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PACS 07.57.-c

THE EXCITATION SPECTRA
OF SINGLET OXYGEN AND PHOTOLUMINESCENCE
OF METHYL PHEOPHORBIDE-a

The correlation between the photoluminescence intensity of a dye-sensitizer (methyl pheophorbide-a, MPP-a) and the efficiency of the singlet oxygen ($^1O_2$) generation is studied. The excitation spectrum of $^1O_2$ emission at 1270 nm has one strong band at 403 nm that coincides with the Soret band of MPP-a and only a weak peak at 667 nm, which practically coincides with the strongest band of the MPP-a excitation spectrum. Therefore, the choice of an excitation wavelength for the efficient $^1O_2$ generation should be based on direct measurements of the excitation spectra of the sensitized $^1O_2$ emission.

K e y w o r d s: singlet oxygen, photodynamic therapy, photosensitization, dye sensitizer, methyl pheophorbide-a.

1. Introduction

In many applications, the efficiency of a dye sensitizer is assessed by the intensity of its visible photoluminescence, because the direct detection of singlet oxygen is rather difficult. We studied the correlation between the photoluminescence (PL) intensity of a dye sensitizer to generate singlet oxygen ($^1O_2$) and the intrinsic singlet oxygen emission at 1270 nm by the example of methyl pheophorbide-a (MPP-a) [1, 2]. Particularly, such study is of interest for the practice of photodynamic therapy (PDT). PDT is a medical technique, which uses a combination of photosensitizing preparation and light to induce a selective damage on the target [3]. Applications of the photodynamic effects mainly explored on the cancer treatment as an alternative to the chemotherapy or radiotherapy procedure. Moreover, the photodynamic methods are already in use either routinely or in experimental studies on several medical fields, such as dermatology, ophthalmology, gastroenterology, cardiology, neonatology, and against fungal and bacterial infections. In PDT under suitable illumination, the photosensitizer can give rise to activated species, which are very reactive to biological environment [4, 5]. Following the light absorption, the photosensitizer molecule promotes the excited singlet state with a short lifetime (ns). The photosensitizer can return to it ground state by emitting a photon (fluorescence) or by internal conversion with energy loss as heat. The intersystem crossing can transfer the excited photosensitizer molecule to a triplet state with a longer lifetime (ms), by increasing the probability of the energy transfer to other molecules [6, 7]. The triplet state of a photosensitizer can react with biomolecules following two mechanisms known as Type I and Type II reactions. Type I reaction involves the electron/hydrogen transfer directly from a photosensitizer producing ions or the electron/hydrogen absorption from a substrate molecule to form free radicals. The second one (Type II reaction) produces the electronically excited highly reactive singlet state of molecular oxygen known as singlet oxygen [8, 9]. In many applications, including PDT, the efficiency of excitation of a dye sensitizer to generate singlet oxygen often assessed by the intensity of dye's visible PL, because the direct detection of singlet oxygen is rather difficult. The generation rate of $^1O_2$ can monitored by measuring the intensity of emission at 1270 nm. Unfortunately, this emission is very faint because the corresponding transition between the singlet excited and triplet ground states is forbidden in the dipole approximation.

