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Photocross-linking and the Cleavage of DNA by Iron Complex-substituted Psoralen

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The iron complex-substituted psoralen (1) having a moiety capable of photochemically cross-linking to DNA and a DNA cleaving moiety was synthesized. The photoreaction of which is noniron complex of 1, with Col E1 plasmid DNA gave the covalently interstrand cross-linked DNA which was cleaved in the presence of $FeSO_4$ and dithiothreitol under air.

Bleomycin is one of the most potent antitumor antibiotic agents known, which strongly binds to DNA and causes not only single strand but also double-strand DNA scission.[1] It was observed that bleomycin does not show mutagenesis[1] although most of the antitumor antibiotics that bind to DNA, such as mitomycins, actinomycins and daunomycins, show mutagenesis or carcinogenesis.[2] The failure of bleomycin to cause mutagenesis may be a result of the killing of cells due to irreparable damage of DNA caused by highly double strand breaks.[1] Few reports have appeared concerning the artificial double-strand DNA-cleavage agents.[3] Dervan has described sequence specific double-strand cleavage of DNA by bis(EDTA-distamycin.Fe^{II}).[3] We now report the synthesis and ability of DNA strand scission of a new type of DNA cleavage agent, iron complex-substituted psoralen (1).

As shown Fig. 1, the molecule was designed according to the following strategy and is essentially composed of following three parts: (A) a moiety capable of photochemically cross-linking to DNA; (B) a DNA cleaving moiety; (C) a linker moiety connecting (A) and (B) having a high binding-affinity to DNA. A psoralen[4] was used as (A), an iron-complexing group as cleaving moiety (B) which has been synthesized as a bleomycin model,[5] and polymethylene-amides as a linker (C).

The psoralen-linked compound (15) was synthesized according to Scheme 1. 8-Hydroxypsoralen (2) was refluxed with bromide (3) in the presence of potassium carbonate in 2-butanone for 48 h to give 4 (91% yield). Hydrolysis of 4 with trifluoroacetic acid (TFA) gave the amine (5) which was treated with the thiazolidine-2-thione derivative (6), prepared by condensation of 3-(N-t-butoxycarbonyl)aminobutanoic acid and 1,3-thazolidine-2-thione with dicyclohexylcarbodiimide (DCC) in the presence of N,N'-dimethylaminopyridine (DMAP),[6] to

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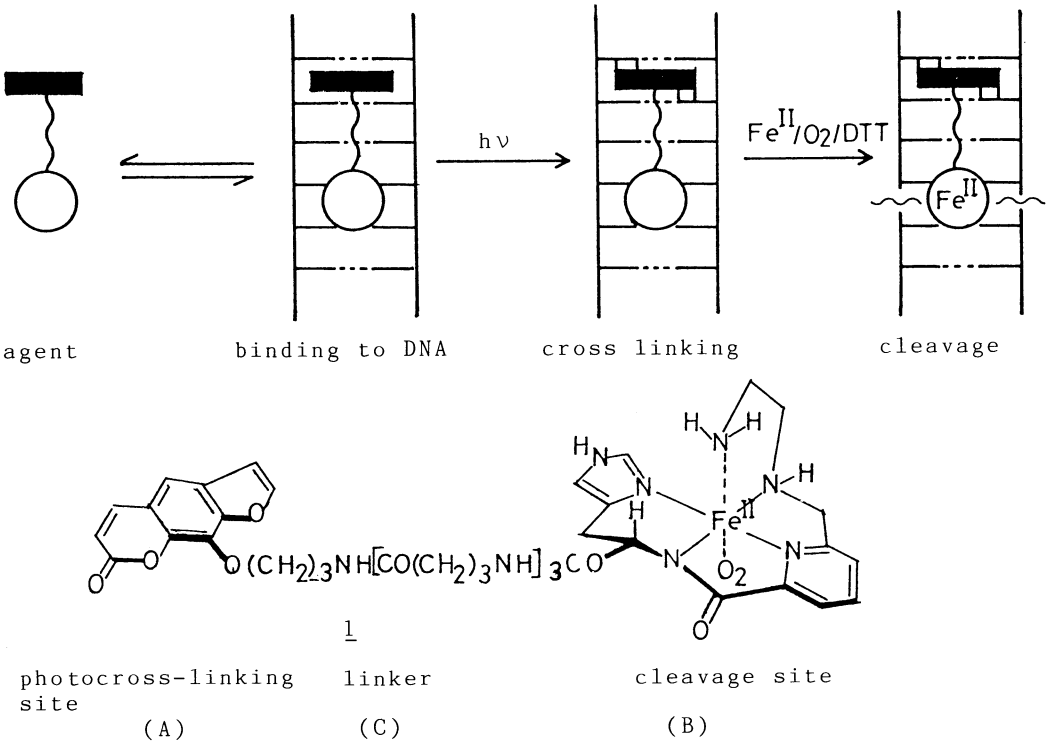

