Chinese Chemical Letters Vol. 13, No. 4, pp 299 – 302, 2002
http://www.imm.ac.cn/journal/ccl/html

DNA Modification with Photochromic Spiro Compounds

Peng ZHANG[1], Ji Ben MENG[1]*, Teruo Matsuura[2], Yong Mei WANG[1]

[1] Department of Chemistry, Nankai University, Tianjin 300071
[2] Emeritus Professor, 21-26 Kawashima-Gondencho, Saikyoku, Kyoto 615-8195, Japan

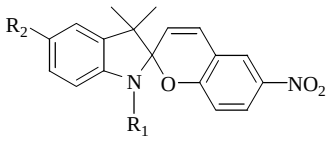
Abstract: The photochromic spiropyrans and spirooxazine having a succinimidyl ester or isothiocyanate pendant group can form covalent products with transaminated DNA. The absorption spectra and solid reflection spectra of modified DNA with these photochromic spiro compounds were investigated.

Keywords: Photochromic spiro compound, modification, DNA**.**

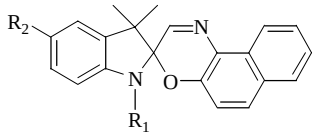
Spiropyrans and spirooxazines are important classes of photochromic materials[1-3]. Recently, modification of biomolecules with photochromic compounds arouses strong interests because such modification could lead to photoswitchable biomaterials which have potential application on information storage and biosignal amplifiers[4,5]. Here we report the modified DNA with photochromic spiropyrans and spirooxazines.

The structures of synthesized photochromic spiro compounds[6] are shown in **Scheme 1**. The compounds with one carboxyl group[7] reacted with N-hydroxysuccinimide in the presence of DCC to form succinimidyl esters **1a** and **2a**, respectively. These esters reacted with various amino acids to give new compounds with a longer chain pendant group compound, which were further converted into **1b-1d** and **2b-2d**, respectively. The compounds **1e** and **2e** with isothiocyanate group were prepared from corresponding comounds with amino group.

Scheme 1



1a-e



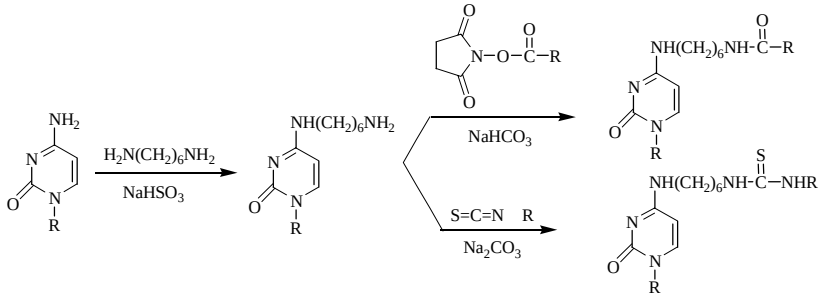
2a-e

a: R_1=$CH_2CH_2COONHS$, R_2=H
b: R_1=$CH_2CH_2(CONHCH_2)_2COONHS$, R_2=H
c: R_1=$CH_2CH_2CONH(CH_2)_5COONHS$, R_2=H
d: R_1=CH_3, R_2=COONHS
e: R_1=CH_3, R_2=NCS
Note：**a**, **b**, **c**, **d** 中 NHS= *N*-succinimidyl

A compound having a reactive group such as isothiocyanate and succinimidyl ester is known to react covalently with DNA transaminated with a diamine[8,9]. The first step

for the modification of DNA is the introduction of reactive amino groups into DNA by transaminating with 1, 6-hexanediamine in the presence of sodium bisulfate. The transamination solution (1 mol/L $NaHSO_3$, 3 mol/L 1, 6-hexanediamine, 5 mmol/L hydroquinone) was titrated to pH 6 by adding concentrated HCl. *Prior* to the transamination reaction, ct-DNA (100 μg in 250 μl 10 mmol/L Tris-HCl pH 7.4, 1 mmol/L EDTA) was denatured at 98°C for 10 min. After denaturation nine volumes of the transamination solution was added. After overnight dialysis against four exchanges of 5 mmol/L sodium phosphate, the DNA solution was centrifuged and further purified by ethanol precipitation. By this reaction, cytosine bases in a single-strand nucleic acids can be converted to N^4-substituted amino derivatives[10,11] as illustrated in **Scheme 2**.

