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Intercalation of New Bispsoralen Derivatives into DNA

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1, 4-Bis[n'-(8-psoralenoxy) alkyl] piperazine (Bis(PsC_n)PIP n = 4, 6, 8), new bispsoralen derivatives, showed efficient intercalation into DNA as measured by fluorescence and circular dichroism.

Key words: Bis(PsC_n)PIP (n = 4, 6, 8), Circular dichroism(CD), Fluorescence

INTRODUCTION

Psoralens (Ps), planar tricyclic furocoumarins, present in numerous plants are photosensitizers of UVA especially from 320 nm to 400 nm, a range at which cellular nucleic acids and proteins are weakly absorbing if any at all [1-3]. Because of their skin-photosensitizing properties, these compounds have been used in the photochemotherpy of the skin disorders such as psoriasis and vitiligo [4,5], and have recently been used in sterilization of human blood fractions for transfusion [6], and sterilization of polymerase chain reaction (PCR) carryover [7]. However, the water solubility of clinically used psoralens, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4, 5', 8-trymethylpsoralen (TMP), is a limiting factor in the treatment of skin disorders and photodetoxification of blood and blood products prior to transfusion. The preparation of highly water-soluble psoralen derivatives is, therefore, very important for effective PUVA therapy [8].

Binding of psoralens to DNA is generally the consequence of two successive events [9]. (1) Intercalation into DNA between the base pairs and, (2) photocycloaddition reaction of 3, 4-pyrone double bond and/or 4', 5'-furan double bond with 5, 6-double bond of thymine.

Bispsoralens have four photoreactive double bonds in a molecule as shown in Fig. 1 and consequently they have a high probability of photobinding to DNA. They can cross-link effectively not only just one double strand of DNA but also crosslink another adjacent double strand of DNA simultaneously leading to multi-strand cross-linked DNA. This will lead to very effective inactivation of DNA function. Most of the bispsoralens linked by polymethylene bridge, however, have very low water solubility limiting the utility of these molecules in clinics. Bispsoralens linked by polymethylene bridges containing piperazine are expected to give high water solubility and easily intercalate into DNA, not only into a double strand DNA, but also into adjacent DNA strands simultaneously and have effective photobiological effects.

We prepared 1, 4-bis[n'-(8-psoralenoxy) alkyl] piperazine (Bis(PsC_n)PIP, n = 4, 6, 8) having two psoralen moieties and investigated the intercalation of these compounds into DNA.

MATERIAL AND METHODS

Instruments. Circular dichroism (CD) experiments were conducted on a Jasco J-715 spectropolarimeter. UV absorption spectra were recorded on a Shimadzu 3100S spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS-50 luminescence spectrometer with a gated photomultiflier tube detector at room temperature.

Materials. 8-Methoxypsoralen (8-MOP) was obtained from Sigma Chemical Co. All the solvents were reagent grade or HPLC grade and purified according to the literature procedure [10]. Spectroscopic grade ethanol, and methylene chloride were purchased from Merck and used as received. 1, 4 - Bis[(4'-(8-psoralenoxy))butyl]piperazine (Bis(PsC_4)PIP), 1, 4 - bis[(6'-(8-psoralenoxy))hexyl]piperazine (Bis(PsC_6)PIP) and 1, 4 - bis[(8'-(8-psoralenoxy))octyl]piperazine (Bis(PsC_8)PIP) were synthesized by the reported procedures [11]. The calf thymus DNA was obtained from Sigma Chemical Co. All the other chemicals were of analytical grade.

