Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 146 (2015) 249–254

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Physicochemical properties of potential porphyrin photosensitizers for photodynamic therapy

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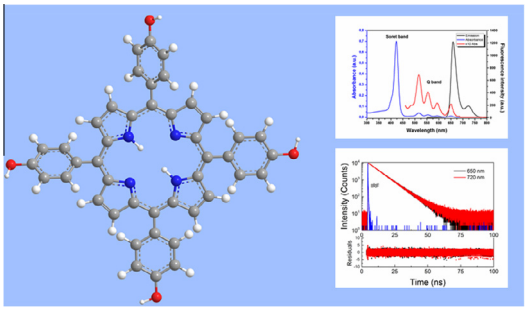
HIGHLIGHTS

- Measurements of absorption and emission spectra of photosensitizers.
- Quantum yields of fluorescence and singlet oxygen generation by dyes.
- Determination of the triplet state lifetime using laser flash photolysis.
- Determination of fluorescence lifetimes using TCSPC method.
- Comparison of results with commercial compounds.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 October 2014
Received in revised form 24 February 2015
Accepted 8 March 2015
Available online 12 March 2015

Keywords:
Photodynamic therapy
Porphyrin
Singlet oxygen
Fluorescence lifetime
Triplet state lifetime

ABSTRACT

This research evaluated the suitability of synthetic photosensitizers for their use as potential photosensitizers in photodynamic therapy using steady state and time-resolved spectroscopic techniques. Four tetraphenylporphyrin derivatives were studied in ethanol and dimethyl sulfoxide. The spectroscopic properties namely electronic absorption and emission spectra, ability to generate singlet oxygen, lifetimes of the triplet state, as well as their fluorescence quantum yield were determined. Also time-correlated single photon counting method was used to precisely determine fluorescence lifetimes for all four compounds. Tested compounds exhibit high generation of singlet oxygen, low generation of fluorescence and they are chemical stable during irradiation. The studies show that the tested porphyrins satisfy the conditions of a potential drug in terms of physicochemical properties.

Introduction

Porphyrins are naturally occurring organic compounds which are involved in a variety of important biological processes. The main element of their chemical structure is the porphyrin ring formed from four pyrrole molecules connected by methine bridges =CH— [1]. Depending on the modification of the ring by the lateral substituents, porphyrin derivatives exhibit different spectroscopic properties. All of these compounds have tendency to aggregate, what influences their physicochemical and spectroscopic properties. Porphyrins and their derivatives due to reduced lymphatic drainage, leaky vasculature, high number of LDL receptors, low interstitial pH and large interstitial space tend to favor accumulation in neoplastic lesions comparing to the surrounding normal

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http://dx.doi.org/10.1016/j.saa.2015.03.076
1386-1425/

tissue [2]. Since they are very effective chromophores of visible light have the ability to produce singlet oxygen and free radicals [3,4]. Due to these unique properties, porphyrins have been successfully used for photodynamic therapy (PDT).

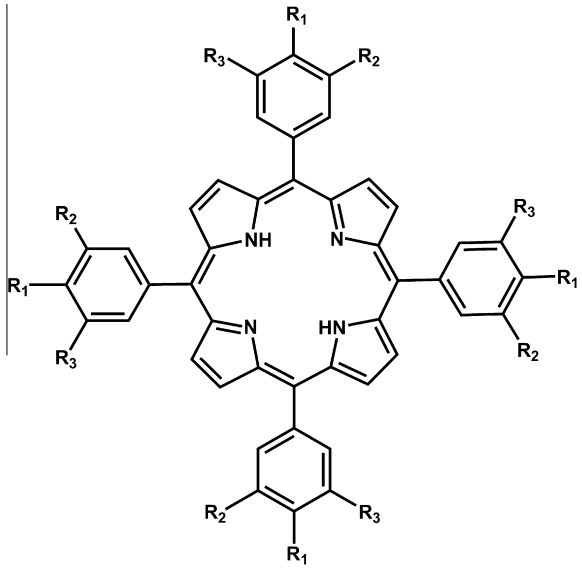
PDT is a successful treatment method for cancer and premalignant conditions that leads to the selective destruction of tumor through photodynamic process [5]. This requires a pro-drug to effectively accumulate in diseased tissues. Often a PDT compounds are called photosensitizers since they are activated by light of a particular wavelength corresponding to the absorption band at lowest energy. Subsequently, in the presence of molecular oxygen light induced reactions (photodynamic process) yield cytotoxic products that damage biologically crucial macromolecules and numerous intracellular structures and lead to the destruction of cancer tissue [6,7]. There are two mechanisms causes destruction of tumor cells in the process of oxidation of the biomolecules and leading to the formation of reactive oxygen species (ROS): free radicals (mechanism type I) and singlet oxygen (mechanism type II). Both are induced simultaneously and dominance depends on a variety of factors (including the type of photosensitizer, its concentration, and the concentration of molecular oxygen in the reaction medium) [8–10].

