of Relaxation Processes in Molecules of Chlorophyll and Related Compounds. In: *Excited Molecules. Kinetics of Transformation* (Krasnovsky A.A., Ed.) Leningrad: Nauka, **1982**. p. 59–74 (in Russ.) [Джагаров Б.М., Гуринович Г.П. Механизмы релаксационных процессов в молекулах хлорофилла и родственных соединений. В кн.: *Возбужденные молекулы. Кинетика превращений* (Красновский А.А., ред.) Ленинград: Наука, **1982**. с. 59–74].
20. Simaremare E.S., Djalil A.D., Tjahjono H.D. *Indonesian J. Pharm.* **2015**, 26, 29–36.
21. Pylina Y.I., Shadrin D.M., Shevchenko O.G., Khudyaeva I.S., Belykh D.V., Velegzhaninov I.O. *Macroheterocycles* **2017**, 10, 279–288.
22. Mazaletskaia L.I., Sheludchenko N.I., Tarabukina I.S., Belykh D.V. *Petroleum Chemistry* **2014**, 54, 309–315.
23. Tarabukina I.S., Pylina Y.I., Velegzhaninov I.O., Startseva O.M., Shadrin D.M. and Belykh D.V. *Butlerov Commun.* **2015**, 43, 18–23.
24. Belykh D.V., Karmanova L.P., Spirikhin L.V., Kuchin A.V. *Russ. J. Org. Chem.* **2007**, 43, 126–134.
25. Krasnovsky A.A., Kozlov A.S. *Journal of Biomedical Photonics and Engineering* **2017**, 3(1), 010302: 1–10 (DOI: 10.18287/JBPE17.03.010302).
26. Krasnovsky A.A., Jr., Benditkis A.S., Kozlov A.S., *Biochemistry (Moscow)* **2019**, 84, 153–163 (DOI: 10.1134/S0006297919020068).
27. Krasnovsky A.A., Jr., Kozlov A.S., Roumbal Ya.V., *Photochem. Photobiol. Sci.* **2012**, 11, 988–997 (DOI: 10.1039/c2pp05350k).
28. Krasnovskii A.A., Jr., Kozlov A.S. *Biophysics* **2014**, 59, 199–205.
29. Krasnovsky A.A., Jr., Kozlov A.S. *J. Photochem. Photobiol. A: Chemistry* **2016**, 329, 167–174 (DOI: 10.1016/j.jphotochem.2016.06.026).
30. Mal'shakova M.V., Pylina Y.I., Frolova L.L., Alekseev J.H., Patov S.A., Shadrin D.M., Kuchin A.V., Velegzhaninov I.O., Belykh D.V. *Macroheterocycles* **2016**, 9, 238–243.
31. Pylina Y.I., Shadrin D.M., Shevchenko O.G., Startseva O.M., Velegzhaninov I.O., Belykh D.V., Velegzhaninov I.O. *Int. J. Mol. Sci.* **2017**, 18, 103 (DOI: 10.3390/ijms18010103).

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