Intercalation studies by circular dichroism (CD). The DNA solutions for circular dichroism (CD) measurements contained 1 $\times 10^{-4}$ M calf thymus DNA and 3.3$\times 10^{-5}$ M bispsoralens in Tris

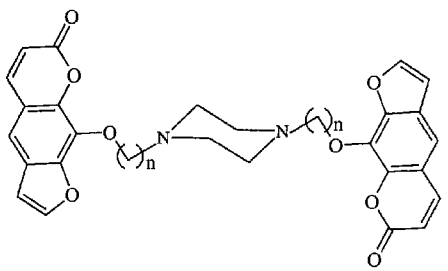

Figure 1. Chemical structure of Bis(PsC_n)PIP, n = 4, 6, 8

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HCl and 0.2 M NaCl solution, pH 7.4 and 5% ethanol as cosolvents.

Intercalation of Bis(PsC$_n$)PIP into calf thymus DNA. The stock solution of DNA is prepared in distilled water (5.0 mg/10 mL) containing 2 mM NaCl. The solutions of 50 μM 8-MOP and Bis(PsC$_n$)PIP (n = 4, 6, 8) are prepared in distilled water containing 3% ethanol. Intercalation of psoralens into DNA was determined monitoring the change of the fluorescence intensities on addition of DNA to each sample.

RESULTS AND DISCUSSION

Intercalation studies by circular dichroism (CD)

When psoralen molecules intercalate into DNA, UV absorbance decreases, the viscosity of the solution increases, and circular dichroism (CD) spectra are affected. As shown in Fig. 2, the CD spectra of calf thymus DNA changed on addition of the three bispsorlens prepared in a similar pattern indicating the formation of similar intercalation complexes between DNA and bispsoralens. Better intercalation is observed from shorter bridging alkyl chains. The positive band at 275 nm is decreased on the addition of psoralens while the negative band at 300 nm is increased [12,13].

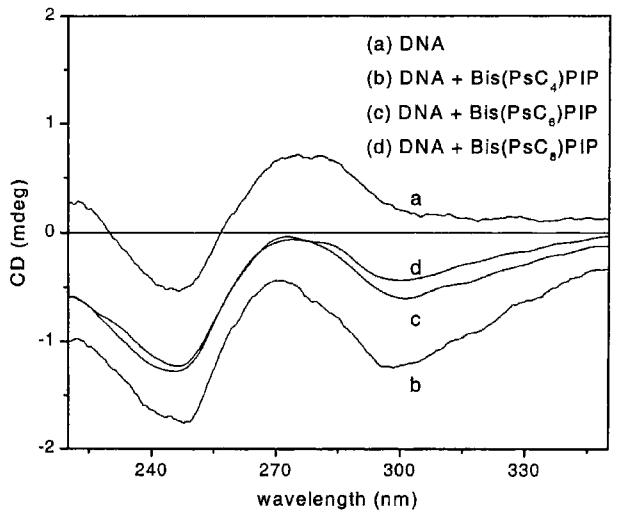

Figure 2. CD spectra of calf thymus DNA in the absence and in the presence of Bis(PsCn)PIP (n = 4, 6, 8). a: DNA alone (1×10^{-4} M), b: DNA (1×10^{-4} M) and Bis(PsC$_4$)PIP (3.3×10^{-5} M), c: DNA (1×10^{-4} M) and Bis(PsC$_6$)PIP (3.3×10^{-5} M), d: DNA (1×10^{-4} M) and Bis (PsC$_8$)PIP (3.3×10^{-5} M).

Fluorescence studies on the intercalation of Bis(PsC$_n$)PIP into calf thymus DNA

It has been reported that addition of DNA changes the fluorescence spectra of psoralens, both the intensity and emission maxima [14,15]. The fluorescence of 8-MOP was reduced as calf thymus DNA is added to the solution but λ_{max} at 500 nm and shape of the fluorescence spectra did not change (Fig. 3(I)). On the contrary, a new maximum at 365 nm

