Photochemical investigation of the triplet state of 3,3′-diethylthiacarbocyanine iodide in the presence of DNA

M. Yu. Anikovsky, A. S. Tatikolov, L. A. Shvedova, and V. A. Kuzmin*

N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 4 ul. Kosygina, 119991 Moscow, Russian Federation. Fax: +7 (095) 137 4101. E-mail: deer@sky.chph.ras.ru

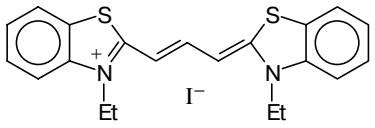
The spectral kinetic parameters of the triplet state of 3,3′-diethylthiacarbocyanine iodide (DTCI) in the presence of DNA were studied by pulse photolysis. The formation of DTCI complexes with DNA increases the quantum yield of the triplet state of the dye. Analysis of the absorption spectra of the ground and triplet states of DTCI at different DNA concentrations in a solution indicates the existence of two types of complexes. Complex formation decreases substantially the quenching rate constant of the triplet state of the dye by dioxygen.

Key words: cyanine dyes, DNA, triplet state, complex formation.

Many photobiological processes, in particular, photodynamic cancer therapy and several cutaneous diseases, are based on the interaction of dyes and related compounds with various biomacromolecules.[1] Complex formation of dyes from various classes with deoxyribonucleic acid (DNA) is of special interest.[2] The double DNA helix is capable of a special interaction with several complex organic molecules, which results in the formation of complexes with high binding constants (10^4—10^7 L mol^{-1}).[3] Acridine Orange, Methylene Blue, Bengal Rose, Ethidium bromide, Thiazole Orange, and other dyes presently find wide application in biochemistry and photobiology due to their capability of forming strong complexes with DNA (the energy being at least 42 kJ mol^{-1}).[3] These complexes are studied first *in vitro*. However, there are grounds to assume that they are also present in biological systems where nucleic acids exist in native states.[3] Presently, the interaction of various classes of dyes with DNA are under intensive studies.[4—7]

Complex formation of polymethine dyes with DNA have been discussed previously.[8—11] These dyes are widely distributed due to their application in laser systems,[12,13] nonlinear optics,[14] as molecular probes,[15] and biomedicine.[16,17] Cyanine dyes are characterized, as a rule, by a high extinction coefficient due to the full π-electron conjugation in the polymethine chain and possess a long-wave absorption maximum, whose position is varied within wide limits depending on the length of the polymethine chain. The lifetimes of the excited singlet state of cyanine dyes (tens and hundreds of picoseconds) and the corresponding quantum yields of fluorescence are low and can depend, to a great extent, on the temperature and viscosity.[18] On photoexcitation cyanine dyes can isomerize due to the rotation about the C=C bonds of the polymethine chain.[19]

The photochemical reactivity of dyes is determined to many respects by the properties of their triplet states. Under standard conditions, the quantum yield to the triplet state of most cyanine dyes is low.[20] However, the recent studies of 3,3′-diethylthiacarbocyanine iodide (DTCI) in the presence of DNA showed that the specific interaction of the double DNA helix with dye molecules increases the quantum yield of its triplet state.[21] This work is devoted to the spectral kinetic study of the triplet state of DTCI in the presence of DNA.



DTCI

Experimental

Absorption spectra of the dye and its complexes with DNA were recorded on a Shimadzu UV-1601 PC spectrophotometer in a cell with an optical path length of 1 cm. To study the triplet state of DTCI and to determine the quenching rate constants of the dye triplet by molecular oxygen, we used a pulse photolysis technique with excitation by a xenon lamp (energy 50 J, pulse duration at the half-height $\tau_{1/2}$ = 7 μs)[22] and a laser pulse photolysis technique with a dye laser, and a nitrogen laser was used as the excitation source (λ_{exc} = 337 nm, W = 0.8 mJ, $\tau_{1/2}$ = 1 ns).[23] The fluorescence and excitation spectra of the dye were studied on an Aminco—Bowman spectrofluorimeter with an R136 photoamplifier. DTCI (Aldrich), DNA from chicken purified by dialysis,[24] and recrystallized sodium anthracene-2-sulfonate were used. The concentration of DNA was determined from the extinction coefficient of a base pair ε = 6700 L mol^{-1} cm^{-1} at the wavelength 250 nm.[25] A phosphate buffer with pH 7 (concentration

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20 mmol L^{-1}) was used as the solvent. Solutions were deaerated on a vacuum setup. In experiments of the singlet state quenching by dioxygen, a manometric setup was used to determine the air pressure in the working cell.

