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Photoinduced electron transfer from electron donor to bis-carbocyanine dye in excited triplet state

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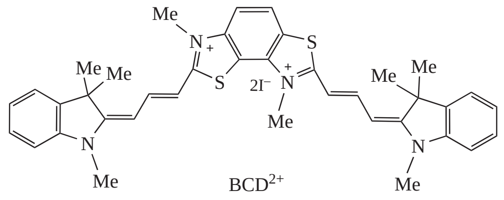
The photoinduced electron transfer from ascorbic acid as a donor to the dication of the bis-carbocyanine dye (BCD^{2+}) in its triplet state was studied by flash photolysis. The differential absorption spectrum of the $BCD^{\bullet +}$ radical cation was recorded and its decay kinetics was measured. Possible mechanisms for the deactivation of this radical were proposed.



BCD^{2+}

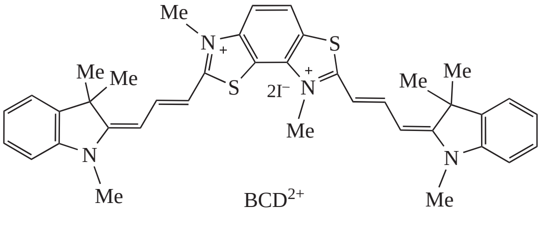
Keywords: photodynamic therapy, bis-carbocyanine dye, flash photolysis, triplet state, electron transfer.

Photodynamic therapy (PDT) is a promising method for the treatment of various diseases, which is already being used in clinics.[1–4] After photoactivation of a photosensitizer (PS), rapid electron transfer (type I reaction) and/or energy transfer (type II reaction) occur between the PS molecule and molecular oxygen. These reactions lead to the formation of cytotoxic reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radical anion ($O_2^{\cdot -}$) and hydroxyl radical ($OH^{\cdot}$).[5–7]

Due to their characteristics, bis-carbocyanine dyes (BCCs) are promising compounds for use in PDT, as they have an intense absorption band in the red spectral region [$\lambda > 600$ nm ($\varepsilon > 10^5$ dm^3 mol^{-1} cm^{-1})], which coincides with the spectral region of the phototherapeutic window.[8] Besides, BCCs have a high quantum yield of the triplet state, which is involved in electron transfer reactions,[9] providing an efficient generation of ROS. Due to their positive charge and relatively high hydrophobicity, BCCs can bind to biomacromolecules, such as DNA and proteins, which guarantees their efficient penetration and accumulation in cells.[10] BCCs have demonstrated high phototoxicity against cancer cells *in vitro*.[11]

Although the participation of BCC in electron transfer reactions has already been demonstrated,[9] the spectral characteristics and the decay kinetics of the resulting BCC radicals have not yet been described.

In this work, we studied the spectral and kinetic properties of a radical formed from 2,7-bis[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propen-1-yl]-3,8-dimethylbenzo-[1,2-*d*:3,4-*d*′]bisthiazolium iodide, a bichromophoric cyanine dye (BCD^{2+}), due to electron transfer from ascorbic acid (AA) to the dye molecule in its excited triplet state.[†]



BCD^{2+}

Upon the flash photoexcitation of 3×10^{-7} M solution of BCD^{2+}, a BCD^{2+} triplet state is formed, characterized by a differential absorption spectrum [Figure 1(*a*)]. In the absence of oxygen, the decay kinetics of the BCD^{2+} triplet state is monoexponential with a lifetime of 350 μs. The triplet state is quenched by oxygen ($k_qO_2 = 2.0 \times 10^9$ dm^3 mol^{-1} s^{-1}) and ascorbic acid (k_qAA = 2.4×10^9 dm^3 mol^{-1} s^{-1}).[13] The interaction with ascorbic acid is accompanied by a change in the differential absorption spectrum [see Figure 1(*a*)], which we associate with the electron transfer from AA to the BCD^{2+} molecule in its triplet state (Scheme 1).[‡]

$$BCD^{2+}(S_0) + h\nu \longrightarrow BCD^{2+}(S_1) \longrightarrow BCD^{2+}(T) \xrightarrow{+AA} BCD^{\cdot +} + AA^{\cdot +}$$

Scheme 1

The photoexcitation of BCD^{2+} in the presence of AA induces distortion of the singlet–singlet absorption spectrum of BCD^{2+}, which at high AA concentrations coincides with the spectrum of a monochromophoric cyanine dye (CD) with a chromophore length equal to the length of a single BCD^{2+} chromophore [Figure 1(*b*)].

[†] BCD^{2+}, synthesized as described,[12] was obtained from the collection of the former GosNIIKhimFotoProekt (the State Design and Scientific Research Institute of the Chemical and Photographic Industry, Moscow, Russia).

[‡] Absorption spectra were recorded using a Shimadzu UV-3101PC spectrophotometer in 1×1 cm quartz cells at room temperature. Propan-1-ol of spectroscopy grade and chemically pure ascorbic acid from Komponent-Reaktiv (Russia) were used. The triplet–triplet absorption spectra as well as the decay kinetics of triplet states and the radical cation were obtained by flash photolysis using quartz cuvettes with an optical path length of 20 cm and Xe lamp photoexcitation at 80 J for 15 μs. Optical glass filter OS-14 (transmission >580 nm) was used for direct photoexcitation in electron transfer experiments. Registration was carried out using a PMT-38 photomultiplier (MELZ, USSR) in the range of 400–700 nm. The extinction coefficients of the BCD^{2+} triplet state and the $BCD^{\cdot +}$ radical cation were calculated by the singlet depletion method. The diffusion rate constant was calculated as described.[18,19] All the solutions were degassed before use.

