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Photochemical properties of new bis-cyanine dye as a promising agent for *in vivo* imaging

Alexey A. Kostyukov,*[a] Anton E. Egorov,[a] Mikhail G. Mestergazi,[a,b] Anna M. Shmykova,[b] Tatyana A. Podrugina,[b] Iouri E. Borissevitch,[c,d] Alexander A. Shtil[a,b] and Vladimir A. Kuzmin[a]

[a] *N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 119334 Moscow, Russian Federation. Fax: +7 499 137 4101; e-mail: aak@sky.chph.ras.ru*

[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

[c] *Departamento de Física, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-901, Brazil*

[d] *Instituto de Física, Universidade Federal de Goiás, Goiânia, GO 74001-970, Brazil*

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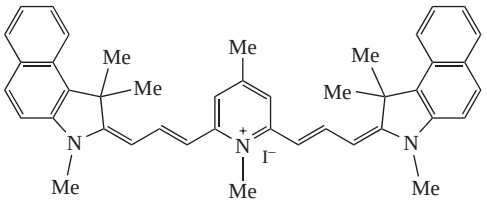
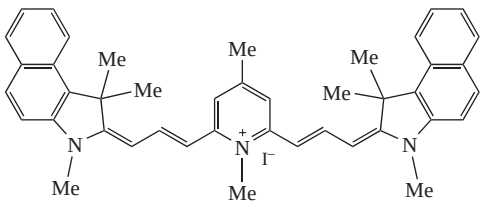
Photochemical properties of a new bis-cyanine dye have been investigated, including spectral kinetic characteristics of singlet and triplet excited states as well as affinity to BSA as a representative blood protein. The results obtained reveal, that the dye is a promising agent for imaging and fluorescent labeling of biomacromolecules.



Keywords: cyanine dye, albumin, binding constant, triplet state, imaging, fluorescence lifetime.

Cyanine dyes (CDs) are widely used as drug delivery agents and fluorescent probes for angiography.[1–3] The advantage of CDs among other fluorophores originates from their ability to form complexes with albumin and accumulate in cancer cells due to their increased metabolism.[4] CDs with fluorescence emission in the NIR range find an application as fluorescent probes because of the low NIR background emission of biomolecules. Conjugation of CDs with porphyrins is beneficial for the design of dual-function agents for theranostics, in these systems porphyrin moiety acts as a photosensitizer, while the CD one is used for fluorescence imaging.[5–8] Note, that CDs themselves can be used as photosensitizers for photodynamic therapy.[9–11] Dyes of bis-carbocyanine class (BCC) are particularly promising, since their structure contains a system of two conjugated chromophores,[12] for which the shift of absorption band to the NIR range is a result of an electron tunneling effect through the central heterocycle and a dipole–dipole interaction between the chromophores.[13,14]



BCD

In this work, a representative of BCC, namely 1,4-dimethyl-2,6-bis-[(1*E*,3*E*)-3-(1,3,3-trimethyl-1,3-dihydro-2*H*-benzo[*e*]indol-2-ylidene)prop-1-en-1-yl]pyridinium iodide (BCD), was synthesized, for the preparation details see Online Supplementary Materials. This dye has a *para*-dimethylpyridine moiety included in a polymethine chain with two benzoindole chromophores capping the chain at both sides. BCD, like other BCC dyes, has a system of two conjugated chromophores.[15] It has an absorption peak at ~670 nm, unlike monochromophoric cyanine dyes having three carbon atoms between two heterocycles, where the corresponding absorption band is at ~550 nm.[16] It is important to investigate the photochemical characteristics of this structural type of dyes with a pyridine moiety, in line with the reported BCC dyes having benzobisthiazole heterocyclic moiety at the center of their structure.[16,17]

