chemistry proceedings

Synthesis of light-harvesting antenna dyads as phototherapeutic agents

M. Eugenia Pérez, M. Elisa Milanesio and Edgardo N. Durantini

IDAS-CONICET, Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, X5804BYA Río Cuarto, Córdoba, Argentina

Abstract: Photodynamic inactivation (PDI) has been proposed as an alternative therapy to combat bacterial infections. This therapy combines a photosensitizer, visible light, and oxygen to produce reactive oxygen species (ROS), which lead to cell death. In this sense, porphyrins are interesting photodynamic agents, however, they present low absorption in the phototherapeutic window. Therefore, the design of new dyads combining tetrapyrrolic macrocycles and light-harvesting antennas can be useful to increase the absorption in visible region.

In this study, two dyads, P-BDP and ZnP-BDP, were synthesized containing a BODIPY unit linked to a free-base porphyrin and its complex with Zn(II) via a 1,3,5-triazine group. The BODIPY moiety in the dyads acts as a light-harvesting antenna, which produces an intramolecular energy transfer to the porphyrin unit. In addition, the Zn(II) in the tetrapyrrolic macrocycle increases the intersystem crossing, favoring the generation of ROS. The absorption spectrum of P-BDP and ZnP-BDP resulted in a linear combination of the spectra of the corresponding monomers. The fluorescence spectra showed a strong decrease in the BODIPY emission along with the increase in the porphyrin unit emission, indicating a deactivation (>99%) of the BODIPY singlet state by porphyrin. The photodynamic activity of both dyads is enhanced with respect to that of their porphyrin monomers. Thus, the new dyads present interesting properties to act as phototherapeutic agents.

1. Introduction

Photodynamic therapy (PDT) represents a modality for the treatment of malignant tumors [1]. This technique is currently being extended to several non-cancerous illnesses, especially those characterized by unwanted cell overgrowth such as various dermatological diseases, benign prostatic hyperplasia, and age-related muscle degenerations. In the last few decades, research and development in this field have allowed for the use of photosensitizers (PSs) in the treatment of infectious diseases. This methodology is called the photodynamic inactivation (PDI) of microorganisms. In this sense, PDI could become a tool solve these types of health problems. PDI therapy is based on three fundamental stages: the preferential accumulation of the PS in the microbial cells, the illumination of the affected area to generate reactive oxygen species (ROS), and the reaction of these intermediates with the macromolecules of the cells, thus causing lethal damage [1].

A variety of macrocycles derived from porphyrins, chlorines, bacteriochlorins, and phthalocyanines have been proposed as potential phototherapeutic agents [1]. In particular, the use of porphyrins has three main advantages: chemical stability due to their aromatic structure, and low toxicity in the dark. Porphyrin synthesis is of utmost importance because it involves a wide spectrum of scientific applications [1]. Usually, porphyrins show poor absorbance in the blue-green region of 450–550 nm, whereas BODIPYs have excellent absorbance and thus may serve as a supplementary pigment for light harvesting, since the excitation energy transfer from BODIPY to porphyrin is often quite efficient [2]. To improve their light-harvesting potential, porphyrins can be derivatized with highly absorbing antenna chromophores, which act as sensitizers of the porphyrin-based excited state. The boron dipyrrin, BODIPY, chromophore is particularly

effective as an antenna group for porphyrins as it combines a high fluorescence quantum yield, a relatively long lifetime, a suitable excited state energy, and excellent photostability [2]. BODIPYs can be covalently bound to porphyrins throughIn the use of cyanuric chloride as a bridging unit. In this work, we describe the facile synthesis of BODIPY-porphyrin dyads using cyanuric chloride as the linker [2].