Results and Discussion

The photochemical properties of DTCI in liquid solutions are well studied.[20,21] In an aqueous solution, this dye has the long-wave absorption maximum at λ_{max} = 560 nm (Fig. 1, *a*, *b*, spectra *1*). The contribution of fluorescence (the quantum yield is 0.026 in the phosphate buffer, pH 7) and intersystem crossing (quantum yield <0.001) to the deactivation of the dye in the excited singlet state is insignificant; the main channel of electron energy degradation is the internal conversion and isomerization (with a quantum yield of 0.25 in methanol[26]) of the initial *trans*-form into the *cis*-isomer. It is known that *trans*—*cis*-photoisomerization of carbocyanine dyes, which is one of the channels of nonradiative deactivation, occurs through the excited singlet state[27] and, according to the Rulliere scheme, has a common channel with internal conversion.[28]

Since at direct photolysis to the absorption band of the dye the signal corresponding to T—T absorption (T is the triplet state) is not observed, to study the properties of the T state of DTCI in an aqueous solution, its triplet levels were populated by energy transfer from the donor to the dye. Sodium anthracene-2-sulfonate was used as the donor (E_T = 14900 cm^{-1}).[29] The corresponding differential spectrum of the T—T absorption of DTCI in water (Fig. 2) is similar to the T—T absorption spectra of the dye in other solvents.[27] The kinetics of the T state deactivation obeys a first-order law with a rate constant of $7 \cdot 10^3$ s^{-1}. The quenching rate constant of the T state of DTCI by oxygen is $1.2 \cdot 10^9$ L mol^{-1} s^{-1}.

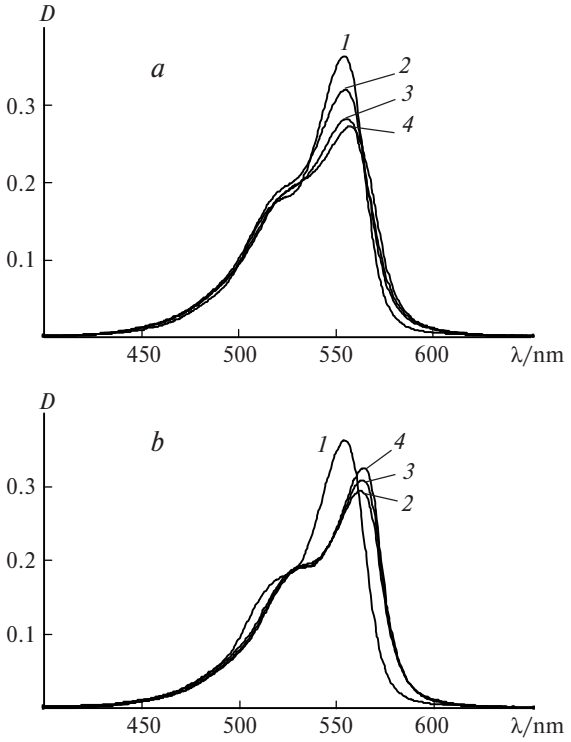

Fig. 1. Absorption spectra of 3,3′-diethylthiacarbocyanine iodide ($4 \cdot 10^{-6}$ mol L^{-1}): (*a*) at low DNA concentrations: C_{DNA} = 0 (*1*), $2.3 \cdot 10^{-5}$ (*2*), $4.9 \cdot 10^{-5}$ (*3*), and $9.4 \cdot 10^{-5}$ mol L^{-1} (*4*); (*b*) at high DNA concentrations: C_{DNA} = 0 (*1*), $2.8 \cdot 10^{-4}$ (*2*), $3.8 \cdot 10^{-4}$ (*3*), and $4.6 \cdot 10^{-4}$ mol L^{-1} (*4*).

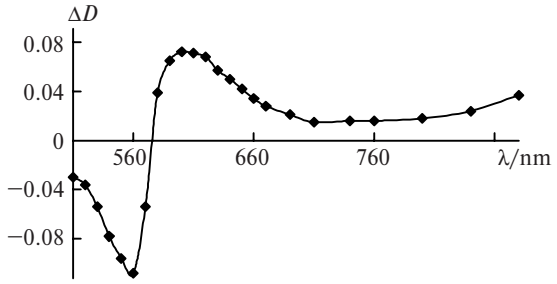

Fig. 2. Differential T—T spectrum of DTCI ($4 \cdot 10^{-6}$ mol L^{-1}) in water obtained by pulse photolysis technique 80 μs after a flash on excitation sensitized by sodium anthracene-2-sulfonate (10^{-6} mol L^{-1}).

The introduction of DNA into the dye solution noticeably changes the absorption spectra of DTCI (see Fig. 1), which indicates the formation of complexes between the dye molecules and biomacromolecules due, probably, to the Coulomb attraction and hydrophobic interactions (the dye is not prone to form strong hydrogen and donor-acceptor bonds with DNA bases).

