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The Influence of Media Polarity and Red Light on Photofrin II and Hypericin

Wpływ polarności środowiska i światła czerwonego na fotofrin II i hiperycynę

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Abstract

Objectives. There was investigated an effect of different medium influence and the red light influence on Photofrin II and hypericin.
Material and Method. Photofrin II and hypericin dissolved in ethanol and PBS were excitated by the red light (λ = 630 nm) and studied by spectroscopy UV/Vis method.
Results. The red light increased value of the sensitizer extinction coefficient (ε). Increasing of a solvent polarity caused absorption bands red shift for Ph II (λ_{PBS} = 370 nm and $\lambda_{Et\text{-}OH}$ = 393 nm) and blue shift for HY (λ_{PBS} = 604 nm and $\lambda_{Et\text{-}OH}$ = 590 nm).
Conclusions. The red light and solvent type have a significant influence on dyes examined in this study. Knowing the chemical features one can determine their behaviour and the way they can bind each other and with other compounds in the cell **(Adv Clin Exp Med 2004, 13, 6, 897–901)**.

Key words: photodynamic therapy, Photofrin II, hypericin, red light absorption, spectroscopy UV/Vis.

Streszczenie

Cel pracy. Głównym celem niniejszej pracy było określenie wpływu polarności różnych rozpuszczalników i światła czerwonego na dwa fotouczulacze: fotofrin II i hiperycynę. Oba fotouczulacze mają szerokie zastosowanie w terapii fotodynamicznej.
Materiał i metody. Fotofrin II i hiperycynę zbadano w etanolu i PBS. Następnie próby wzbudzano światłem czerwonym i zbadano absorpcyjnie metodą spektroskopii UV/Vis.
Wyniki. Czerwone światło powodowało wzrost wartości współczynnika ekstynkcji fotouczulaczy (ε). Wraz ze zwiększającą się polarnością rozpuszczalnika pasma absorpcji wykazywały red shift (przesunięcie pasma w stronę fal dłuższych), dla Ph II (λ_{PBS} = 370 nm i $\lambda_{Et\text{-}OH}$ = 393 nm) oraz blue shift (przesunięcie pasma w stronę fal krótszych) dla HY (λ_{PBS} = 604 nm i $\lambda_{Et\text{-}OH}$ = 590 nm).
Wnioski. Poznanie wpływu zarówno czerwonego światła, jak i typu środowiska, w którym znajduje się fotouczulacz ma znaczący wpływ na przebieg terapii fotodynamicznej. Dzięki poznaniu tych właściwości chemicznych hematoporfiryn można określić ich zachowanie i sposób łączenia się z innymi związkami w komórce **(Adv Clin Exp Med 2004, 13, 6, 897–901)**.

Słowa kluczowe: terapia fotodynamiczna, fotofrin II, hiperycyna, absorpcja światła czerwonego, spektroskopia UV/Vis.

Photodynamic therapy (PDT) is a medical method of tumour treatment that employs the combination of light and a drug to get a cytotoxic or modifying effect to cancerous tissue. A drug – photosensitizer – is introduced into the body and accumulates preferentially in tumours cells. In this

Abbreviations: Ph II – Photofrin II; HY – hypericin; Hp – hematoporfirins; PBS – phosphate buffered saline; UV – ultraviolet; Vis – visible; NIR – near infrared; EtOH – ethanol.

study, the authors used Photofrin II (Ph II) and hypericin (HY) [1]. They are derivatives of hematoporfirins (Hp). These dyes are generally used in photodynamic method and their role is very significant in tumour recognising and therapy [2]. The principle of PDT involves administration of a photosensitizer and illumination of the tumour area with the light of an appropriate wavelength to excite the sensitizer to its triplet state. Illuminated hematoporfirins can undergo many changes. They have a strong absorption band at about 400 nm (the Soret band) and a number of absorption bands at longer wavelengths up to 630 nm [3, 4]. One of the fundamental conditions the Ph II and HY must perform in PDT is to induce their long-term photosensitivity (red light, near infrared – NIR). These photosensitizers have the strongest absorption peak at the visible part of the spectrum (400–800 nm). In PDT one uses the range of wavelength 600÷800 nm, because in that range there is the deepest tissue penetration (so called: optical therapeutic window) [3, 5]. This range of wavelength is the least absorbed by the water and endogenous dyes. According to their chemical structure, most lipophilic photosensitizers tend to aggregate in aqueous media as a result of the propensity of the hydrophobic skeleton to avoid contact with water molecules. This state determines the efficacy of the drug *in vivo* by decreasing its bioavailability and limiting its capacity to absorb light [6–8]. Clinical and preclinical PDT research confirm that wavelength $\lambda = 630$ nm activates Ph II and HY and absorption peak at this point suits to monomers of Photofrin II.