Scheme 2



The second step for the modification of DNA is covalent reaction of the transaminated DNA with photochromic compounds. We prepared the transaminated DNA in this manner which was treated with succinimidyl ester **1a** in the presence of $NaHCO_3$ in aqueous DMSO for three hours at room temperature[12] to give modified DNA designated as **1a**-DNA. Modified DNA was further purified by ethanol precipitation. Other modified DNA, **1b**-, **1c**, **1d**, **2a**-, **2b**-, **2c**-, **2d**-DNA, were prepared in the same manner. Another type of modified DNA, **1e**-DNA and **2e**-DNA, were prepared by the treatment of the transaminated DNA with isothiocyanate **1e** and **2e** in the presence of Na_2CO_3 in aqueous DMSO. For this reaction, pH was kept at about 10 and it took at least 24 hrs at room temperature.

The modified DNA, thus obtained are water-soluble and their physical properties, are similar to native DNA. Their absorption spectra were measured in aqueous DMSO solution and in the solid state. As shown in **Table 1**, the modified DNA exhibited a new absorption peak at 400 — 440 nm besides 260 nm peak which is characteristic for natural DNA. The new absorption peaks are observed at much longer wavelength (305 — 345 nm) than those of the corresponding parent spiro compounds.

We prepared modified DNA using different amounts compounds (**1a**-**e** and **2a**-**e**) in the reaction with the transaminated DNA and measured their absorption spectra. It was found that the intensity of the absorption maximum at 400 — 440 nm increased with the increase of the amount of the compound in the reaction. When the concentration of the compound increased to some extent, the intensity of the absorption maximum did not increase any more. It showed that modified photochromic compound became saturated. The result of **1b**-DNA is illustrated in **Figure 1**.

Table 1 The maximum absorption (λ_{max}) of the modified DNA and the corresponding parent compound in DMSO: H_2O =5:1 (v/v)

Compd.	λ_{max} (nm)	Modified DNA	λ_{max} (nm)	Compd.	λ_{max} (nm)	Modified DNA	λ_{max} (nm)
1a	335	**1a**-DNA	400	**1b**	339	**1b**-DNA	420
1c	338	**1c**-DNA	420	**2a**	336	**2a**-DNA	405
2b	338	**2b**-DNA	418	**2c**	338	**2c**-DNA	420
1d	340	**1d**-DNA	440	**2d**	345	**2d**-DNA	442
1e	305	**1e**-DNA	436	**2e**	310	**2e**-DNA	438

The photochromic behavior of modified DNA was examined with solid reflection spectroscopy. **Figure 2** shows the spectra of **1b**-DNA before and after UV-irradiation. The former shows a maximum at 420 nm which is the same as that in solution. The absorption maximum of the open colored form appeared at 586nm having a red shift of 30 nm compared with the parent compound **1b**. **Table 2** shows the maximum absorption of the open colored form of the modified DNA and corresponding parent spiro compounds after UV-irradiation.