With the purpose for revealing the composition and nature of the DTCI complexes with DNA, we studied the dependence of the spectral properties of the dye at its unchanged concentration (C_{DTCI} = $4 \cdot 10^{-6}$ mol L^{-1}) on the DNA concentration varied within $2 \cdot 10^{-5}$—$4.5 \cdot 10^{-4}$ mol L^{-1} (see Fig. 1). When the DNA concentration increases from $2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$ mol L^{-1}, the observed extinction coefficient of the dye decreases due to the broadening of its absorption band (see Fig. 1, *a*), which is usually ascribed to the aggregation of the dye molecules on the DNA surface.[30,31] At higher DNA concentrations ($(2.5—4.5) \cdot 10^{-4}$ mol L^{-1}), a substantial (~10 nm) shift of the absorption band of the dye to the longer wavelengths and an increase in the extinction coefficient are observed (see Fig. 1, *b*), which is resulted, most likely, from the decomposition of the aggregates due to the intercalation of the dye molecules between the base pairs of the double DNA helix. The intercalated dye exists in the hydrophobic molecular environment different substantially from an aqueous medium, which results in the bathochromic shift of the absorption band. It also possesses strong fluorescence, unlike aggregated and non-complex forms, because the appeared steric hindrances prevent the formation of photoisomers. Analysis of the fluorescence excitation spectra of dye in the

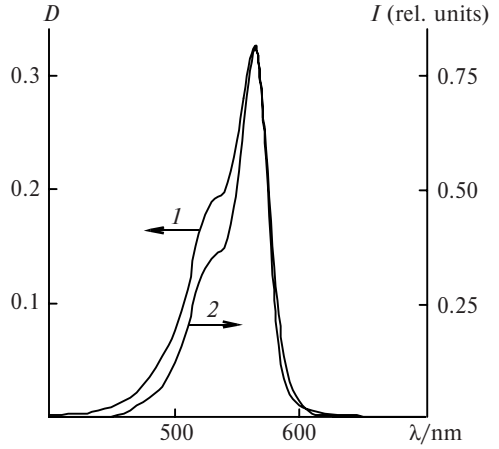

Fig. 3. Absorption spectrum of DTCI at a high concentration of DNA ($4.6 \cdot 10^{-4}$ mol L^{-1}) (*1*) and fluorescence excitation spectrum of DTCI at a low concentration of DNA ($2 \cdot 10^{-5}$ mol L^{-1}) (*2*); DTCI concentration $4 \cdot 10^{-6}$ mol L^{-1}.

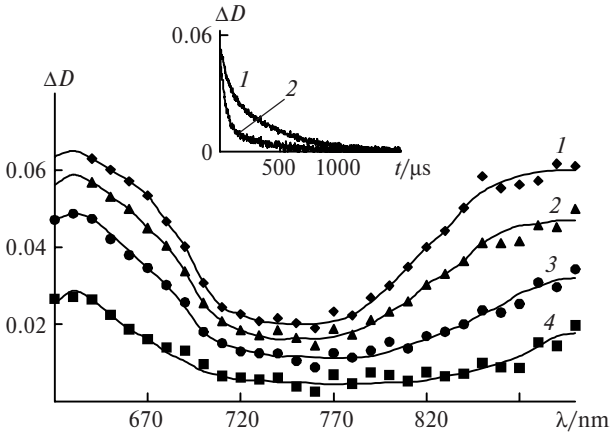

Fig. 4. Differential T—T spectra of DTCI ($4 \cdot 10^{-6}$ mol L^{-1}) measured 84 (*1*), 114 (*2*), 194 (*3*), and 634 μs (*4*) after a flash at a concentration of DNA of $7 \cdot 10^{-4}$ mol L^{-1}. Insert: the decay kinetics of the triplet state DTCI at λ = 650 (*1*) and 820 nm (*2*) in the presence of DNA.

presence of DNA shows the presence of intercalated molecules at both high and low DNA concentrations: the absorption spectra are broadened, whereas the fluorescence excitation spectra remain narrow and correspond to the intercalated dye (Fig. 3). The aggregation of cyanine dye molecules on DNA molecules, which precedes or occurs simultaneously with intercalation, has previously been found for pseudo-isocyanine[30] and bi- and trichromophoric cyanines.[31]

The introduction of DNA into a solution increases the quantum yield of the triplet state of the dye, which enables the detection of the T—T absorption upon direct photoexcitation. It is known that the direct population of triplet manifolds of polymethine dyes due to intersystem crossing occurs under the conditions that hinder *trans—cis*-photoisomerization[29] because these two processes of degradation of the first excited singlet state compete with each other. Therefore, the increase in the quantum yield of the triplet state of DTCI can be explained by a decrease in the mobility of the benzthiazole fragments of the dye molecules bound to DNA, which hinders photoisomerization.