Following the absorption of light, the photosensitizer, initially at ground state (PS^0) is activated to a short-living excited state ($^1PS^*$) that may convert to a long-lived state ($^3PS^*$). The main role of the singlet state in the photosensitization process is to act as a precursor of the triplet state. This triplet state is the photoactive state, which may generate cytotoxic species by undergoing two main reactions: energy transfer and electron or hydrogen transfer. The energy transfer can produce free radicals or superoxide ions resulting from hydrogen transfer. The second type of reaction involves the interaction between oxygen and the triplet state of the sensitizer to go between formation of singlet oxygen which is generally the main cytotoxic species in PDT. Therefore the PDT efficiency depends on the chemical features of used photosensitizers [5, 7, 9].

The main purpose of this study was to check the influence of the red light ($\lambda = 630$ nm) on both photosensitizers (Photofrin II and hypericin) in solvents of different polarity and for different concentration of the dye.

Material and Methods

Chemicals

Hypericin and phosphate buffered saline (PBS, pH 7.2 at 25°C; sodium chloride 150 mM, sodium phosphate 150 mM) were purchased from Sigma-Aldrich. Photofrin II® was purchased from QLT (Vancouver, CA). Ethanol (EtOH) (Absolute ≥ ≥ 99,8% v/v) was obtained from Fluka. Photosensitizers were dissolved in two different media: ethanol and PBS. The authors prepared different concentrations (respectively for ethanol and PBS) for Photofrin II: 15, 20, 25, 30, 35 μM and for hypericin: 5, 10, 15, 20, 25 μM. [9]. (All solutions were prepared three times.)

Sensitization of Dyes

Photofrin II and hypericin solutions were exposed to the red light ($\lambda = 630$ nm) for 15 min. Both photosensitizers were irradiated using a fiber illuminator (OPTEL Opole, 150 W). The illuminator emits visible light from the range 400÷780 nm. During sensitizers excitation the red glass filter – 630 nm was used. With this filter the lamp delivers a power density of 15.6 $W \cdot cm^{-2}$.

Spectroscopic Measurements

All solutions were measured at room temperature in 1 cm pathlength quartz cuvettes. Absorption spectra were taken using a Jasco V-530 UV/Vis spectrophotometer. (All measurements were repeated three times.)

Results

The spectroscopy of porphyrins is highly conditioned by their microenvironment. Fig. 1 and Fig. 2 explain that the red light at $\lambda = 630$ nm causes excitation of Photofrin II and hypericin and an absorption peak at this point corresponds with their monomers. Absorption of illuminated Ph II and HY solutions in PBS and Ph II solutions in EtOH is weaker than in not illuminated solutions. However, absorption of HY solutions in EtOH is stronger than for not illuminated solutions. Both photosensitizers are more stable in EtOH solutions than in water solutions. In contrast to EtOH solutions, in water environment the authors observed precipitation.

The red light ($\lambda = 630$ nm) decreases value of extinction coefficient (ε) for absorption peaks of

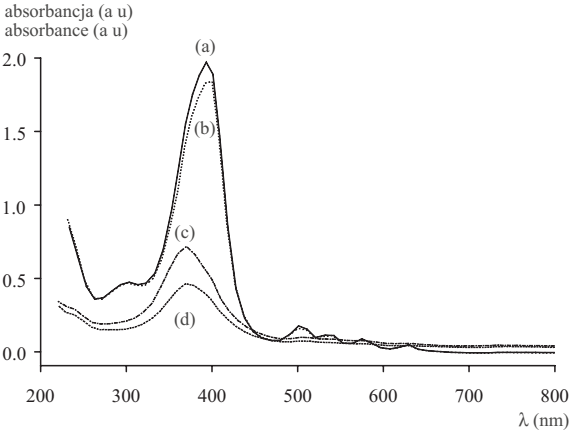

Fig. 1. Absorption spectra from solutions of 30 μM. Photofrin II in EtOH (99.8%) not illuminated – curve (a), illuminated – curve (b) and in PBS (sodium chloride 150 mM, sodium phosphate 150 mM) not illuminated – curve (c), illuminated – curve (d)

