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Spectroscopical study of bacteriopurpurinimide–naphthalimide conjugates for fluorescent diagnostics and photodynamic therapy

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ABSTRACT

Two novel bis(chromophoric) dyads **ABPI–NI1** and **ABPI–NI2** containing 1,8-naphthalimide and bacteriopurpurinimide units linked by *p*-phenylene-methylene (**ABPI–NI1**) and pentamethylene (**ABPI–NI2**) spacers were prepared to test their ability to be used in the design of effective agents for both photodynamic therapy (PDT) and fluorescent tumor imaging. Photophysical studies revealed that the emission from the naphthalimide chromophore in both conjugates was partially quenched due to resonance energy transfer between the photoactive components. Compound **ABPI–NI2** with more sterically flexible oligomethylene group demonstrated higher fluorescence intensity as compared with that for **ABPI–NI1**.

1. Introduction

Cancer is known to be one of the deadliest diseases of our time and throughout the world. Current treatment modalities for patients afflicted by cancer include surgery, radiation therapy, chemotherapy, and a relatively novel option called photodynamic therapy [1,2]. Surgery is used to excise abnormal growths and surrounding tissue, but the procedure is invasive and may be complicated by relapse of the cancer if not all of the tumor cells are removed. Chemotherapy employs different cytotoxic chemicals to attack or block specific cellular and molecular mechanisms that aid tumor growth. Unfortunately, patients on chemotherapeutics suffer from side effects due to adverse drug toxicities. Radiation uses ionizing energy to attack neoplastic cells, but it is nonspecific and may cause damage to surrounding healthy tissue, which can lead to the occurrence of secondary cancers. PDT uses a drug known as a photosensitizer, light, and oxygen to destroy tumors and their surrounding vasculature. PDT has several advantages in that (i) there is no systemic, organ, or tissue toxicity, (ii) it is noninvasive, and (iii) it can be used repeatedly as a primary or adjuvant treatment [3,4].

Porphyrin-based photosensitizing agents for PDT possess unique advantages due to their ability to be retained in tumors and to produce cytotoxic singlet oxygen upon exposure to an appropriate wavelength of light. UV/Vis absorption spectra of porphyrins contains strong π–π^* transition around 400 nm (Soret band) and satellite Q-bands in the visible region between 600 and 800 nm, which coincidently drop into "therapeutic window" where tissues exhibit the best light penetration ability [2]. Therefore only Q-bands are useful for PDT. To date, many porphyrins have been obtained to test their efficiency in PDT and some of them are currently at different stages of clinical or preclinical trials [5–9].

In addition to therapy, the difference in concentration of the photosensitizer in malignant and normal tissue could be the basis of fluorescent imaging. Noninvasive in nature, fluorescent imaging instruments are simple and not expensive to operate and can allow precise assessment of the location and size of a tumor, providing information on its invasiveness in adjacent tissue [10,11]. Unfortunately, porphyrins and most of the tumor-avid long-wavelength photosensitizers (e.g. chlorins and bacteriochlorins) have very small wavelength differences between their NIR absorption and emission bands (Stokes shifts). Such an inherent property limits application of these molecules for imaging. To overcome this difficulty a new approach have been recently developed by Pandey

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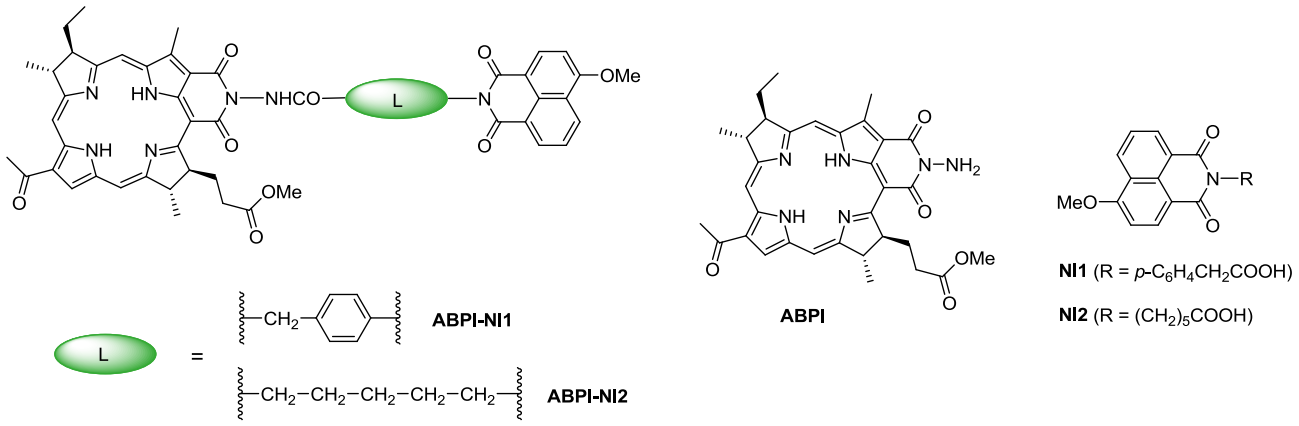

Scheme 1.

and co-workers [12,13], who offered the construction of tumor-specific imaging agents by conjugating of organic fluorophores with appropriate photophysical properties to porphyrin-based compounds. Thus, the excitation of the labeled fluorescent species is expected to produce the strong emission signal, which could be used to monitor tumor responses to treatment. Further excitation by an other light wavelength corresponding to the absorption maxima of porphyrin leads to formation of singlet oxygen responsible for cancer cell damage.