2. Results and Discussion

Spectra of PL of MPP-a dissolved in chloroform ($6 \times \times 10^{-5}$ M) and the band of sensitized $^1O_2$ emission were recorded with a VIS IR spectrophotome-
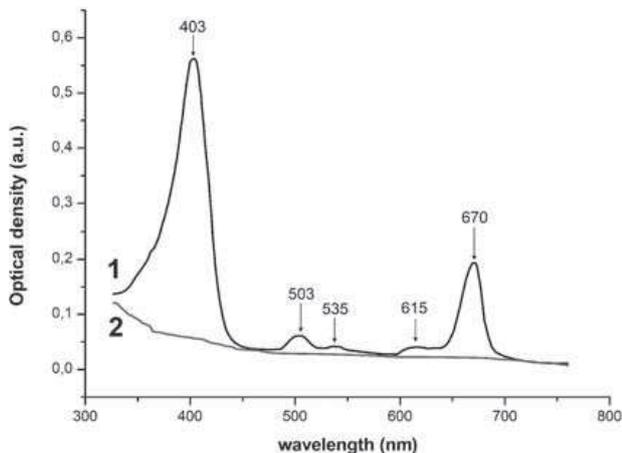
Fig. 1. Absorption spectra of the MPP-a dissolved in chloroform (6×10^{-5} M) before (*1*) and after (*2*) irradiation by a laser at 405 nm
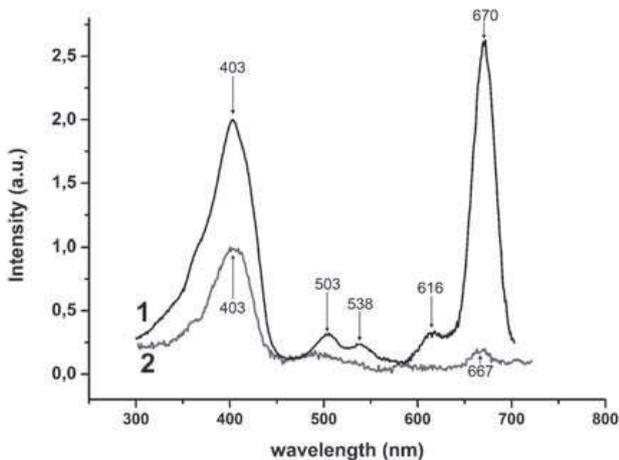

Fig. 2. Excitation spectra of intrinsic photoluminescence of MPP-a dissolved in chloroform (6×10^{-5}) (*1*) and $^1O_2$ emission (*2*)

ter based on a wide-aperture double monochromator equipped with a cooled photodiode InGaAs (IGA-30 EOS, USA). The obtained spectrum of PL of MPP-a was similar to that presented in [2].

We study the correlation between the rates of degradation of photoluminescence of MPP-a in visible and sensitized $^1O_2$ emission during irradiation by a semiconductor laser operating at 405 nm. We found that the intensity of PL in visible and the intensity of $^1O_2$ emission decrease, but the degradation rate of PL of the dye is by a factor of $1.5 \div 2.5$ less than that of the emission of singlet oxygen. This shows that the
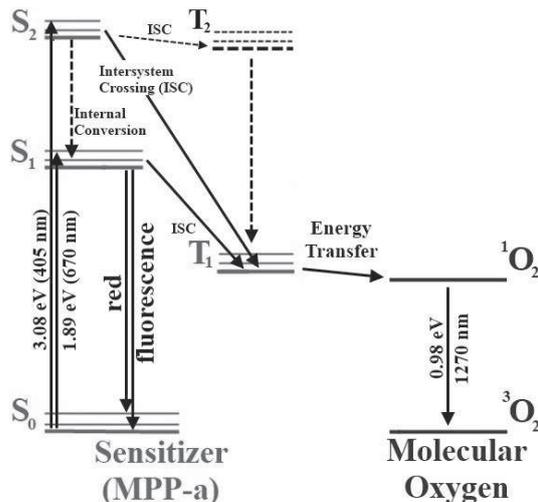

Fig. 3. Energy levels diagram of photosensitization of $^1O_2$ emission by MPP-a

dye efficiency assessment based on the intensity of its visible PL is acceptable.

One can assume that the degradation of PL of the dye occurs due to the degradation of the dye itself under excitation laser irradiation. To prove this assumption, the absorption spectra of MPP-a dissolved in chloroform before and after laser irradiation were recorded with a Specord UV VIS spectrophotometer. The obtained absorption spectrum of MPP-a is similar to that presented in [2], and all its bands completely disappeared after irradiation when visible PL cannot be more observable (Fig. 1).

We also measured the excitation spectra of intrinsic PL of MPP-a at 730 nm and the sensitized emission of singlet oxygen at 1270 nm under the same conditions of excitation with a UV-Vis spectrophotometer Varian Cary Eclipse (VCE). The excitation spectra of intrinsic photoluminescence of MPP-a were recorded using the standard option of VCE. To register an extremely faint sensitized $^1O_2$ emission, we used a cooled InGaAs photodiode covered with a set of filters providing a narrow band of transmission centered at 1270 nm. The photodiode detector was inserted to the cuvette section of VCE directly next to the sample. The photodiode was connected to the input of its own registration system independent of the VCE fluorescence intensity registration. The registration of $^1O_2$ emission was synchronized in time with the wavelength scanning of an excitation monochroma-

tor of VCE. In the excitation spectrum of intrinsic PL of MPP-a, one can observe two strong bands at 403 nm and 670 nm and three week bands at 503 nm, 538 nm, and 616 nm.