Ryc. 1. Widma absorpcyjne roztworów 30 μM fotofrinu II. Roztwory fotofrinu II w etanolu (99,8%): krzywa (a) nienaświetlane; krzywa (b) naświetlane. Roztwory fotofrinu II w PBS (chlorek sodu 150 mM, ortofosforan sodowy 150 mM): krzywa (c) nienaświetlane; krzywa (d) naświetlane

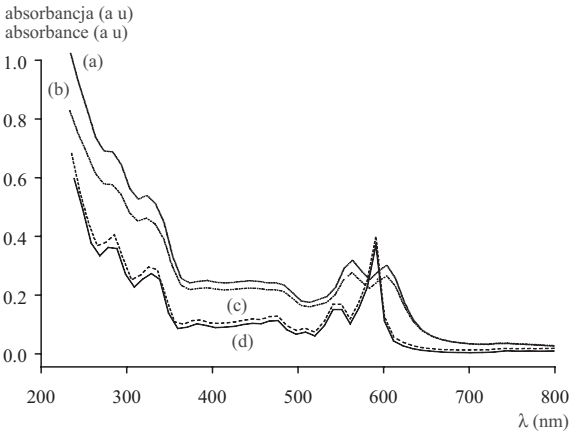

Fig. 2. Absorption spectra from solutions of 20 μM hypericin in PBS (sodium chloride 150 mM, sodium phosphate 150 mM) not illuminated – curve (a), illuminated – curve (b) and 5 μM in EtOH (99.8%) not illuminated – curve (d), illuminated – curve (c)

Ryc. 2. Widma absorpcyjne roztworów 20 μM hiperycyny w PBS (chlorek sodu 150 mM, ortofosforan sodowy 150 mM): krzywa (a) nienaświetlane; krzywa (b) naświetlane. Widma absorpcyjne roztworów 5 μM hiperycyny w etanolu (99,8%): krzywa (c) naświetlane; krzywa (d) nienaświetlane

both dyes solutions. The decrease of ε is about 5000÷10 000 mol · l^{-1} · cm^{-1}. There is a red shift of the Soret band with the increasing solvent polarity (λ_{PBS} = 370 nm and λ_{EtOH} = 393 nm). With increasing polarity increases ε, it is over 60 000 mol · l^{-1} · · cm^{-1} (for EtOH solutions) (Fig. 3 and Fig. 4).

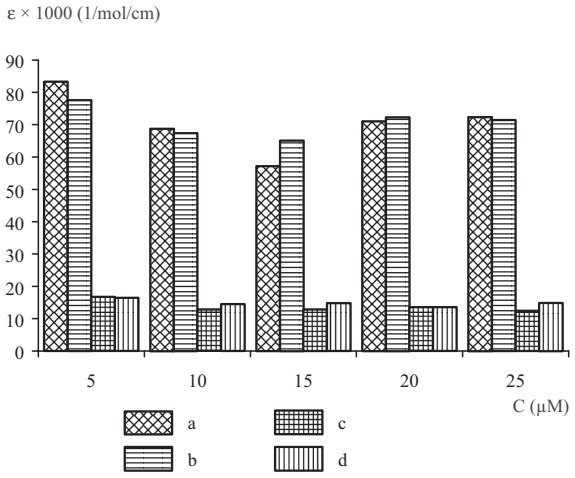

Fig. 3. The relation between the extinction coefficient (ε) and the concentration of hypericin (C). The sensitizer extinction coefficient (ε) for hypericin solutions in EtOH illuminated, (a) absorption peak at λ = 630 nm and not illuminated, (b) peak at λ = 604 nm, ε for hypericin solutions in PBS illuminated, (c) absorption peak at λ = = 630 nm and not illuminated, (d) peak at λ = 590 nm

Ryc. 3. Zależność między współczynnikiem ekstynkcji (ε) i stężeniem hiperycyny (C). Współczynnik ekstynkcji dla roztworów naświetlanych hiperycyny w etanolu, (a) absorpcja dla długości fali λ = 630 nm; ε dla roztworów w etanolu nienaświetlanych, (b) pik absorpcji dla długości fali λ = 604 nm; ε dla roztworów naświetlanych hyperycyny w PBS, (c) pik absorpcji dla λ = 630 oraz roztworów nienaświetlanych, (d) pik absorpcji dla λ = 590 nm

