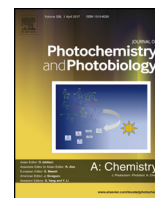




Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Invited paper

Phototransformation of cyanine dye with two chromophores. Effects of oxygen and dye concentration



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ARTICLE INFO

Article history:

Received 22 April 2017

Received in revised form 29 July 2017

Accepted 28 August 2017

Available online 30 August 2017

Keywords:

Cyanine dye with two chromophores

Phototransformation

Visible light

Effect of dye concentration

Effect of oxygen

Structure of photoproduct

ABSTRACT

Phototransformation of a cyanine dye with two chromophores (biscyanine dye, BCD) subject to visible light in phosphate buffer was studied using optical absorption and fluorescence spectroscopic techniques. The effects of the dye concentration and molecular oxygen were demonstrated. The monoexponential dependence of optical absorption of the BCD solution on the irradiation time was observed. The increase of the characteristic phototransformation time with the dye concentration is explained in terms of the dye aggregation. The explanation is confirmed by experiments in the presence of SDS micelles, where the concentration effect is absent, since at binding with SDS micelles BCD does not aggregate. The dye phototransformation rate increase in the presence of oxygen is explained in terms of singlet oxygen formation due to energy transfer from the dye excited triplet state to molecular oxygen. Singlet oxygen was detected, and its quantum yield was determined by an indirect chemical method. The effect of singlet oxygen on the BCD phototransformation was confirmed by reduction of its phototransformation rate in the presence of 2,5-dimethylfuran, a known singlet oxygen water soluble quencher. Singlet oxygen may destroy the π -conjugation of the BCD chromophores. A possible structure of the dye photoproduct is proposed based on the similarity of its spectral characteristics and those of monochromophoric cyanine dye and confirmed by quantum chemical modelling.

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1. Introduction

Cyanine dyes are widely applied in biology as absorption and fluorescence probes due to their high optical absorption and intensive fluorescence in the visible spectrum region and high affinity to cell structures [1,2]. Cyanine dye absorption spectra can easily be shifted to red and near infrared by modifying their chemical structure, for instance, by increasing the π -conjugated chromophore chain length. This stimulates the interest to cyanine dye application as photosensitizers (PS) in photodynamic therapy

(PDT) of cancer and as fluorescence probes (FP) in cancer fluorescence diagnostics (photodynamic diagnostics, PDD) [3–6]. Unfortunately, a significant increase in the length of the π -conjugated chromophore chain responsible for shifting the optical absorption, at the same time increases the chain flexibility reducing fluorescence and triplet state quantum yields, and the chemical and photochemical stability of the molecule, as well. Moreover, photoisomerization specific for cyanine dyes [7–10] opens one more channel for the excitation energy dissipation, reducing excited state lifetimes and quantum yields even more and thus reducing the cyanine dye efficacy in PDT and PDD. The class of cyanine dyes with two chromophores linked by central heterocycle (biscyanine dyes, BCD) is free from these disadvantages, because the absorption spectra red shift is achieved due to the dipole–dipole chromophore interaction and electron tunneling through

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central heterocycle [11,12] rather than the increase of the chromophore chain lengths. BCD has molar absorption coefficients $\epsilon > 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ in the 600–700 nm range, high affinity to cell membranes and biomacromolecules [13,14], and high triplet state quantum yields [15]. Recently, high efficiency of BCD in photo-deactivation of B16F10 murine and C8161 human melanoma cells *in vitro* has been demonstrated [16], which considers BCD as promising compounds for PDT.

PS can be subject to phototransformation during PDT treatment. This process can compete with photodynamic outcome by decreasing PS concentration and hence its capability to induce cell death, thus reducing the treatment efficacy. In addition, the photoproducts may be toxic causing adverse reactions in the patient organism. On the other hand, since phototransformation occurs in parallel with the PDT process, it seems possible to control the PS phototransformation and/or formation of photoproducts during the treatment, preventing damages [17]. Deeper understanding of these processes is required justifying the study of the PS phototransformation.

The principal mechanism of PS photoactivity in PDT is associated with formation of active molecular oxygen excited state, singlet oxygen, which may, in turn, attack the PS molecule itself, thus inducing its transformation. Singlet oxygen formation at cyanine dye light excitation has been demonstrated in a number of studies and the transformation of the cyanine dye π conjugated chromophore chain due to singlet oxygen attack, formed at cyanine dye excitation has been demonstrated, as well [18–25]. However, for BCD this process has not yet been studied.

Another important factor, which can affect the PS phototransformation, is its aggregation, specific for the most of organic PS molecules in aqueous media. Aggregation reduces lifetimes and quantum yields of PS excited states, thus reducing their photoactivity and singlet oxygen production [26,27]. Aggregation increases with the compound concentration and, therefore, reduction of the PS photoactivity and its phototransformation rate may be expected with the PS concentration increase. On the other hand, aggregation may present alternative ways for PS phototransformation, such as photodimerization, etc., thus increasing the phototransformation rate.