Figure 1 The absorption spectra of **1b**-DNA which were obtained by the reaction with different amounts of compound **1b** (increase amounts of substrates from 0-5)

Figure 2 The solid UV-Vis reflection spectra of **1b**-DNA before and after irradiation

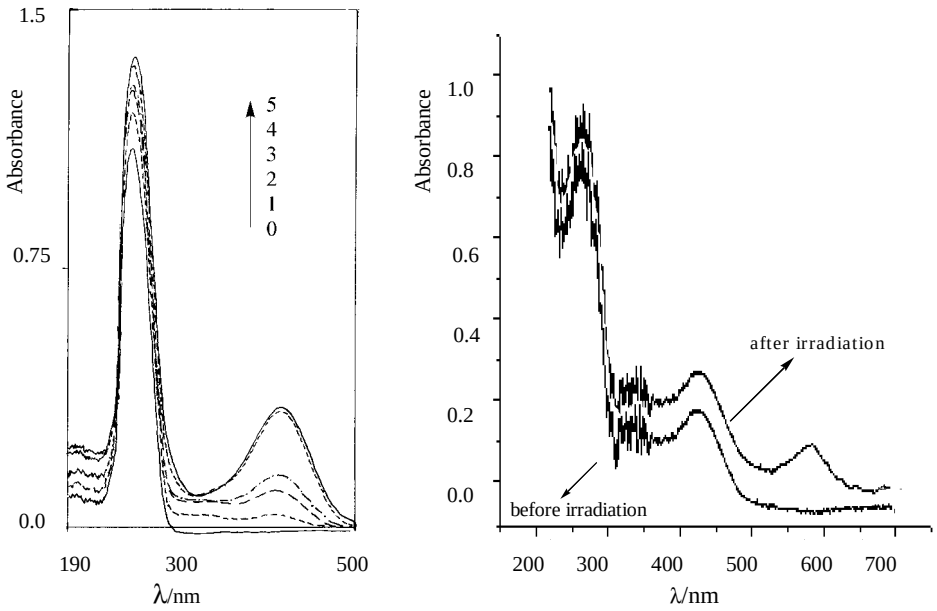


Table 2 The maximum absorption of the open colored form of modified DNA and the corresponding parent spiro compounds after UV-irradiation in solid state

Compd.	λ_{max} (nm)	Modified DNA	λ_{max} (nm)	Compd.	λ_{max} (nm)	Modified DNA	λ_{max} (nm)
1a	558	**1a**-DNA	582	**2a**	569	**2a**-DNA	596
1b	556	**1b**-DNA	586	**2b**	567	**2b**-DNA	595
1c	552	**1c**-DNA	582	**2c**	567	**2c**-DNA	592
1d	544	**1d**-DNA	576	**2d**	555	**2d**-DNA	585
1e	542	**1e**-DNA	570	**2e**	547	**2e**-DNA	580

Acknowledgment

This study was supported by the National Natural Science Foundation of China (No. 29872015, 29832030).

References and Note

1. H. Durr, H. Bouas-Laurent, Eds., *Photochromism and System*, Elsevier, Amsterdam, **1990**, 12.
2. R. C. Bertelson, *Mol. Cryst. Liq. Cryst.*, **1994**, *246*, 1
3. J. D. Winkler, C. M. Bowen, V. Michelet, *J. Am. Chem. Soc.*, **1998**, *120*, 3237.
4. I. Willner, S. Rubin, *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 367.
5. I. Willner, *Acc. Chem. Res.*, **1997**, *30*, 347.
6. Synthesis of these compounds will be published elsewhere.
7. X. L. Li, Y. Wang, T. Matsuura, J. Meng, *Heterocycles*, **1999**, *51*, 2639.
8. P. Hurskainer, P. Dahlén, J. Ylikoski, M. Kwiatkowski, H. Siituri, T. Lövgren, *Nucleic Acids Res.*, **1991**, *19*, 1057.
9. A. H. Al-Hakin, R. Hull, *Nucleic Acids Res.*, **1986**, *14*, 9965.
10. D. E. Draper, L. Gold, *Biochemistry*, **1980**, *19*, 1774.
11. D. E.Draper, *Nucleic Acids Res.*, **1984**, *12*, 989.
12. J. B. Randolph, A. S. Waggoner, *Nucleic Acids Res.*, **1997**, *25*, 2923.

Received 16 July, 2001