2. Materials and Methods

2.1. Equipment and chemical substances

Proton nuclear magnetic resonance spectra were achieved on a FT-NMR Bruker Avance DPX400 spectrometer (Bruker BioSpin, Rheinstetten, Deutschland). Absorption spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan), while fluorescence spectra were carried out on a Spex FluoroMax spectrofluorometer (Horiba Jobin Yvon Inc, Edison, NJ, USA). Compounds from Sigma-Aldrich (Milwaukee, WI, USA) were used as received. Silica gel thin-layer chromatography (TLC) plates 250 microns were purchased from Analtech (Newark, DE, USA) and silica gel 60 (0.040-0.063 mm, 230-400 mesh) from Merck (Darmstadt, Germany).

2.2. Synthesis

BODIPYs **BDP1** and **BDP2** were synthesized according with the methodologies reported [3,4].

Porphyrin **P1**, **P2** and dyads **P-BDP** and **ZnP-BDP** were obtained as previously described [1,2].

2.3. Spectroscopic and photodynamic studies

Spectroscopic characterizations and photodynamic properties were determined in *N*,*N*-dimethylformamide (DMF) [1].

3. Results and Discussion

3.1. Synthesis of dyads P-BDP and ZnP-BDP

In this study, two dyads, P-BDP and ZnP-BDP, were synthesized containing a BODIPY unit linked to a free-base porphyrin and its complex with Zn(II) via a 1,3,5-triazine group. Chlorine substitution can be temperature controlled to operate in a staggered manner [5]. In this way, the mono-substitution of chlorine occurs below 0 °C, the di-substitution occurs at room temperature and the tri-substitution occurs above 60 °C. First, an amino-porphyrin was bound to 1,3,5-triazine in 80% yield. In a second step, this structure was reacted with an amino-BODIPY to obtain a dyad (25%). Finally, the third chlorine atom of the triazine unit was substituted by *N*,*N*-dimethylethylenediamine in THF (98%).

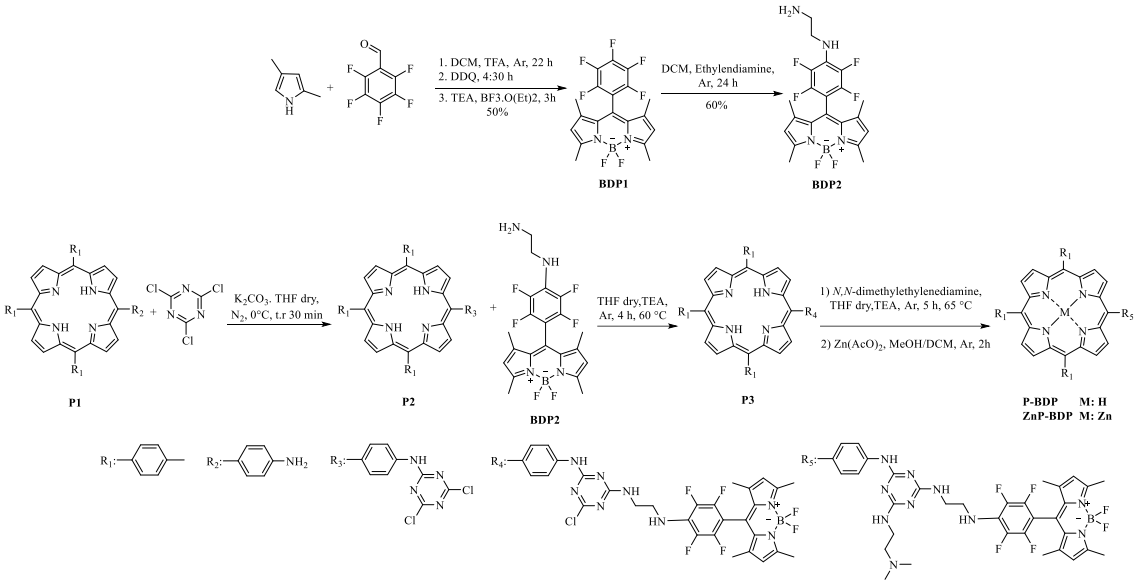

Figure 1. Synthesis of dyads **P-BDP** and **ZnP-BDP**.