In this work, we report on the study of the BCD phototransformation under visible light and accompany spectral changes as a function of the BCD concentration in phosphate buffer (PBS) in the presence and absence of oxygen. The study was conducted using optical absorption and fluorescence spectroscopy techniques. The singlet oxygen formation following BCD photoexcitation was demonstrated and its quantum yield was determined through relative chemical method. The effect of singlet oxygen on the BCD phototransformation was confirmed by reduction of its phototransformation rate in the presence of 2,5-dimethylfuran (DMF), a known singlet oxygen water soluble quencher [28]. The quantum chemical model was applied to elucidate observed changes in the BCD absorption spectrum. The observed reduction of the BCD phototransformation rate due to growth of its concentration was explained via its aggregation, the explanation being confirmed by the absence of this effect at BCD binding with SDS micelles where BCD does not aggregate.

2. Materials and methods

The bichromophoric cyanine dye used, benzo[1,2-*d*:4,5-*d'*] bithiazolium,2,6-bis[3-(1,3-dihydro-1-ethyl,3,3-dimethyl-2H-indol-2-ylidene)-1-propenyl]-3,7-diethyl (Fig. 1), is one of a series of BCD synthesized by Dr. Felix Mikhailenko at the Institute of Organic Chemistry of the Academy of Science of Ukraine and available from the GOSNIKHIMFOTOPROEKT collection. The BCD

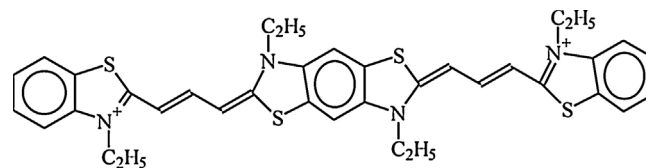


Fig. 1. Bichromophoric cyanine dye (BCD) structure.

purity degree was controlled by High Pressure Liquid Chromatography, optical absorption and fluorescence spectroscopy methods.

BCD stock solution was prepared in ethanol in concentrations controlled by optical absorption using molar absorption coefficient $\epsilon = 2.90 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at $\lambda = 633 \text{ nm}$ [13]. Experimental solutions were prepared by adding aliquots of the BCD stock solution into a particular volume of the phosphate buffer (7.5 mM, pH 6.8).

The solution was irradiated in a 1 cm length quartz cell at constant mixing by magnetic stirrer using a high-pressure xenon 75 W lamp with color glass filters with light transmission at $\lambda > 600 \text{ nm}$; a 2 cm length quartz cell filled with water was positioned in front of the test cell to prevent thermal effects.

To eliminate the oxygen effect, oxygen was removed from the cell by nitrogen bubbling. Phototransformation was monitored by solution absorption spectra at preset time intervals.

Singlet oxygen formation was confirmed, and its quantum yields (Φ_{Δ}) in ethanol and PBS were determined with a relative chemical method by addition of 1,3-diphenylisobenzofuran (DPBF) to ethanol and uric acid (UA) to buffer as singlet oxygen quenchers and methylene blue (MB) as the reference compound [29,30]. UA and DPBF degradation were controlled by absorption band reduction at 290 nm for UA and 416 nm for DPBF, respectively. Concentrations of the quenchers were reduced to $\sim 3 \times 10^{-5} \text{ M}$ to avoid chain reactions induced by them in the presence of singlet oxygen. PS solutions containing quenchers were prepared in the dark and radiated by a LED at 632 nm.

To confirm the singlet oxygen effect on the BCD phototransformation various aliquots of water soluble singlet oxygen quencher 2,5-dimethylfuran (DMF, quenching constant $k_q = 1.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) were added into the BCD solution.

To confirm the effect of aggregation on the BCD phototransformation, irradiation of BCD was realized in the presence of 20 mM of sodium dodecyl sulphate (SDS), forming micelles, since BCD bound with these micelles does not aggregate.

All reagents were analytical grade from Sigma-Aldrich. All experiments were performed at 24 °C.

Absorption spectra were monitored using Beckman Coulter DU 640 spectrophotometer, and fluorescence spectra were controlled by Hitachi F-7000 spectrofluorimeter.

Geometry optimizations in the quantum chemical model were made by the HF/3-21G model. Vertical electronic transitions were determined by the semi-empirical ZINDO1/S method [12]. All calculations were done using Gaussian 98 software [31].

3. Results and discussion

The BCD absorption spectrum in aqueous solutions at concentrations below 10^{-6} M is characterized by intense absorption within the 450–700 nm range with the maximum at 633 nm (Fig. 2A). In this concentration range, the BCD absorbance is proportional to its concentration, the profile of absorption spectra being virtually unchanged. The authors associate this spectrum with the dye monomeric form. At higher concentrations, the spectrum is deformed and a new absorption band appears with the maximum at 597 nm (Fig. 2A), which is associated with the formation of the dye aggregates [13]. The position of this absorption band in a wavelength region shorter than that of the

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