In the BCD–albumin complex formation, a dye molecule incorporates into the hydrophobic pocket of the protein, and as a result, the more rigid structure of BCD is formed. Therefore, the contribution of vibrational relaxation in BCD is lowered, which leads to a more than sixfold increase in the fluorescence quantum yield. The value of an equilibrium binding constant for the complex with albumin K_b was found to be 1.5×10^5 dm^3 mol^{-1} (Figure 1),[†]

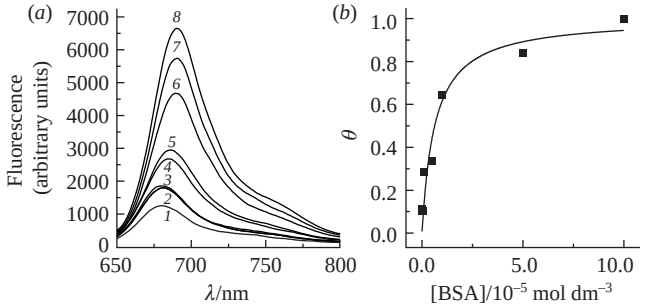


Figure 1 (*a*) Fluorescence spectra of 5×10^{-7} M solution of BCD at different albumin concentrations: (*1*) 0, (*2*) 5×10^{-8}, (*3*) 1×10^{-7}, (*4*) 5×10^{-7}, (*5*) 1×10^{-6}, (*6*) 5×10^{-6}, (*7*) 1×10^{-5} and (*8*) 5×10^{-5} mol dm^{-3}. (*b*) Fraction θ of bound BCD dye *vs.* albumin concentration.

[†] Equilibrium binding constant K_b for the BCD–albumin complex formation was obtained from fluorescence measurements at λ_{em} = 690 nm using the following equation:

$$F - F_0 = F_{max}[BSA]/(K_b + [BSA]),$$

which was typical for cyanine dyes.[17,19] The fluorescence lifetime of BCD in phosphate buffer saline was 0.2 ns, while for the rigid structure of BCD–albumin complex an increase in the lifetime up to 1.7 ns was observed.

Under direct photoexcitation of BCD in *n*-propanol, the triplet state formation was not detected, which indicated a higher rate of internal conversion to the ground state compared with the intersystem crossing rate. Taking into account the experimental setup sensitivity, the BCD triplet state quantum yield φ_T was estimated to be $\leqslant 10^{-4}$,‡ which allowed one to suggest an absence of photodegradation through the triplet state.

As a result, it may be concluded that BCD reveals high photostability under the direct red light photoexcitation.

Triplet state of BCD was detected by the triplet–triplet energy transfer method using the anthracene triplet as energy donor with $E_T = 14700$ cm^{-1} as the following. UV-A excitation (320–380 nm) of anthracene and BCD solution in *n*-propanol resulted in the triplet–triplet energy transfer accompanied by bleaching of the main BCD absorption band at 500–680 nm as well as the formation of BCD triplet state with absorption band at 700–1000 nm (Figure 2). The value of the triplet–triplet energy transfer constant in *n*-propanol $k_{TT} = 2.2 \times 10^9$ dm^3 mol^{-1} s^{-1} was close to the value for the diffusion constant, taking into account spin-statistical factor. This demonstrated efficiency of the energy transfer process in the liquid phase. The rate of the triplet state k_T was found to be 1.8×10^3 s^{-1}. Direct laser photoexcitation did not reveal photobleaching of BCD, which allowed one to conclude that the internal conversion process was faster than the light source setup resolution time (5 ns). In the presence of oxygen, the sensitized triplet state of BCD was quenched with the constant $k_q = 2 \times 10^9$ dm^3 mol^{-1} s^{-1}. Photoexcitation of degassed solution of the BCD–albumin complex (with 3×10^{-6} M and 2×10^{-4} M concentrations, respectively) in phosphate buffer saline did not lead to the triplet state formation as determined by an absence of intermediates in the triplet state absorption region.