The desired therapeutic effect is related to a number of specific physical, chemical and biological properties of the photosensitizer [8,11]. An ideal photosensitizer should be chemically pure, stable, selectively accumulate in target tissue, have a short time interval between administration and maximum accumulation in the tissue and be rapidly cleared from the body after therapy. The maximum absorption of the photosensitizers should correspond to the optical window between 600 nm and 850 nm [8]. In this optical range tissue penetration is quite high and the energy of triplet state is sufficient enough for singlet oxygen production. It is also important that photosensitizers administered to the patient should have an amphiphilic nature. This ensures that the transportation in the circulatory system takes place without aggregation and with effective penetration through the lipid layer of cell membrane. Furthermore, the photosensitizers should be safe for the patient and their injection must not cause any toxic effects, allergic reactions or other side-effects. An important parameter of the photosensitizer due to its accumulation and subsequent removal from tissue is optimal fluorescence quantum yield. The fluorescence emission may be used to monitor these processes due to its high sensitivity. In order to succeed in the treatment, another key feature is high efficiency of the ROS generation. Most of today's commonly used dyes are far from being ideal [8–10,12,13].

Such specific requirements for potential photosensitizers used in PDT make difficult to find compounds which would satisfy all of them simultaneously. Furthermore, due to the different morphometric structures of various neoplasms (presence of connective tissue, tumor vasculature, etc.), a single dye cannot be effective for treating different types of cancer. Therefore, it is important to search for new photosensitizers which would have the desired properties for photodynamic therapy.

Depending on the environment in which the test compound is dissolved its photophysical properties may change. In most cases shift of the absorption bands, change in their shape, intensity and tendency to aggregate are observed. Determination of the effect of the environment on test compounds is essential in photodynamic therapy process. This allows to predict how the photosensitizer will behave in contact with other compounds in the cell [14,15].

The aim of the study was to determine the physicochemical characteristics of the four compounds from the porphyrin group as potential photosensitizers used for PDT. These compounds are derivatives of tetraphenylporphyrin (TPP): 5,10,15,20-tetrakis(3-hydroxyphenyl)-porphyrin (Porphyrin **1**); 5,10,15,20-tetrakis (4-hydroxyphenyl)-porphyrin (Porphyrin **2**); 5,10,15,20-tetrakis (3-methoxy-4-hydroxyphenyl)-porphyrin (Porphyrin **3**) and 5,10,15, 20-tetrakis(3,5dimethoxy-4-hydroxyphenyl)-porphyrin (Porphyrin **4**). Their structures are shown in Fig. 1. We evaluated the effect of different substituents and various polar environments (ethanol and DMSO) on spectroscopic parameters that we expect are crucial role for their PDT properties. In our previous publication we have estimated certain parameters in toluene, chloroform and methanol [16]. Additionally measured parameters and collected information allowed us to draw conclusions regarding the usefulness and effectiveness of tested compounds in photodynamic therapy. The obtained results will be compared against a commercial photosensitizer – TPP. This compound is not applied in photodynamic therapy, but it is widely used as a standard in conducting experiments regarding to determine the physicochemical properties of photosensitizers.



Porphyrin 1: R_1=H; R_2=OH; R_3=H
Porphyrin 2: R_1=OH; R_2=R_3=H
Porphyrin 3: R_1=OH; R_2=H; R_3= OCH_3
Porphyrin 4: R_1=OH; R_2=R_3= OCH_3
TPP: R_1=R_2=R_3=H

Fig. 1. Structures of tested porphyrins.

Experimental

Materials

Tested compounds are tetraphenylporphyrin derivatives and were synthesized at the Institute of Chemistry University of Silesia. Detailed description of the synthesis is presented in Rojkiewicz et.al. [16]. 5,10,15,20-tetraphenyl-21H,23H-porphyrin (TPP), phenalenone (Phe) and Ludox were purchased from Sigma Aldrich Company. Ethanol, dimethyl sulfoxide (DMSO) and toluene were obtained from POCH S.A.

Methods

Absorption, emission and excitation spectra

Absorption spectra were recorded using Hitachi UV–VIS spectrophotometer U-1900. Spectra were collected from 300 nm to 800 nm in 2 nm steps. Emission and excitation spectra were obtained using Hitachi F-7000 spectrofluorometer from 550 nm to 800 nm and 300 nm to 650 nm in 1 nm steps, respectively.