The decay kinetics of the triplet state of DTCI in the presence of DNA is biexponential (Fig. 4). The differential spectra of the T—T absorption of DTCI with different time resolutions are presented in Fig. 4. Since the free dye is virtually absent from the system under these conditions, two observed components in the decay kinetics of the triplet state can be attributed to two different populations of the dye molecules bound to DNA. One of these populations is formed, probably, by monomers (and in part by aggregates) of the dye, which is rather strong bound to the DNA surface, perhaps, in grooves of DNA molecules (complex **1**). Another population corresponds to intercalated monomers of the dye (complex **2**). Note that two components in the decay kinetics of the triplet state were also observed at low DNA concentrations. It can be assumed that the formation of these two types of complexes occurs simultaneously. The ratio of the concentration of complex **2** to that of complex **1** is low at low DNA concentrations where the contribution of the intercalated dye molecules to the corresponding absorption spectra is low. It is most likely that the formation of complex **2** requires a special sequence of nucleotides in the DNA molecule favorable for dye intercalation. To answer this question, a series of experiments with synthetic oligonucleotides with different structures should be performed. With an increase in the DNA concentration, the number of intercalation centers, where complex **2** can be formed, increases, and the equilibrium is shifted toward the formation of complex **2**. Thus, complex **2** is more energetically favorable than complex **1**, probably, due to the stronger hydrophobic interaction with adjacent base pairs.

The differential spectra of T—T absorption of two components related to different complexes were obtained by the global kinetic analysis of the T—T absorption decay in the framework of the biexponential model using the nonlinear optimization algorithm Generalized Reduced Gradient (Fig. 5). The spectrum of the long-lived component differs insignificantly from the T—T absorption spectrum of DTCI in water obtained by T—T transfer (see Fig. 2). It can be assumed that the long-lived triplet component (the decay rate constant is $8.6 \cdot 10^2$ s^{-1}) corresponds to complex **1** because the dye molecules on the surface (in grooves) of DNA molecules do not experience substantial perturbations and the spectrum of their triplet state should be similar to such a spectrum in the absence of DNA. The short-lived triplet component (the decay rate constant is $1.4 \cdot 10^4$ s^{-1}) is probably related to the intercalated dye molecules, which are in close contact with DNA bases due to the restricted space between DNA base pairs accessible for the intercalated dye molecules. The short lifetime of this component can be due to conformational perturbations

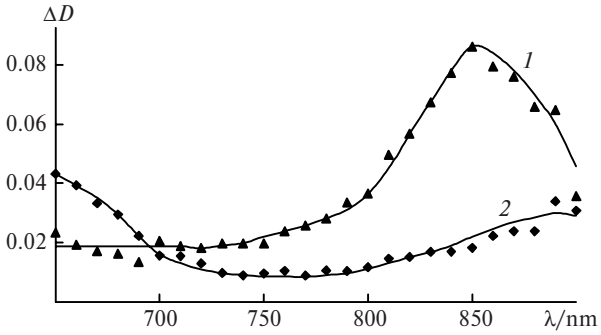

Fig. 5. Differential T—T spectra of two triplet components of DTCI ($4 \cdot 10^{-6}$ mol L^{-1}) in the presence of DNA ($7 \cdot 10^{-4}$ mol L^{-1}): short-lived (*1*) and long-lived components (*2*).

and the interaction of the triplet dye molecule with the nearest DNA base, for example, *via* the charge transfer mechanism, which accelerates T—S conversion to the ground state. The same factors result in an increase in the intensity of the T—T absorption in the long-wave region and the appearance of a new band at ~850 nm.

The reactivity of the T state of the dye with respect to molecular oxygen in the presence of DNA was also studied. Using the global kinetic analysis, we obtained the quenching rate constants of two T components of DTCI by oxygen, which were $1.5 \cdot 10^8$ and $4.5 \cdot 10^8$ L mol^{-1} s^{-1} for the short- and long-lived components, respectively, which is much lower than the quenching rate constant of the triplet state of DTCI by oxygen in the absence of DNA. The interaction with the DNA molecules prevents the oxygen access to the DTCI molecules.

The difference between the quenching rate constants of the fast and slow components confirms indirectly the above assumptions on the nature of the complexes. The access of the O_2 molecules to the dye molecules is more hindered compared to the dye molecules, which are in the grooves. Thus, the quenching of the short-lived triplet component, which presumably belongs to the intercalated dye, is less efficient. Note in conclusion that the formation of the triplet state during complex formation with DNA in an air-saturated medium can be accompanied by the generation of singlet oxygen, which damages DNA.

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