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PHOTOISOMERIZATION OF DIETHYLOXADICARBOCYANINE IODIDE IN DNA AND PROTEIN#

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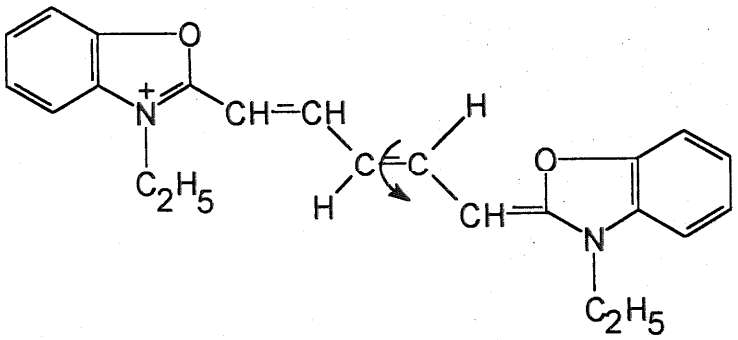
Abstract--Photoisomerization of 3,3'-diethyloxadicarbocyanine iodide (DODCI) is studied in aqueous solution in the presence of salmon sperm DNA and bovine serum albumin (BSA). It is observed that in aqueous solutions DNA and BSA offer extremely high friction to the isomerization of DODCI, like a highly viscous liquid. As a result, the isomerization of DODCI is completely suppressed inside DNA and BSA.

INTRODUCTION

Photoisomerization of organic molecules containing conjugated double bonds plays a fundamental role in many chemical and biological process, including the primary photochemical step of the vision process [1-15]. The isomerization about the double bond is identified as the main nonradiative pathway in the excited states of polyenes [3,5,11]. The friction imparted by several interfaces to this important photophysical process has been the subject of many recent studies [3,14]. To study an interface between a polar and a nonpolar media, the most suitable probes are those which contain a charged or highly polar end and a rather long nonpolar hydrocarbon chain, e.g. the rod shaped laser dye molecule, 3,3'-diethyloxadicarbocyanine iodide (DODCI, Scheme 1) [3,11]. Several groups have earlier studied the photophysical process of DODCI thoroughly in many solvents and established that the main nonradative pathway in the excited state of DODCI is the photoisomerization process [11]. The isomerization of DODCI has been studied at various interfaces, such as the air-water interface [3], lipids [13], sol-gel glass [15], microemulsions [14a] and micelles [14c]. The recent time resolved surface second harmonic generation (SSHG) experiments have demonstrated that for the air-water interface, the friction against the photoisomerization is different for different probes [3].

#Dedicated to Professor M.V. George on his 70th birthday.



Scheme 1.
Structure of DODCI.

While, for DODCI, at the air-water interface, the isomerization is faster compared to that in the bulk water, for the nearly planar malachite green, it is slower at the air-water interface [3]. We have, earlier, showed that the photoisomerisation of DODCI, inside the water pool of a microemulsion, is nearly three times slower compared to that in ordinary bulk water [14a]. More recently, we have observed that the photoisomerisation of DODCI is markedly slower at various micellar interfaces compared to bulk water [14c]. Relative to bulk water, in cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and triton X-100 (TX) micelles, the rate of photoisomerization decreases by a factor of 20, 7 and 8 times respectively. The viscosity dependence of the photoisomerisation process has been the subject of many studies [4-6]. At very high viscosity, the rate of photoisomerization becomes inversely proportional to the viscosity of the medium (Smoluchowski limit). Assuming that the Smoluchowski limit and the same "slip/stick" boundary condition hold for the highly viscous solvent, *n*-decanol and the micelles and comparing the isomerization rates of DODCI in the three micelles with that in *n*-decanol, we estimated the microviscosities of CTAB, SDS and TX to be 70.0 ± 20, 24.5 ± 2 and 26.0 ± 2 cP, respectively [14c]. The steady state and time resolved optical anisotropy also depends on the microviscosities of a medium [16-17]. However, in the host-guest complexes, rotational motion of the probe, guest molecule becomes coupled with the slower motion of the host, (e.g. DNA, protein, micelles etc.). This often leads to a multiexponential anisotropy decay, from which it is difficult to extract a meaningful value of the microviscosity [13,16-17].