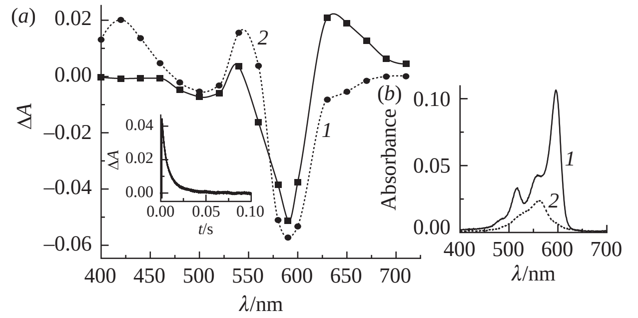


Figure 1 (*a*) Differential triplet–triplet absorption spectra of 3×10^{-7} M solution of BCD^{2+} in propan-1-ol at 200 μs after the flash photoexcitation (*1*) in the absence and (*2*) in the presence of 1×10^{-2} M ascorbic acid. The inset shows the decay kinetics of the $BCD^{\cdot+}$ radical cation at 420 nm in 6×10^{-7} M solution of BCD^{2+} in the presence of 1×10^{-2} M ascorbic acid. (*b*) Absorption spectra of 4×10^{-7} M solution of BCD^{2+} in propan-1-ol after photolysis (*1*) in the absence and (*2*) in the presence of ascorbic acid.

The interaction of chromophores in bichromophoric dyes causes a splitting of the energy levels of the singlet state of the dye. As a result, two absorption bands appear in the optical absorption spectrum of the dye, one of which is shifted towards the blue and the other towards the red, in comparison with the corresponding monochromophoric dye.[8] The observed changes in the absorption spectrum of BCD^{2+} indicate that after recombination of the dye radical, one of its chromophores appears to be destroyed, probably due to a break in the π-conjugation chain. This disrupts the interaction between the BCD^{2+} chromophores, and only one 'surviving' chromophore is responsible for the absorption spectrum. A similar effect was observed in the phototransformation of one of the BCCs in the presence of molecular oxygen,[14] where the break in the chromophore π-conjugation chain was caused by the reaction with singlet oxygen formed by energy transfer from the same BCC molecule in the triplet state to molecular oxygen. In this work, oxygen was removed from the solution *in vacuo*, so the observed effect can only be associated with the reaction between the $BCD^{\cdot+}$ radical cations.

The molar absorption coefficients for the BCD^{2+} triplet state ($\varepsilon_{630} = 9.6 \times 10^4$ dm^3 mol^{-1} cm^{-1}) and the $BCD^{\cdot+}$ radical cation ($\varepsilon_{420} = 7.8 \times 10^4$ dm^3 mol^{-1} cm^{-1}) are calculated as the limits at the maximum singlet–singlet absorption bleaching, taking into account that for the singlet–singlet absorption ε_{590} is 2.5×10^5 dm^3 mol^{-1} cm^{-1}.

The decay kinetics of the $BCD^{\cdot+}$ corresponds to the second order reaction [see Figure 1(*a*), inset]. This process cannot be caused only by the reverse electron transfer between the formed $BCD^{\cdot+}$ and $AA^{\cdot+}$ radicals (Scheme 2).

$$BCD^{\cdot+} + AA^{\cdot+} \longrightarrow BCD^{2+} (S_0) + AA$$

Scheme 2

In a completely reversible recovery process, both the dye and the donor should be present in the solution at the initial concentrations, and the absorption spectrum should be unchanged. The fact of changes in the optical absorption spectrum indicates the existence of some other mechanism of $BCD^{\cdot+}$ decay kinetics, in addition to the reverse electron transfer.

Since the BCD^{2+} molecule carries two positive charges in its structure, its radical cation possesses a charge of +1 and can accept one electron. Therefore, we believe that a reaction between two $BCD^{\cdot+}$ radicals, accompanied by irreversible destruction of the dye chromophore (Scheme 3), may also occur.

However, we cannot exclude some other processes that may be responsible for the $BCD^{\cdot+}$ radical decay. Among them, we should mention the dimerization of the dye. This process, described for monochromophoric cyanine dyes,[15] leads to the rupture of the chromophore π-conjugation chains of both radicals that participate in the reaction. It should exclude the conjugation of the two residual chromophores. Moreover, the distance between these chromophores should be large enough, reducing the dipole–dipole interaction between them. Therefore, in the case of bichromophoric dyes, the absorption spectrum of the dimer formed during radical dimerization should be close to that of the individual chromophore.

$$BCD^{\cdot+} + BCD^{\cdot+} \longrightarrow BCD^{2+} (S_0) + CD (S_0)$$

Scheme 3

The experimental decay kinetics constant $2k$ for $BCD^{\cdot+}$ is 1.3×10^9 dm^3 mol^{-1} s^{-1}. The calculated diffusion rate constant k_{diff} for propan-1-ol at room temperature is 3.4×10^9 dm^3 mol^{-1} s^{-1}. The results show that the decay kinetics of $BCD^{\cdot+}$ is practically controlled by diffusion, which indicates its high efficiency.

The generation and decay of the $BCD^{\cdot+}$ radical cation were investigated. The interaction between the radical cation and molecular oxygen can lead to the formation of cytotoxic superoxide anion.[16,17] The capability of the BCD^{2+} molecule to act as a PS *via* an electron transfer mechanism may be important in subsequent detailed studies of PDT mechanisms.

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