Thus, despite a significant inhibition of the internal conversion process detected from the increase in fluorescence, there is no elevation of the quantum yield of intersystem crossing for the dye–albumin complex. In contrast to the BCC dyes with a benzobisthiazole central heterocyclic moiety, BCD does not generate a triplet state after direct photoexcitation. This can be explained by the greater tendency to fast photoisomerization for the BCD molecule due to the compact size of the central heterocyclic fragment as well as the efficient vibrational relaxation.

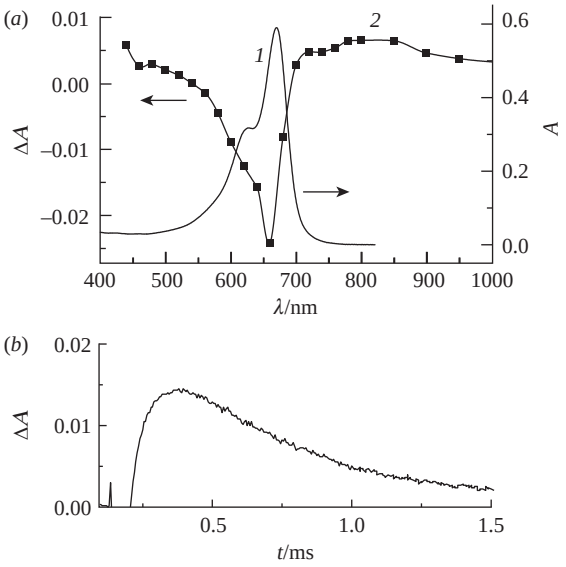


Figure 2 (*a*) Singlet and triplet state absorption spectra of BCD: (*1*) absorption spectrum of 3×10^{-6} M solution of the dye, (*2*) differential spectrum of transient species (triplet state) originated from 3×10^{-6} M solution of BCD in *n*-propanol in the presence of 1×10^{-5} M anthracene at 350 μs after the UV-A excitation flash. (*b*) Kinetics of triplet state decay at 720 nm.

The BCD triplet state can be generated by the triplet–triplet energy transfer from anthracene as a donor, thus the estimation of the upper limit for the triplet energy level is 14700 cm^{-1}. BCD molecules form noncovalent albumin complex with a high value of binding constant and an increase in fluorescence. Generally, the photostability, high affinity to albumin and the absence of triplet state formation for BCD under direct photoexcitation conditions along with high fluorescence of the dye–albumin complex make this compound a potential agent for imaging and fluorescent labeling of biomacromolecules.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.07.012.

References

1 A. Hagen, D. Grosenick, R. Macdonald, H. Rinneberg, S. Burock, P. Warnick, A. Poellinger and P. M. Schlag, *Opt. Express*, 2009, **17**, 17016.
2 T. Desmettre, J. M. Devoisselle and S. Mordon, *Surv. Ophthalmol.*, 2000, **45**, 15.
3 J. A. Cardillo, R. Jorge, R. A. Costa, S. M. T. Nunes, D. Lavinsky, B. D. Kuppermann, A. C. Tedesco and M. E. Farah, *Br. J. Ophthalmol.*, 2008, **92**, 276.
4 A. S. Tatikolov, *J. Photochem. Photobiol., C*, 2012, **13**, 55.
5 K. A. Zhdanova, A. V. Ezhov, N. A. Bragina and A. F. Mironov, *Mendeleev Commun.*, 2018, **28**, 626.
6 A. F. Mironov, K. A. Zhdanova and N. A. Bragina, *Russ. Chem. Rev.*, 2018, **87**, 859.
7 N. S. James, P. Joshi, T. Y. Ohulchanskyy, Y. Chen, W. Tabaczynski, F. Durrani, M. Shibata and R. K. Pandey, *Eur. J. Med. Chem.*, 2016, **122**, 770.
8 N. J. Patel, Y. Chen, P. Joshi, P. Pera, H. Baumann, J. R. Missert, K. Ohkubo, S. Fukuzumi, R. R. Nani, M. J. Schnermann, P. Chen, J. Zhu, K. M. Kadish and R. K. Pandey, *Bioconjugate Chem.*, 2016, **27**, 667.
9 L. Jiao, F. Song, J. Cui and X. Peng, *Chem. Commun.*, 2018, **54**, 9198.