In the present study, we wish to extend our earlier studies to estimate the friction towards the isomerization process of a polyene in aqueous solution in the presence of deoxyribonucleic acid (DNA) and a protein, bovine serum albumin

(BSA). Due to the inherent negative charge of the phosphate groups, DNA exerts a strong electrostatic attraction for the DODC cation and consequently, DODC readily binds with DNA. For BSA, the binding of DODCI occurs due to the hydrophobic effect. The main advantage of using photoisomerization as a probe for complex biological assemblies is due to the fact that since it involves motion of one half of the probe against another, it remains unaffected by the overall motions of the DNA double helix or the peptide chains of a protein. In contrast, in optical anisotropy studies, the bending and twisting motion of the macromolecular chains of DNA and proteins, become superimposed on the orientational motion of the probe [13,16]. Very recently using temperature dependent steady state Stokes shift of intercalated acridine orange dye, Berg *et al.* [21a] showed that DNA exhibits diffusive and viscous dynamics characteristic of a fluid while Young *et al.* [21b] applied molecular dynamic simulation to understand the local dielectric environment of DNA. This inspired us to estimate the microviscosities in the DNA and protein microenvironments. It may be noted that though dynamics of several photophysical processes, such as electron transfer [18-19], rotational relaxation [16-17], and proton transfer [22] have been studied inside the DNA there are relatively few reports on isomerization dynamics inside DNA or any protein, except of course the studies on the visual chromophore [1]. Since the vision process involves photoisomerization of retinyl polyenes covalently attached to a protein, it is of fundamental importance to understand how the microenvironment of a biological macromolecule e.g. DNA or a protein affects the isomerization process [1]. In this article we report our preliminary results on the isomerization dynamics in DNA and protein environments.

EXPERIMENTAL SECTION

Laser grade DODCI (Exciton) was used as received. Salmon sperm DNA (sigma) and BSA (sigma) were also used without purification. Quantum yields are measured using reported quantum yield of DODCI in water [14]. For fluorescence lifetime measurements, the sample was excited at 570 nm, by the fundamental of a synchronously pumped Rhodamine 6G dye laser (Coherent 702-1) pumped by a cw mode locked Nd:YAG laser (Coherent Antares 76s). The fluorescence decays were recorded, at magic angle polarization, using a Hamamatsu MCP PM tube (2809U). The response time of the setup for excitation at the fundamental laser wavelength, 570 nm, is $\approx$100 ps. The fluorescence decays were analysed using the global lifetime analysis software (Photon Technology International). All the steady-state and time-resolved measurements were carried out at 20 °C, using doubly distilled and autoclaved water in a tris buffer solution of pH = 7.4.

RESULTS

On addition of both DNA and BSA to an aqueous solution of DODCI, the absorption and emission spectra of DODCI exhibit a red shift of about 10 nm and the quantum yields of emission, ϕ_f, of DODCI increase nearly two times (Figure 1 and Table 1). This suggests considerable interaction between DODCI and DNA as well as, BSA. While the red shift indicates binding of DODCI with DNA and BSA, the increase in ϕ_f suggests suppression of the nonradiative photoisomerization process of DODCI on binding to DNA and BSA. The inhibition of the photoisomerization process of DODCI is also manifested in the marked slowing down of the fluorescence decay of DODCI on binding to DNA and BSA. In aqueous solution with and without buffer, the fluorescence decay of DODCI is single exponential with lifetime 680 ± 40 ps at 20 °C. However, in the presence of both DNA and BSA, the fluorescence decay of DODCI becomes biexponential with a fast component very similar to that corresponding to the free DODCI molecules in water and a nearly 4 times slower

