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DNA Changes Dramatically Photoisomerization of Arylstilbazolium Ligands

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Photochemical *cis-trans* isomerization plays a major role in many photobiological processes, such as vision, ATP synthesis and other allied processes [1,2]. It has been also applied in photostimulation of the interaction affinity of small molecules with supramolecular systems and biopolymers that provided the basis for the development of future optoelectronical devices and novel analytical systems [3]. The semirigid structure of a DNA duplex makes it an attractive organized medium for creating an artificial photochemical reaction system.

Recently, we have shown, that stilbazolium ligands organized on the DNA can undergo photoisomerization [4–6]. Moreover, DNA affected this process due to the preferences in binding affinity towards particular isomers [5]. On the other hand, Bhattacharyya *et al.* [7] concluded from lifetime measurements that photoisomerization of the laser dye 3,3′-diethyloxadicarbocyanine iodide is completely suppressed when bound to salmon sperm DNA. A possible explanation of this discrepancy may involve isomerization pathway through unbound ligand, which remains equilibrated with bound species.

Here we report on the preliminary HPLC studies of photoisomerization of stilbazolium ligands: 2-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene **(1)**, 9-[2-(N-methylpyridinium-4-yl)vinyl]phenanthrene (**2**) and 9-[2-(N-methylpyridinium-4-yl)vinyl]anthracene (**3**), in the presence of Calf Thymus DNA.

The stilbazolium derivatives **1**– **3** were obtained as *trans* isomers by the conventional aldol condensation [8,9]. Calf thymus DNA (CT-DNA) was purchased from Sigma and was used without further purification. Samples for photoisomerization experiments (40 μM ligand, 0 or 1 mM DNA) were irradiated at selected wavelengths in a cell compartment of spectrofluorimeter (Shimadzu RF 5000) equipped with the 150 W Xe lamp. When irradiation was finished, the DNA matrix was separated by centrifugation in the presence of $MgCl_2$ in ethanol. Aliquot of 10 μl of the supernatant solution was analyzed on a LaChrom HPLC system (Hitachi) equipped with an ODS 3 column (Gl Science Inc.) with gradient elution (acetonitrile/water mobile phase with ammonium acetate as an ion-pairing agent). Chromatogram was monitored at the isosbestic point of ligand and concentrations of *cis* and *trans* isomers were determined from peak areas. Determination of a photostationary state composition (*pss*) was performed using simple relationship *pss* = [*cis*]/[*trans*], where [*cis*] and [*trans*]

denote concentrations (peak areas) of isomers at photoequilibrated mixture (long irradiation times).

In the absence of DNA only two photoproducts (*cis* and *trans* isomers) were detected for all compounds. Isomerization of DNA bound ligands gave the same products for compounds **2** and **3**. Figure 1 shows typical chromatogram of isomeric mixture of ligand **3** obtained in the presence of DNA and spectra of HPLC peaks recorded with a diode array detector. The spectra of peaks are consistent with those reported for pure *cis* and *trans* isomers of **3** [8]. Only in case of 2-naphthyl derivative **1**, additional peaks were found in chromatogram. The absence of strong absorption band of ethylenic bond in the spectra of these peaks (330–400 nm range) suggested that they might represent cyclobutane photodimers. HPLC-MS analysis of unknown photoproducts supported this conclusion (mass spectrum with strong signals at m/z = 239 (100%) and 477 (25%)). DNA-groove binding ability of ligand **1** [5] explains formation of photodimers, since due to the binding cooperativity, two molecules arranged in a face-to-face fashion can be located in the minor groove of DNA. The striking increase in selectivity of DNA to produce photoproducts not observed in homogeneous solution indicates a degree of topological control of ligand reactivity by DNA. Such phenomena were previously observed in the AOT reversed micelles [10] and clay layers [11].

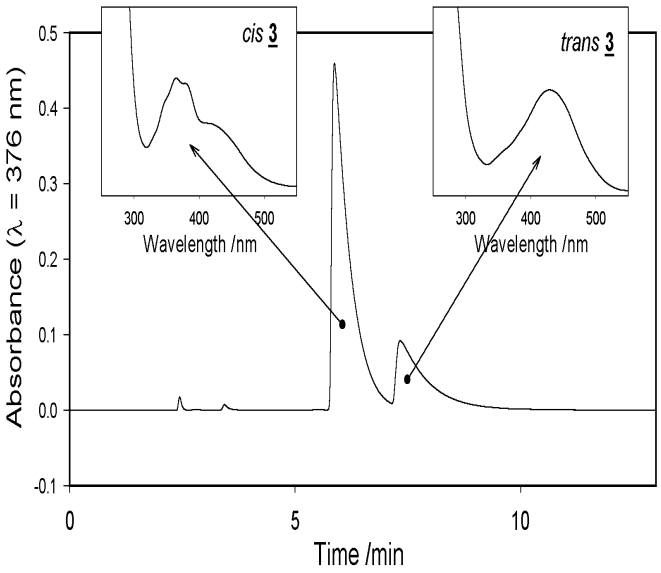

Figure 1. HPLC chromatogram of the isomeric mixture of ligand **3** irradiated in the presence of DNA (40 μM ligand, 1 mM DNA) for 30 min. Irradiation and monitoring wavelengths were 459 and 376 nm, respectively. Insets show absorption spectra of photoproducts recorded by a diode array detector.

Values of *pss* in the presence of DNA were 0.6, 0.75 and 3.8 for ligands **1**, **2** and **3**, respectively. Corresponding results for free ligands were 3.1, 7.8 and 1.8. Free ligands show *cis* isomer-rich *pss* values that can be explained by the fundamental photoisomerization relationship $pss = \frac{\varepsilon_T}{\varepsilon_C} \frac{\phi_{TC}}{\phi_{CT}}$. Remarkable high values of *pss* are due to the significantly higher molar absorptivities of *trans* isomers at irradiation wavelengths ($\varepsilon_T > \varepsilon_C$). Lower values of *pss* observed in the presence of DNA for ligands **1** and **2** are in accordance with expectation and seem to reflect DNA binding affinity preferences. Similar phenomena were previously reported for distilbazolium ligands [4,9]. Only in case of ligand **3**, DNA enhances *trans*→*cis* isomerization despite that the binding factor prefers *trans* isomer. These results are very surprising since anthryl derivative **3** possesses similar DNA-binding preferences as compounds **1** and **2**. Explanation of peculiar behavior of ligand **3** may involve different mechanism of isomerization than in case of ligands **1** and **2** with the involvement of energy transfer processes. The anthracene moiety exhibits intriguing photochemical behavior and plays an important role in the energy transfer including triplet and charge transfer states [12]. As we have reported before, an intramolecular charge transfer excited state is responsible for the photoisomerization of free ligand **3** [8].

Concluding, presented results showed that DNA could affect the *trans-cis* photoisomerization of bound ligand by simple differentiation between structurally dissimilar isomers (binding affinity), or by the interplay of binding factor and an additional photophysical process (energy transfer). To our knowledge, this is first evidence that DNA can mediate photoisomerization against the binding affinity preferences. Further studies including quantum yield determination are in progress.

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