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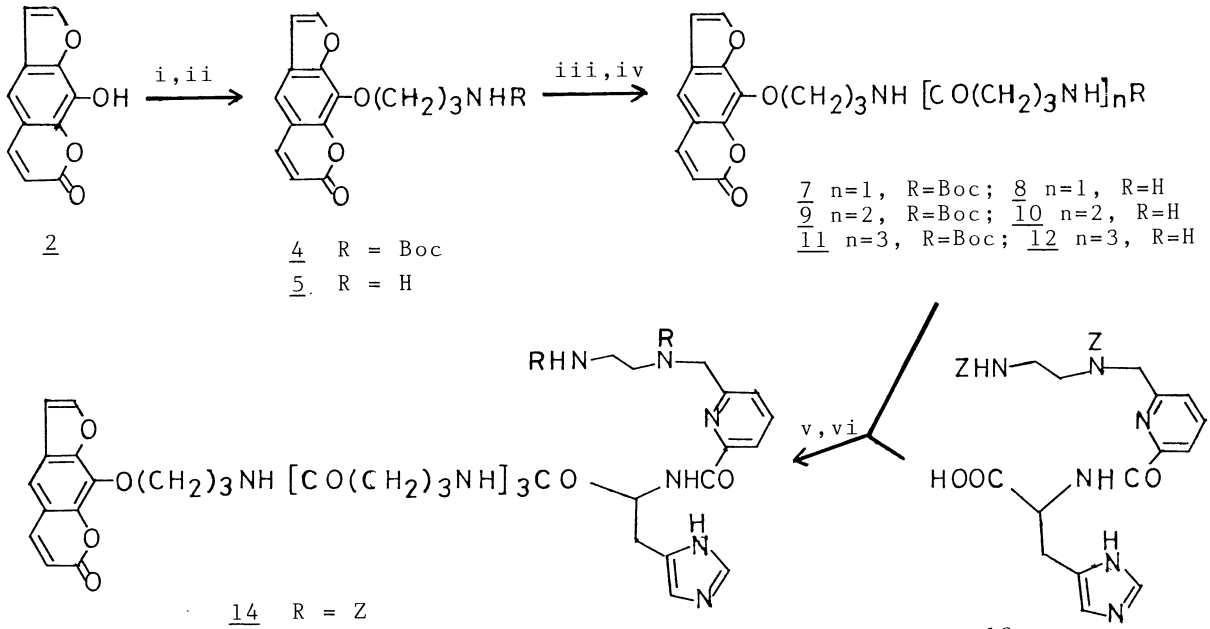
Fig. 1. Mechanism of photocross-linking and the cleavage of DNA, and structure of iron complex-substituted psoralen (1).



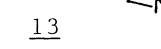
2

4 R = Boc
5 R = H

7 n=1, R=Boc; 8 n=1, R=H
9 n=2, R=Boc; 10 n=2, R=H
11 n=3, R=Boc; 12 n=3, R=H

14 R = Z
15 R = H
1 iron complex of 15

13

3 $Br(CH_2)_3NHBoc$
6 

Reagents: i, 3, K_2CO_3, 2-butanone, reflux; ii, TFA, CH_2Cl_2; iii, 5 with 6; iv, 7 to 8, 9 to 10, 11 to 12, TFA, CH_2Cl_2; 8 to 9, 10 to 11, with 6; v, DCC, HOBT; vi, TFA, thioanisole.

Boc; t-butoxycarbonyl
Z; benzyloxycarbonyl

Scheme 1.

give 7 (94%). Twice repeating of the hydrolysis and amide formation of 7 with 6 gave 11 (92% from 7). Hydrolysis of 11 with TFA gave amine 12 which was treated with 13[5] and DCC in the presence of 1-hydroxybenzotriazole (HOBT)[7] to give 14 (40%). Hydrolysis of 14 with TFA in the presence of thioanisole[8] at room temperature for 15 h gave 15 (63%) which was purified by XAD-2 column chromatography. The UV spectum of 15 was characteristic of psoralen at 250, 265 and 296 nm and the fast atom bombardment mass spectrum showed a molecular ion at m/z 829 $(M+H)^+$ corresponding to $C_{41}H_{52}N_{10}O_9$.[9]

The determination of the photosensitized cross-linking of 15 and 8-methoxypsoralen (16) with λDNA was performed according to the ethidium fluorescence assay.[10] As shown in Fig. 2, 15 and 16 at 10^{-4} M concentration by irradiations were bound with covalently cross-linking to λ DNA over 80% for 30 and 60 min, respectively. The results of the photoreactions indicate that 15 could have a higher binding-affinity for double-stranded DNA than 16.