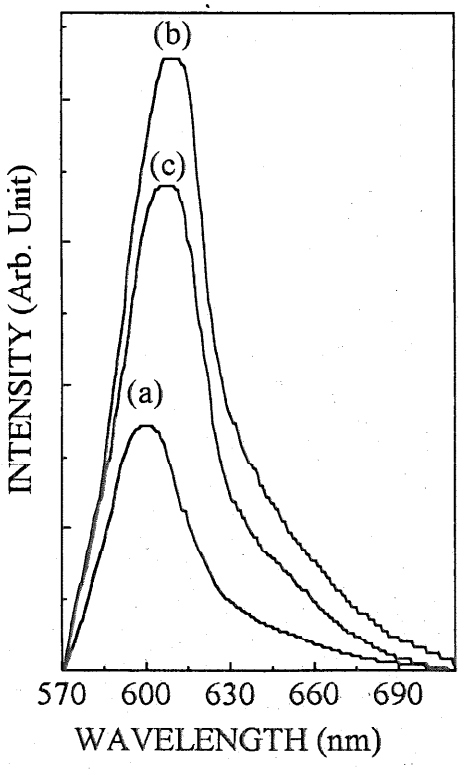


Figure 1. Emission spectrum of 1×10^{-6} M DODCI in (a) water (b) 1 mg/ml salmon sperm DNA in tris buffer and (c) 150 μM BSA in tris buffer, pH = 7.4.

Table 1
Steady state emission properties of 1×10^{-6} M DODCI.

Medium	λ_{em}^{max} (nm)	ϕ_f^{obs} [a]
Water	600	0.30
DNA	615	0.65
BSA	610	0.60

[a] ± 5%.

component of 2.8 ± 0.05 ns for DNA and 2.3 ± 0.05 ns for BSA. Though the bound form may have a large number of lifetimes due to the slight variations in its various environments we restricted ourselves to a biexponential analysis to get an average picture. The slower component is assigned to the DODCI molecules bound to DNA and BSA. It may be recalled that in the presence of lipids DODCI exhibits similar biexponential decay with components 0.68 ns and 1.76 ns which are assigned respectively to the free and bound forms [13]. The amplitude of the slow decay increases from 0.36 in BSA (0.48 in DNA) at 595 nm to 0.53 in BSA (0.82 in DNA) at 700 nm (Table 1). Evidently at the blue end (550 nm) only the free DODCI molecules with fast decay (680 ps) contribute to the decay while the decay at the red end (700 nm) is dominated by that of the bound form with long lifetime. Unfortunately since with our Rhodamine 6G dye laser we excited the sample at 570 nm we could not record the decay at the blue end of the emission spectrum at 550 nm. Figure 2 depicts the fluorescence decays of DODCI in water in the absence and in the presence of DNA and BSA. Table 1 and 2 summarize the emission quantum yield (ϕ_f), lifetime (τ_f) and emission maxima (λ_{em}^{max}) of DODCI in DNA and BSA.

Once the lifetime (τ_f^b) of the bound DODCI molecules (2.8 ns in the case of DNA and 2.3 ns in the case of BSA) is ascertained the rate constant (k_{iso}^b) of the nonradiative photoisomerization process of the bound DODCI molecules can be estimated using the relation, $k_{iso}^b = (\tau_f^b)^{-1} - k_r^b$. The rate constant of the radiative decay (k_r) increases slightly with increase in the refractive index (n) of the medium [11,13]. In going from methanol (n = 1.33) to *n*-decanol (n = 1.44), k_r of DODCI increases slightly from 0.39×10^9 s^{-1} to 0.43×10^9 s^{-1} [11a]. It is difficult to estimate the local refractive index of the microenvironment of the bound DODCI molecules. If we assume it to be in between those of methanol and decanol, the rate constant of the radiative decay rate, of bound DODCI (k_r^b) can be taken as $(0.42 \pm 0.2) \times 10^9$ s^{-1}. If we substitute this value for k_r^b the rate constant (k_{iso}^b) of the nonradiative photoisomerization process of the bound DODCI molecules is calculated to be nearly zero. In other words, the photoisomerization process of DODCI is completely suppressed in DNA or BSA.