The overall view of the PL excitation spectrum of MPP-a displayed in Fig. 2 is in rather good agreement with its absorption spectrum shown in Fig. 1 and with the PL excitation spectrum in Fig. 4.4. in [2]. In the excitation spectrum of PL of MPP-a, the bands at 403 nm and 670 nm dominate, and the band at 670 nm has a major peak intensity. At the same time, the band with a maximum at 403 nm dominates in the excitation spectrum of the emission of singlet oxygen, and only a weak peak can be seen at 667 nm, i.e. in a very close vicinity of 670 nm. This means the effective excitation of PL of the dye-sensitizer in the visible spectrum does not always evidence the effective excitation of molecular oxygen, i.e. the effective generation of singlet oxygen. Therefore, the common opinion that a PDT dye has to have an absorption band in the "windows of transparency" of typical tissues (600–900 nm) for the best one-photon PDT activation is not undisputable.

Main transitions involved in the formation of spectra shown in Figs. 1 and 2 and in the process of singlet oxygen photosensitization are summarized in Fig. 3.

On the left, we present the Jablonski diagram adapted to MPP-a with regard for the assignment of transitions in similar pheophorbide derivatives [4, 5]. The transition from the S_0 ground state of MPP-a molecule to the S_2 excited state forms the so-called Soret band at 403 nm in absorption and excitation spectra.

The $Q_y(0, 0)$ absorption transition from the S_0 ground state of MPP-a molecule to the S_1 excited state forms the band at 670 nm in absorption and excitation spectra. Back transitions from the excited S_1 state to the S_0 ground state contribute to the red photolumenscence of MPP-a. Three weak absorption peaks at 503, 535, and 615 nm (Fig. 1) and the corresponding peaks at 503, 538, and 616 nm in the excitation spectra (Fig. 2) may be assigned to $Q_x(0, 1)$, $Q_x(0, 0)$, and $Q_y(0, 1)$ transitions not shown in Fig. 3 [4].

The right side of Fig. 3 displays the triplet ground state ($^3O_2$) and the lowest singlet excited state ($^1O_2$) of molecular oxygen. The $^1O_2$ state is populated by the energy transfer from the triplet T_1 state of MPP-a molecule. The rate of $^1O_2$ generation depends both

on the rate of energy transfer and the T_1 state population. The T_1 state of MPP-a can be populated by the intersystem crossing (ISC) from S_1 and S_2 states. In addition, the T_1 state may be efficiently populated due to transitions from the T_2 state that may be populated, in turn, by ISC from the S_2 state. From the comparison of excitation spectra of the PL of MPP-a in the visible and sensitized emission of singlet oxygen, it follows that the probability of transitions $S_1 \rightarrow S_0$ is significantly greater than that of $S_1 \rightarrow T_1$.

3. Conclusion

The effective excitation of PL of the dye-sensitizer in the visible spectrum does not always evidence the effective excitation of molecular oxygen. The efficiency of the singlet oxygen generation cannot be directly connect with the intensity of visible photoluminescence of the dye-sensitizer. A choice of the dye-sensitizer excitation band to generate singlet oxygen with regard for only the dye-sensitizer PL intensity may be incorrect. The selection of an excitation wavelength for the efficient $^1O_2$ generation should be based on direct measurements of the excitation spectra of sensitized $^1O_2$ emission.

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СПЕКТРИ ЗБУДЖЕННЯ СИНГЛЕТНОГО КИСНЮ
І ФОТОЛЮМІНЕСЦЕНЦІЯ МЕТІЛФЕОФОРБІДУ-а

Р е з ю м е

Досліджено кореляцію між інтенсивністю фотолюмінесцен-
ції барвника-сенсибілізатора метілфеофорбіду-а (MPP-a) і
ефективністю генерації синглетного кисню ($^1O_2$). Спектр
збудження емісії $^1O_2$ при 1270 нм має одну сильну сму-
гу, при 403 нм збігається зі смугою Соре MPP-a і тільки
слабкий пік при 667 нм практично збігається з самою ін-
тенсивною смугою спектра збудження MPP-a. Тому вибір
довжини хвилі збудження для ефективної генерації $^1O_2$ по-
винен бути заснований на прямих вимірах спектра збудже-
ння сенсибілізованої емісії $^1O_2$.