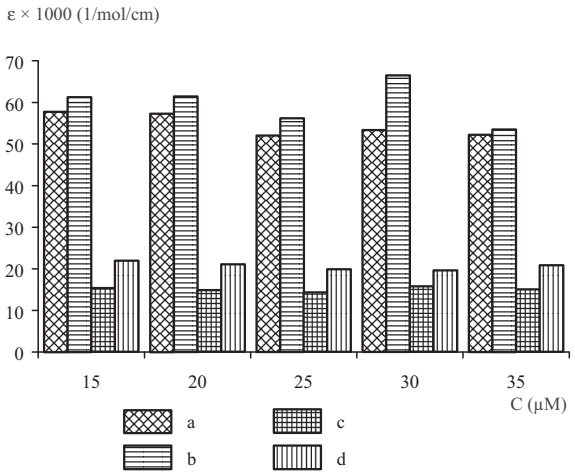

Fig. 4. The relation between the extinction coefficient (ε) and the concentration of Photofrin II (C). The sensitizer extinction coefficient (ε) for Photofrin II solutions in EtOH illuminated, (a) absorption peak at λ = 393 nm and not illuminated, (b) peak at λ = 393 nm, ε for Photofrin II solutions in PBS illuminated, (c) absorption peak at λ = 370 nm and not illuminated, (d) peak at λ = 370 nm

Ryc. 4. Zależność między współczynnikiem ekstynkcji (ε) i stężeniem fotofrinu II (C). Współczynnik ekstynkcji dla roztworów naświetlanych fotofrinu II w etanolu, (a) absorpcja dla długości fali λ = 393 nm; ε dla roztworów w etanolu nienaświetlanych, (b) pik absorpcji dla dłuości fali λ = 393 nm; ε dla roztworów naświetlanych fotofrinu II w PBS, (c) pik absorpcji dla λ = 370 oraz roztworów nienaświetlanych, (d) pik absorpcji dla λ = 370 nm

After exposing the dyes in PBS to the red light, interactions of aromatic bonds between molecules are decayed and electrostatic interactions only exist. The value of the extinction coefficient is much lower, ε is about 10 000÷20 000 mol · l^{-1} · · cm^{-1} (Fig. 3 and Fig. 4). It means that HY and Ph II aggregates are disintegrated on single molecules [1, 6, 10].

Presented explorations demonstrate the influence of ethanol, PBS and the red light on Ph II and HY spectra. Absorption spectra (Fig. 1 and Fig. 2) of dyes solutions after the red light irradiation ($\lambda_{excitation}$ = 630 nm) are presented in this study. They show that in lower concentration (5–15 μM) absorption bands are weaker than in higher concentration (25–35 μM). It is related to photosensitizer's property of aggregating in higher concentration [11].

Discussion

This study may contribute to explanation of molecular mechanism phototherapy in this range of radiation and play a significant role in diagnostic and tumour therapy. The efficiency of PDT depends on the chemical features of used photosensitizers [5, 7–9].

Excited hematoporfirins can undergo many changes. They reveal a spectral change that is blue and red shift of the Soret band. These changes can be interpreted as an interaction between photosensitizers (and derivatives) and model biological macromolecules – proteins, nucleic acids and polysaccharides [7, 8]. Therefore, these spectral changes are also the evidence that in some cases there is a formation of highly ordered aggregated structures [4, 9]. Other studies that were investigated on macrocyclic photosensitizers confirm presented experiment and also other spectroscopic and fluorescent research [4, 7, 9].

In conclusion, presented data demonstrate the basic chemical features of commonly used photosensitizers: Photofrin II and hypericin. The red light (λ = 630 nm) excites photosensitizers. This excitation wave is the most proper because has the deepest penetration of the tissue ("therapeutic window"). The authors also observe stronger activity of the dyes in more polar solvent – EtOH (99.8%), especially for Photofrin II. Fig. 1 shows high values of absorbance for Ph II in EtOH, twice higher in contrast to Ph II in PBS. The same effect is visible in the Fig. 3 and Fig. 4, the relation between the extinction coefficient and the concentration. The values of ε for solutions in EtOH are even four times higher than ε values for solutions in PBS. This indicates better efficacy of PDT in environment of higher polarity.

These studies are significant to know the mechanism of photodynamic therapy. Having known dye behaviour, one can make a provision for further therapy, one can plan and establish the exact conditions of the treatment.

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