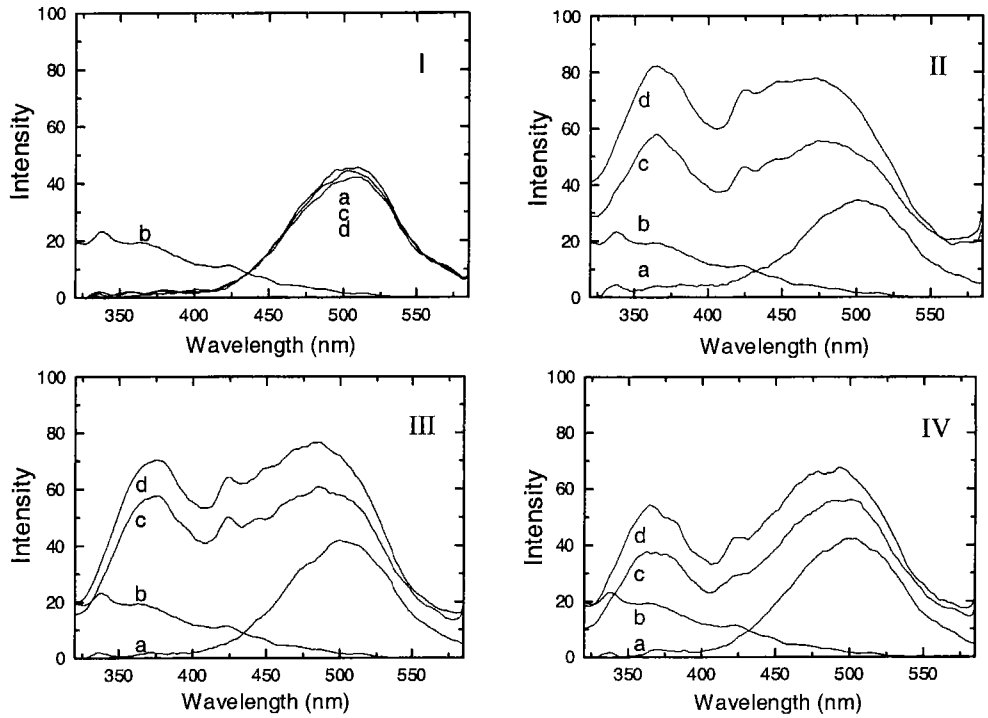

Figure 3. The effects of calf thymus DNA on the fluorescence spectra of psoralens. A new emission peak appeared at 370 nm due to the formation of bispsoralen - DNA molecular complex with λ_{ex} at 300 nm. I: 8-MOP, II: Bis(PsC$_4$)PIP, III: Bis(PsC$_6$)PIP, IV: Bis(PsC$_8$)PIP. a: psoralen alone (50 μM), b: DNA (5 μg /10 mL), c: psoralen + 25 μL DNA, d: psoralen + 50 μL DNA.

appeared along with 500 nm peak in the fluorescence spectra of bispsoralens as calf thymus DNA is added indicating the formation of the excited complex (exciplex) between the excited state psoralens and the ground state DNA (Fig. 3). When DNA concentration is increased, the new emission maximum at 365 nm is red-shifted by 2-3 nm and λ_{max} at 500 nm is blue shifted by 5-30 nm. As the alkyl chain is shortened ($n = 8 \rightarrow 6 \rightarrow 4$), the overall fluorescence intensity is increased but the relative intensity around 500 nm is decreased and blue shifted on the increase of DNA concentration. The results indicate more efficient intercalation of bispsoralens into DNA than monopsorlens and exciplexes are formed between Bis(PsC_n)PIP (n = 4, 6, 8) and DNA. The intercalation efficiency was in the order of Bis(PsC_4)PIP > Bis(PsC_6)PIP > Bis(PsC_8)PIP. The high efficiency of intercalation and exciplex formation should lead to high quantum yield of photoaddition of bispsoralens to DNA and consequently high efficiency of DNA inactivation on irradiation [16-18].

In conclusion, the Bis(PsC_n)PIP (n = 4, 6, 8) containing the piperazine moiety show high water solubility and more efficient intercalation into DNA than 8-MOP.

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