3.2. UV-visible absorption spectroscopic properties of dyads P-BDP and ZnP-BDP

The absorption spectrum of P-BDP and ZnP-BDP resulted in a linear combination of the spectra of the corresponding monomers (Table1). The spectrum of the P-BDP dyad consists of an intense *Soret* band around 420 nm that coincides with the spectrum of the reference porphyrin P2 and three *Q* bands of lower intensity between (λ= 530-675 nm), all of them characteristic of porphyrins free base. In addition, it presents a band of greater intensity at approximately 518 nm, which corresponds to the transition to the first excited state (S_0-S_1), and a band of less intensity around 480 nm, associated with the vibrational structure, which are characteristic of the BODIPY fragment of P-BDP. The absorption spectrum of ZnP-BDP consists of an intense *Soret* band around 427 nm, and two *Q* bands of lower intensity between (λ= 560-600 nm), that coincides with the spectrum of the reference porphyrin ZnP2, and a band at approximately 518 nm characteristic of the BODIPY fragment. The fluorescence spectra showed a strong decrease in the BODIPY emission along with the increase in the porphyrin unit emission, indicating a deactivation (>99%) of the BODIPY singlet state by porphyrin. The photodynamic activity of both dyads is enhanced with respect to that of their porphyrin monomers. Thus, the new dyads present interesting properties to act as phototherapeutic agents.

Table 1. Spectroscopic and photodynamic properties in DMF.

PS	Absorption λ_{max} (nm)	Fluorescence λ_{max} (nm)	Φ_{Δ}[a]
P-BDP	420 514 554 598 653	524 656 720	0.40±0.04
P2	420 517 559 598 653	656 720	0.45±0.04
BDP1	514	528	0.01±0.001
ZnP-BDP	427 514 561 603	524 610 661	0.76±0.07
ZnP2	427 561 603	618 661	0.48±0.04

[a] quantum yield of $^1O_2$.

4. Conclusions

The BODIPY moiety in the dyads acts as a light-harvesting antenna, which produces an intramolecular energy transfer to the porphyrin unit. In addition, the Zn(II) in the tetrapyrrolic macrocycle increases the intersystem crossing, favoring the generation of ROS. In turn, the tertiary amine in the triazine structure can acquire a positive charge at physiological pH by increasing binding to microbial cells.

The UV-visible absorption spectrums of **P-BDP** and **ZnP-BDP** are essentially a linear combination of the spectra of the monomeric units that make it up. The fluorescence emission of BODIPY is strongly deactivated when it is part of the dyad. The intensity of fluorescence in the porphyrin bands increases. This result shows a strong effect of EnT from BODIPY towards the tetrapyrrolic porphyrin macrocycle.

The photodynamic activity of the **P-BDP** and **ZnP-BDP** dyad indicates that it is capable of efficiently producing $^1O_2$ ($^1\Delta_g$) therefore, there is a significant contribution of Type II mechanisms to the photodynamic action in DMF. It is enhanced with respect to **P2** and **ZnP2** due to the antenna effect exerted by the BODIPY unit. This effect allows lower concentrations of photosensitizer to be used to produce a desired result, which is beneficial for PDI applications.

References

1. Pérez, M.E.; Durantini, J.E.; Reynoso, E.; Alvarez, M.G.; Milanesio, M.E.; Durantini, E.N. Molecules, 2021, 26, 5877.
2. Lazarides, T.; Charalambidis, G.; Vuillamy, A.; Réglier, M.; Klontzas, E.; Froudakis, G.; Kuhri, S.; Guldi, D. M.; Coutsolelo A. G. Inorg. Chem. 2011, 50, 8926-8936.
3.
Scanone, A. C.; Santamarina, S. C.; Heredia, D. A.; Durantini, E. N.; Durantini, A. M. ACS Appl. Bio Mater. 2020, 3, 1061−1070
4. Vives, G.; Giansante, C.; Bofinger, R.; Raffy, G.; Del Guerzo, A.; Kauffmann, B.; Batat, P.; Jonusauskas, G.; McClenaghan N. D. Chem. Commun., 2011, 47, 10425–10427
5. Grzegorz, B. Tetrahedron 2006, 62, 9507-9522.