The cleavage of Col E1 supercoiled covalently closed circular (CCC) DNA(form I) with 15 and 16 to closed circular (CC) (form II) and linear (form III) DNA were carried out as follows. A solution of 15 or 16 in Tris bufffer (pH 8.0) was irradiated at 360 nm[11] at 0 °C for 45 min, and the unreacted psoralen and EDTA in the solution were removed by ultrafiltration using a microconcentrator with Tris buffer. The two cross-linked DNA preparations were reacted in the presence of $FeSO_4$, dithiothreitol (DTT) under air at 0 °C for 30 and 60 min. The reacted DNA was analyzed by means of agarose gel electrophoresis and fluorescence densitometry with ethidium bromide. The photocross-linked cccDNA (form I') of 15 or 16 moved more slowly, with tailing, than the noncross-linked form I which shows no tailing, but faster than form II and from III on the gels. As shown in Table 1, the

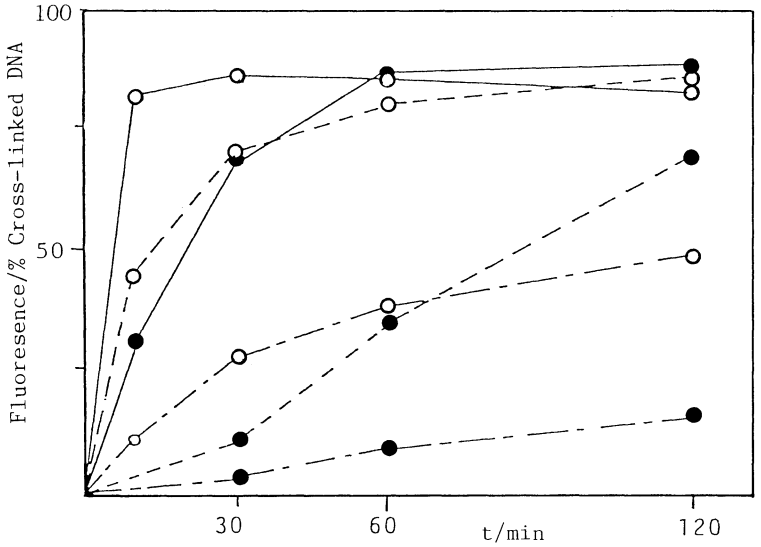


Fig. 2. Photocross-linking of λDNA with 15 and 16. Reactions were performed at 18 °C in a volume of 250 μl buffered at pH 7.0 with 40 mM potassium phosphate, contained 24 μg of λDNA, and were irradiated at 360 nm. Fluoresence reading are those after the heat denaturation and cooling cycle. ○, 15 ; ●, 16 ; ——, 10^{-4}M; – – –, 10^{-5}M; —·—, 10^{-6}M.

results of cleavages of the psoralen-cross-linked DNA were indicated that i) at < 2.5 x 10^{-5} M concentration of 15, the presence of $FeSO_4$ and DTT caused only a small increase in the cleavage (runs, 3 and 4); ii) at > 5.0 x 10^{-5} M cencentrations of the substrates, the rate of (15)-cross-linked DNA cleavage increased greater than that of (16)-cross-linked DNA (runs, 6-8 and 11-13), although certain cleavage of the psoralen-cross-linked DNA was occurring during the ultrafiltration (runs, 5 and 9).

Table 1. Photocross-linking and the Cleavage of DNA Using 15 and 16[a)]

Run	Compound	[b)] Concentration of 15 or 16 x10^{-5}M	Concentration of $FeSO_4$ x10^{-5}M	DTT x10^{-3} M	Time min	Ratio of Forms[c)] % I	II	III
1	---[d)]	---	---	---	---	81	19	0
2	15	1.0	---	1.0	30	28	67	5
3		1.0	1.0	1.0	60	25	71	4
4		2.5	1.0	1.0	60	24	67	9
5		5.0	---	---	60	46	47	7
6		5.0	1.0	1.0	30	8	82	10
7		5.0	1.0	1.0	60	0	84	16
8		5.0	5.0	1.0	60	0	80	20
9	16	5.0	---	---	60	52	43	5
10		5.0	---	1.0	60	35	61	4
11		5.0	1.0	1.0	30	35	60	5
12		5.0	1.0	1.0	60	22	73	5
13		5.0	5.0	1.0	60	14	76	10

a) Photoreactions were performed with 100 W high pressure arc lamp at 360 nm[12)] under argon for 45 min at 0 °C. The reaction solutions contained 6.0 µg of Col E1 plasmid DNA and 15 or 16 in a total volume of 150 µl buffered with 40 mM NaOAc /1 mM EDTA(pH 8.0). The unreacted psoralen and EDTA were removed by ultrafiltration using centrifugal microconcentrator "CENTRICON" (AMICON) with buffer (40 mM Tris/5 mM NaOAc)(pH 8.0). The cleavage reactions of the cross-linked DNA were performed at 0 °C in the presence or absence of $FeSO_4$ and /or DTT under air.

b) Initial concentration of 15 or 16 in the photoreactions.

c) Forms I, II, and III were analyzed by agarose (0.9%) gel electrophoresis and quantitated by densitometry after ethidium bromide staining.

d) Purchased Col E1 plasmid DNA contained 0.6 µg.

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