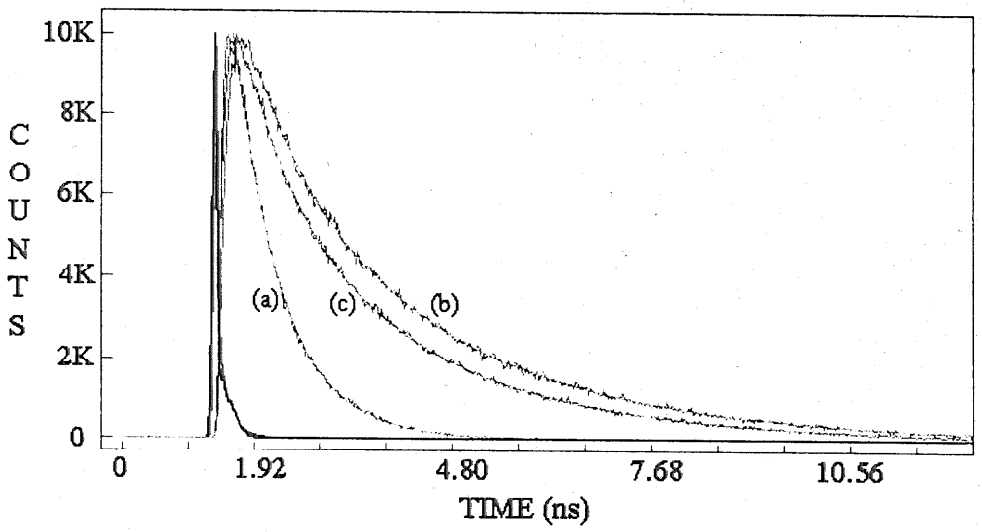


Figure 2. Decay of 1×10^{-6} M DODCI in (a) water, (b) 1 mg/ml salmon sperm DNA in tris buffer and (c) 150 μM BSA in tris buffer, pH = 7.4. λ_{ex} = 570 nm, λ_{em} = 625 nm. (1K = 1000 counts).

Table 2
Fluorescence decay parameters of DODCI at different wavelengths.

Medium	Emission Wavelength (nm)	A_1	τ_1 (ns)	a_2	τ_2 (ns)
Water	625	1.00	0.68		
DNA	595	0.52	0.68	0.48	2.8
	625	0.11	0.68	0.89	2.8
	700	0.18	0.68	0.82	2.8
BSA	595	0.64	0.68	0.36	2.3
	625	0.46	0.68	0.54	2.3
	700	0.47	0.68	0.53	2.3

DISCUSSION

Several groups have established earlier that the main nonradiative pathway of DODCI is the isomerization process [11]. The present work indicates that the isomerization process of DODCI bound to DNA or BSA is almost completely suppressed due to extremely high local viscosity.

For some probes, the activation barrier for the isomerisation and hence, the isomerisation dynamics has been shown to be affected by the polarity of the media [5,8,11]. For *trans*-stilbene, Hicks *et al.* observed that the slope of the isoviscous plots of $\ln(k_{iso})$ against 1/T decreases with increase in the viscosity and they attributed this to the reduction of the barrier for isomerisation, at higher viscosity and lower polarity [8]. However, Velsko and Fleming have demonstrated that for DODCI in alcoholic solvents the isoviscous plots of $\ln(k_{iso})$ against 1/T in alcoholic solvents do not show much variation of the slope with increase in viscosity which indicates that the effect of polarity on the barrier height, is relatively unimportant in the case of DODCI [11a-b]. Waldeck and coworkers have analyzed this issue, in considerable detail, and concluded that a barrier for the isomerization process can be extracted only for media, such as nitriles, where the solvent relaxation time is very much faster than the excited state lifetime [5]. In media where the solvation dynamics is slow, incomplete solvation obscures observation of a well defined barrier [5]. Recently various groups using different techniques independently reported that in many organized and biological assemblies (proteins [23a-c], cyclodextrins [23d], microemulsions [20a] or micelles [20b], vesicles [20c] etc.) the solvent relaxation is several thousand times slower compared to bulk water. Considering the of lack of polarity dependence of the rate of isomerization of DODCI [11a-b] and the observed slow solvation dynamics in such organized environments [20,23], it appears that the complete suppression of the isomerization dynamics of DODCI in DNA and BSA do not originate from the reduced local polarity of the DNA and BSA microenvironment.

CONCLUSION

In summary, photoisomerization dynamics of the positively charged dye, DODCI, is observed to be completely suppressed when it binds to DNA and BSA. This indicates exceedingly high microviscosity of the BSA and DNA. Obviously more experiments are needed, to conclusively establish the exact mechanism of the increased friction in the DNA and BSA microenvironments and to ascertain the exact location of the probe DODCI molecule in the DNA or the BSA, or whether binding of DODCI causes denaturation of DNA. However, it seems that the increased friction is not due to the reduced polarity of these microenvironments. It will be interesting to see through temperature dependent studies how DNA melting affects the isomerization process and how the isomerization is affected when such a probe is covalently bound to a particular site of a protein. We will address some of these issues in our future work.

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