where [BSA] was the concentration of bovine serum albumin, F_0 was the fluorescence intensity of BCD in the absence of albumin, F was the intermediate BCD fluorescence intensity at different albumin concentrations and F_{max} was the fluorescence intensity of BCD completely bound with albumin.[18] The K_b value was calculated using the hyperbolic extrapolation of experimental data employing an OriginPro 2015 software.

‡ Absorption spectra were recorded using a Shimadzu UV-3101PC spectrophotometer (Japan) in 1 × 1 cm quartz cells. Fluorescence spectra were obtained and the measurements of fluorescence lifetime carried out employing a FluoTime 300 spectrometer (PicoQuant, Germany). *n*-Propanol of spectroscopy grade from Komponent-Reaktiv (Russia) as well as phosphate buffer saline (pH 7.4) and BSA from Sigma-Aldrich (USA) were used. Transient species decay kinetics data and absorption spectra for excited states 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 filters OS-14 and SZS-26 (580–750 nm transmission) were used in direct photoexcitation, and UFS-6 filters (320–380 nm transmission) were employed for the anthracene-to-BCD energy transfer experiments. Data were recorded in the range of 440–1000 nm using an OPT-101 photodiode (Burr-Brown Corporation, USA). All the solutions were degassed before use. Experiments in the nanosecond time range were carried out using an LKS80 laser flash photolysis setup (Applied Photophysics, UK) with photoexcitation (5 ns, $\lambda_{exc} = 665$ nm) by a Quantel Brilliant Nd:YAG laser (France) with a MagicPRISM module (Opotek, USA). The samples were purged with argon for 20 min before use.

10 B. Ciubini, S. Visentin, L. Serpe, R. Canaparo, A. Fin and N. Barbero, *Dyes Pigm.*, 2019, **160**, 806.
11 X. Yang, J. Bai and Y. Qian, *Spectrochim. Acta, Part A*, 2020, **228**, 117702.
12 F. A. Schaberle, V. A. Kuz'min and I. E. Borissevitch, *Biochim. Biophys. Acta, Gen. Subj.*, 2003, **1621**, 183.
13 F. A. Schaberle, S. E. Galembeck and I. E. Borissevitch, *Spectrochim. Acta, Part A*, 2009, **72**, 863.
14 C. Zhong, D. Bialas, C. J. Collison and F. C. Spano, *J. Phys. Chem. C*, 2019, **123**, 18734.
15 A. I. Kiprianov, *Russ. Chem. Rev.*, 1971, **40**, 594 (*Usp. Khim.*, 1971, **40**, 1283).
16 É. R. Silva, A. L. S. Pavanelli, L. B. Mostaço, F. A. Schaberle, S. E. Galembeck, P. J. Gonçalves, R. Costa e Silva, L. P. Ferreira, T. D. Nekipelova, A. A. Kostyukov, A. S. Radchenko, A. A. Shtil, V. A. Kuzmin and I. E. Borissevitch, *J. Photochem. Photobiol., A*, 2017, **349**, 42.
17 A. S. Radchenko, A. A. Kostyukov, A. A. Markova, A. A. Shtil, T. D. Nekipelova, I. E. Borissevitch and V. A. Kuzmin, *Photochem. Photobiol. Sci.*, 2019, **18**, 2461.
18 D. Phillips, *Prog. React. Kinet.*, 1997, **22**, 175.
19 V. A. Kuzmin, T. D. Nekipelova, T. A. Podrugina, G. V. Golovina, A. A. Kostyukov, V. V. Temnov, I. A. Doroshenko, E. V. Radchenko, V. A. Palyulin and N. S. Zefirov, *Photochem. Photobiol. Sci.*, 2016, **15**, 1377.

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