The work discussed herein describes the study of spectral properties of novel dyad compounds **ABPI–NI1** and **ABPI–NI2** (Scheme 1) bearing *N*-aminobacteriopurpurinimide (ABPI) and naphthalimide (NI) chromophores aimed to determine their ability to be used in the design of effective agents for both PDT and tumor imaging. Bacteriopurpurinimide type photosensitizer was chosen since the presence of fused imide cycle in the macrocyclic system is known to enhance pigment stability and result in the red shift of longwavelength Q_y-band in the absorption spectrum compared to chlorins and bacteriochlorins [14]. Yet, in spite of their promising optical properties **ABPI** and its derivatives exhibit high light-induced cytotoxicity on A-549 human adenocarcinoma cells [15]. As a basic fluorophore responded for the imaging effect we used 1,8-naphthalimide. Due to strong emission in the visible region, large Stokes shifts and excellent photostability compounds of this type are considered to be good candidates for the construction of fluorescent probes [16,17], organic electroluminescent devices [18], laser dyes [19] and optical brighteners [20]. Moreover, the utility of naphthalimides as fluorescent markers in cells has also been demonstrated [21,22].

2. Materials and methods

2.1. Synthesis of the compounds

For the synthesis of conjugates **ABPI–NI1** and **ABPI–NI2**, *N*-aminobacteriopurpurinimide **ABPI** was prepared from Bacteriochlorophyll *a* by following the literature procedure [23,24], which was afforded from the frozen biomass containing *Rhodobacter Capsulatus*. Reaction of **ABPI** with acyl chlorides derived from **NI1** and **NI2** gave the target compounds. The experimental details concerning the synthesis of **ABPI–NI1**, **ABPI–NI2** and naphthalimides **NI1** and **NI2** are described in Supplementary Information data file.

2.2. Steady-state and time-resolved optical measurements

The absorption spectra were taken on a Varian-Cary 5G spectrophotometer. The fluorescence quantum yield measurements were performed using a Varian-Cary 5G spectrophotometer and a FluoroMax-3 spectrofluorimeter. Spectral measurements were carried out in air-saturated solutions at 20 ± 1 °C. Acetonitrile (Aldrich) and dimethylsulfoxide (Spectro) of spectrophotometric grade were used. The concentrations of studied compounds were of about 0.5–1.0 × 10^{-5} M. All measured fluorescence spectra were corrected for the nonuniformity of detector spectral sensitivity. Quinine sulfate in 1*N* H_2SO_4 (φ^{fl} = 0.55) [25] and Coumarin 481 in acetonitrile (φ^{fl} = 0.08) [26] were used as a reference for the fluorescence quantum yield measurements. Fluorescence lifetimes were determined according to the method described earlier [27].

2.3. Computational details

The three dimentional structures of conjugates **ABPI–NI1** and **ABPI–NI2** were built with MOPAC program package using PM6 semiempirical method [28]. The calculations were performed at optimized geometries, which reached gradient variations less than 0.01 kcal/mol. The solvent effect was included in geometry optimizations following the «COnductorlike Screening Model» (COSMO) implemented in MOPAC. A dielectric constant of ε = 40 and a refraction index of solvent (n) such that n^2 = 2 were used.

3. Results and discussion

The conjugates of bacteriopurpurinimide derivatives with the fluorescent dyes, in which the linker groups separate two photoactive components, can be considered as bis(chromophoric) systems, where the optical characteristics of the individual chromophores might be pertrubed to some extent as a result of mutual energy/electron transfer process or exciplex formation originating from the feasible stereoelectronic properties of the excited state. Regarding the application of conjugates in PDT, one could reveal that the occurrence of such additional deactivation pathways, non-radiative in nature, would impair singlet oxygen sensitization or, alternatively, quench the emission of the fluorophore dye.

In order to avoid the decrease in emission efficiency of naphthalimide unit in the designed conjugates caused by fluorescence resonance energy transfer (FRET) to bacteriopurpurinimide, we followed the rule, according to which the FRET-process could occur whenever the emission spectrum of a donor fluorophore (naphthalimide) overlaps with the absorption spectrum of acceptor (**ABPI**) [29]. As it can be seen from Fig. 1, the absorption sectrum of **ABPI** in acetonitrile contains the interval between 440 and 520 nm, where the absorption values are relatively low. At the same time, it is well-known that photophysical properties of 1,8-naphthalimides are strongly dependent on the nature of the substituent at C-4 involved in the charge transfer interaction with carboximide moiety and, in general, the derivatives with alkoxy